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Fabio Bagnoli
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Staphylococcus aureus

Microbiology, Pathology, Immunology, Therapy and Prophylaxis

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Declaration of Interest

Fabio Bagnoli and Rino Rappuoli are employees of GSK Vaccines and own GSK stocks. Fabio Bagnoli owns patents on *S. aureus* vaccine candidates. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Authorship

Fabio Bagnoli and Rino Rappuoli were involved in the conception and design of the book and approved its content before publication.

Preface

Staphylococcus aureus is a leading pathogen in surgical site, intensive care unit, and skin infections as well as health-care associated pneumonias. These infections are associated with an enormous burden of morbidity, mortality and increase of hospital length of stay and patient cost. *S. aureus* is impressively fast in acquiring antibiotic resistance and multidrug resistant strains are a serious threat to human health. It has been recently estimated that deaths attributable to antibiotic resistant infections will exceed the ones caused by cancer by 2050 (https://amr-review.org/Publications). *S. aureus*, was included among the ESKAPE pathogens (*Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) recognized as the leading cause of antibiotic-resistant infections occurring worldwide in hospitals. Due to resistance or insufficient effectiveness, antibiotics and bundle measures leave a tremendous unmet medical need worldwide. In addition there are no licensed vaccines or immunotherapies on the market despite the significant efforts done by public and private initiatives.

This book includes 16 chapters spanning from basic Microbiology and Immunology aspects to Pathology of key disease manifestations as well as a review of current standard of care. Furthermore, front-edge discoveries on therapeutic and prophylactic approaches alternative to antibiotics are reviewed.

Given the complexity of the Microbiology of this pathogen we decided to give significant emphasis to this aspect. We started describing conventional and molecular diagnostics-based identification methods of *S. aureus* in the microbiology laboratory. Rapid and more informative typization tests are likely to represent a significant benefit for improving clinical practice and containing the emergence of antimicrobial resistance. Methicillin-resistant *S. aureus* (MRSA) is a global issue causing increase of mortality and the need to use last-resource antibiotics. Predominant clones circulating worldwide and the associated antibiotic resistance are described.

Sugar and protein surface structures of the bacterium are comprehensively discussed. These components play key roles in cell viability, virulence and evasion of host defences. The major surface polysaccharides include the capsular

polysaccharide (CP), cell wall teichoic acid (WTA), and polysaccharide intercellular adhesin/poly-β(1–6)-N-acetylglucosamine (PIA/PNAG). They play distinct roles in colonization and pathogenesis and are being explored as targets for antimicrobial interventions.

Surface proteins have very diverse functions (e.g., adhesion, invasion, signalling, conjugation, interaction with the environment and immune-evasion). They have been categorized into distinct classes based on structural and functional analysis. We provide the defining features associated with cell wall-anchored surface proteins and a framework for their categorization based on the current knowledge of structure and function.

On top of surface virulence factors, *S. aureus* secretes pore-forming toxins that kill eukaryotic immune and non-immune cells. Here we provide an update on the various toxins, the identification of its receptors on host cells, and their roles in pathogenesis.

S. aureus pathogenicity is driven by the wealth of virulence factors and its ability to adapt to different environments. The latter is due to the presence of complex regulatory networks fine-tuning metabolic and virulence gene expression. One of the most widely distributed mechanisms is the two-component signal transduction system (TCS) that can reveal an environmental signal and trigger an adaptive gene expression response. It encodes a total of 16 conserved pairs of TCS that are involved in diverse signalling cascades ranging from global virulence gene regulation such as quorum sensing by the Agr system, the bacterial response to antimicrobial agents, cell wall metabolism, respiration and nutrient sensing. Herein we give an overview of the current knowledge on TCS and its influence on virulence gene expression.

The versatility of *S. aureus* is reflected by the wide range of disease that it can cause. It's a leading cause of bacteraemia, infective endocarditis, osteomyelitis, pneumonia, indwelling medical device related infections, as well as skin and soft tissue infections (SSTIs). SSTIs are among the most common infections worldwide. They range in severity from minor, self-limiting, superficial infections to life-threatening diseases requiring all the resources of modern medicine. They have variable presentations ranging from impetigo and folliculitis to surgical site infections (SSIs). Here we describe the anatomical localization of the different SSTI associated with *S. aureus*, the virulence factors known to play a role in these infections, their current epidemiology as well as the standard of care and potential prophylaxis.

Musculoskeletal infections, bacteremia and infective endocarditis associated to *S. aureus* infections are very difficult to treat and important causes of morbidity and mortality. Osteomyelitis can cause long-term relapses and functional deficits and bacteremia and infective endocarditis are associated with excess mortality when compared to other pathogens. Although considerable advances have been achieved in their diagnosis, prevention and treatment, the management remains challenging and impact on the healthcare system is still very high.

S. aureus can also infect several animal species (e.g., cattle, poultry and pigs) and transmission from animals to humans and vice versa has been observed. This

represents an important threat to public health, as animal strains can adapt to the human population and spread additional antibiotic resistance.

Medical need associated to *S. aureus* infections is enhanced by raising prevalence of multidrug resistant strains and acquisition of resistance to last resort antibiotics. Therefore, alternative medical interventions are urgently needed. Vaccines certainly represent one of the most important options. Unfortunately a correlate of protection against *S. aureus* is not known and this represents a significant issue for developing vaccines. Herein, we review what is known and unknown about innate and adaptive immunity against this complex pathogen. We provide an overview on the major cell types involved in innate immune defence and major differences of the immune response during colonization versus infection. Although the contribution of adaptive immunity against *S. aureus* is not yet clear, there are accumulating evidence both from animal models and from human data that T cell- and B cell-mediated adaptive immunity can control the infection. Unfortunately *S. aureus* has evolved several mechanisms to manipulate innate and adaptive immune responses to its advantage. Indeed, it expresses factors able to interfere with many critical components of the immune system and hamper proper immune functioning. In recent years research, including structural and functional studies, has fundamentally contributed to our understanding of the mechanisms of action of the individual factors.

In addition to the lack of a known correlate of protection, failure of developing an effective vaccine against this pathogen is likely due to several other reasons. Indeed. all attempts so far targeted single antigens, contained no adjuvants and efficacy trials were performed in severely ill subjects. We show the link between Phase III clinical trial data of failed vaccines with their preclinical observations and we provide a comprehensive evaluation of potential target populations for efficacy trials taking into account key factors such as population size, incidence of *S. aureus* infection, disease outcome, primary endpoints as well as practical advantages and disadvantages.

The last chapter provides an overview of a promising new therapeutic approach. Lysins are a new class of anti-infectives derived from bacteriophage, which cleave cell wall peptidoglycan causing immediate bacterial lysis. Importantly, lysins have high specificity for the pathogen and low chance of bacterial resistance.

In conclusion, this volume gives a comprehensive overview of the Microbiology, Pathology, Immunology, Therapy and Prophylaxis of *S. aureus* reviewing recent findings and knowledge on very diverse arguments and at the same time linked to each other. That is the uniqueness behind a book like this and the added value towards a search in literature databases.

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Carriage, Clinical Microbiology and Transmission of *Staphylococcus aureus*

Anna Aryee and Jonathan D. Edgeworth

Abstract *Staphylococcus aureus* is one of the most important bacterial pathogens in clinical practice and a major diagnostic focus for the routine microbiology laboratory. It is carried as a harmless commensal in up to two-thirds of the population at any one time predominantly not only in the anterior nares, but also in multiple other sites such as the groin, axilla, throat, perineum, vagina and rectum. It colonizes skin breach sites, such as ulcers and wounds, and causes superficial and deep skin and soft tissue infections and life-threatening deep seated infections particularly endocarditis and osteomyelitis. *S. aureus* is constantly evolving through mutation and uptake of mobile genetic elements that confer increasing resistance and virulence. Since the 1960s, hospitals have had to contend with emergence of methicillin-resistant *S. aureus* (MRSA) strains that spread better in hospitals than methicillin-susceptible *S. aureus* (MSSA) and are harder to treat. Since the 1980s, distinct community MRSA strains have also emerged that cause severe skin and respiratory infections. Conventional identification of MSSA and MRSA in the microbiology laboratory involves microscopy, culture and biochemical analysis that for most samples is straightforward but slow, taking at least 48 h. This delay has significant consequences for individual patient care and public health, through inadequate or excessive empiric antibiotic use, and failure to implement appropriate infection control measures for MRSA-colonized patients during those first 48 h. This unmet need has driven development of rapid molecular diagnostics that either complement or replace conventional culture techniques in the laboratory, or can be placed in the clinical environment as point-of-care (POC) devices. These new technologies provide results to clinicians anything from within an hour to 24 h, depending on sample and clinical setting, and should transform management of patients with *S. aureus* and other bacterial diseases; however, uptake is often slow due to the disruptive effect of new technologies, costs of transition and uncertainty

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of the optimal solution given successive advances. More evidence of the health economic, clinical and antimicrobial resistance benefit will help support introduction of these new technologies. Finally, preventing MRSA transmission has been a priority for healthcare organizations for many years. There have been significant recent reductions in transmission following local and national campaigns to re-enforce basic and heightened infection control interventions such as universal hand hygiene, barrier nursing, decolonization and isolation of MRSA-colonized patients detected through routine culture or screening policies. Developments in whole genome sequencing are providing greater insight into reservoirs and routes of transmission that should help better target interventions to ensure sustainable control of endemic strains and to identify and prevent emergence of new strains.

Contents

1 Clinical Microbiology

Staphylococcus aureus is a facultative anaerobe belonging to the genus *Staphylococcus* within the family of *Staphylococcae*. It is one of the most commonly identified clinically significant bacteria in a routine microbiology laboratory, and its identification by traditional techniques is a straightforward, albeit slow process, which is becoming more rapid with the introduction of molecular techniques.

Upon receipt of samples in the laboratory, Gram staining can be performed on sterile site samples such as pus and deep respiratory specimens to identify the presence of bacteria by light microscope. Staphylococci appear as irregular small clusters of Gram-positive cocci and traditionally no further information is available to the clinician on the first day. Samples are cultured on blood agar for 18–24 h when *S. aureus* colonies appear glistening, smooth and translucent, often with a golden pigment. Presumptive colonies are confirmed as *S. aureus* at this point using the techniques described below, although plates are usually re-incubated for a

further 24 h to detect slower growing colonies. Antibiotic susceptibility testing can also be set up on colonies identified at 24 h. By 48 h, colonies are approximately 1–2 mm in diameter and often exhibit a small zone of β-haemolysis. Thus, in a traditional laboratory, the clinician can expect to be told if staphylococci are present in important sterile site samples on the day of sample collection, whether *S. aureus* is present in the sample the following day, and receive a final report with antibiotic susceptibilities the day after.

A variety of biochemical tests are used to identify *S. aureus* colonies based on production of coagulase and deoxyribonuclease, presence of *S. aureus* specific antigens or the ability to ferment mannitol. The tube coagulase test is the traditional gold standard for discriminating between *S. aureus* and other staphylococci, usually referred to as coagulase-negative staphylococci (CoNS). This is a clinically important distinction because CoNS are rarely pathogenic in the absence of prosthetic material upon which they can reside in biofilm, although it is recognized that some CoNS are coagulase positive and some coagulase-negative *S. aureus* isolates have been reported (Vandenesch et al. 1993). The slide coagulase test is a more rapid test based on the presence of clumping factor, but up to 15 % of *S. aureus* isolates are negative. Latex agglutination tests detecting protein A, clumping factor and other surface antigens are also sensitive although less specific due to cross-reactivity with various CoNS.

Antimicrobial susceptibility testing is set up at the same time as identification of *S. aureus* using a number of culture-based methodologies. Disc diffusion testing is often used to assess simultaneous susceptibility to a variety of antibiotics. A key focus is to distinguish between methicillin-susceptible and methicillin-resistant *S. aureus*. This can be done using an oxacillin or cefoxitin disc, which has been shown to be an accurate surrogate marker for methicillin resistance (Skov et al. 2006). Antibiotic susceptibilities can also be performed using commercially available automated platforms such as the Vitek®2, BD Phoenix™ or MicroScan WalkAway systems.

An additional important focus for the microbiology laboratory is the specific detection of methicillin-resistant *S. aureus* (MRSA) in screening swabs from carriage and clinical sites, particularly the anterior nares, to identify colonized patients and institute infection control precautions (Coia et al. 2006). Many laboratories inoculate screening swabs directly onto selective agar, particularly chromogenic agars that provide a presumptive positive identification of MRSA within 24 h of sample receipt in the laboratory (Nahimana et al. 2006; Denys et al. 2013). Excluding presence of MRSA requires a further 24 h, and presumptively positive samples should be confirmed by antimicrobial susceptibility testing.

The analysis of blood cultures differs from other samples. 10–15 ml of blood is inoculated into media bottles immediately after collection from the patient and sent to the laboratory where they are placed into automated incubators. Positive cultures are flagged when bacterial growth is detected usually by continuous monitoring of changes in pH due to CO_2 production. The time taken for automated systems to detect bacteria depends on the number of bacteria in the sample (which can be up to 200 CFU/ml for endovascular infection down to <10 bacteria per ml of blood) and

the initial viability of bacteria that may either be intracellular or dormant. For *Staphylococcus aureus* bacteriaemia (SAB), over 80 % of positive culture bottles flag within 24 h (Khatib et al. 2005). Gram staining is performed on an aliquot of a flagged bottle to identify staphylococci, although this information has only limited clinical benefit because CoNS are more frequently identified in blood cultures. Conventionally, flagged blood culture media is plated onto agar that provides identification and disc diffusion susceptibility testing results the following day.

1.1 Introduction of Rapid Molecular Detection Methodologies

The slow nature of culture and biochemical-based detection methods means that identification and antimicrobial susceptibility of *S. aureus* only becomes available about 48 h after initial key clinical management decisions are made, and this is recognized as a major clinical and public health problem. For the individual patient, if serious *S. aureus* infection was not clinically suspected, then the patient may not be started on appropriate initial antibiotic therapy, particularly if the *S. aureus* is methicillin resistant, and this delay has been associated with higher mortality in some studies (González et al. 1999; Soriano et al. 2000). At a population level, uncertainty about whether an acute illness is bacterial or the likely antimicrobial susceptibilities prompts empiric treatment with broad-spectrum antibiotics to cover a range of potential bacterial causes including MRSA. This presents a public health problem due to overuse of empiric antibiotics that drives antibiotic resistance. There is also delay in identifying MRSA-colonized patients and instituting infection control precautions, which increases the potential for nosocomial transmission.

These unmet clinical needs have driven the development of rapid molecular diagnostics throughout the patient pathway to speed up time to detection and reporting of pathogenic bacteria including *S. aureus*. In the laboratory, these molecular methods can either enhance traditional culture-based processing or completely replace culture-based techniques.

1.2 Enhancing Culture-based Techniques

This involves rapid laboratory-based molecular analysis of *S. aureus* colonies or flagged positive blood culture bottle after initial culture of specimens for 24 h or more. Many laboratories have introduced matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS), which identifies bacterial colonies by analysing the protein composition of the bacterial cell (Wieser et al. 2012). This new technology has transformed species identification in

microbiology laboratories allowing bacterial identification within minutes: it is cheaper, more accurate and usually faster than biochemical-based methodologies and can replace most traditional biochemical tests. It was initially applied to bacterial colonies but has also been successfully applied to aliquots of blood culture sample that have flagged as positive (Mestas et al. 2014). Results are available within an hour although identification of Gram-positive bacteria is less effective than Gram-negative bacteria (78 % vs. 90 %). Additionally, rapid latex agglutination tests can be performed on single colonies or positive blood culture bottles to detect PBP2a as a marker of MRSA (Brown and Walpole 2001; Chapin and Musgnug 2004).

There have also been advances in the rapid nucleic acid based detection of organisms including MSSA and MRSA from flagged positive blood culture bottles (Opota et al. 2015). The Cepheid Xpert system uses PCR to identify *S. aureus* and MRSA direct from positive blood culture samples in about 2 h. PCR correlated with culture results in 80/82 (97.5 %) flagged blood culture bottles containing GPC in clusters by microscopy (Ratnayake and Olver 2011). Nanosphere's Verigene Gram-positive blood culture test allows rapid identification of both MSSA and MRSA from positive blood culture samples in less than 3 h. Mono-microbial bacterial isolates were correctly identified in 147 of 148 flagged blood culture bottles containing Gram-positive bacteria (38 MRSA or MSSA) (Beal et al. 2013). The FilmArray Blood Culture ID panel identifies 24 organisms and 3 antibiotic resistance genes including *S. aureus* and *mec*A in positive blood culture samples in approximately 1 h. The FilmArray correctly identified 19 *S. aureus* isolates from 167 mono-microbial flagged blood culture bottles and 156 (91.6 %) isolates overall (Altun et al. 2013). These technologies reduce by about 24 h the time to provide clinicians with a *S. aureus* identification and methicillin susceptibility result from blood cultures.

Rapid non-nucleic acid-based technologies are also under development. Accelerate diagnostics have a platform that uses automated digital microscopy and high-resolution growth analysis to provide identification and antimicrobial susceptibility data from blood and other sterile samples in approximately 5 h. It correctly identified all 77 MRSA and 54 MSSA mostly reference isolates in one study (Price et al. 2014a). Specific Technologies are developing a system that detects mixtures of volatile organic compounds using a colorimetric array integrated into a blood culture bottle, allowing faster detection and identification than traditional methods, although it does not provide susceptibility data (Lim et al. 2014).

1.3 Replacing Culture-based Techniques

Technologies are being developed to provide bacterial identification and genotypic prediction of antimicrobial susceptibilities directly on primary samples. Some are designed to analyse clinical samples including blood cultures and include a broad

range of bacteria. For example, Abbott's Iridica system identifies over 750 bacteria and 4 antibiotic resistance genes (including mecA) from a range of samples including whole blood and respiratory specimens in under 6 h. The Mobidiag Prove-It™ Bone & Joint StripArray system identifies over 30 Gram-positive and Gram-negative bacterial species and various genotypic resistance determinants including the *mecA* gene from synovial fluid, bone biopsy and tissue in 3.5 h from DNA extraction. In one study, 8 of 38 prosthetic joint infection samples culture positive for *S. aureus* were also identified by PCR and there was one additional PCR positive sample in a patient who had received antibiotics before sample collection that was culture negative (Metso et al. 2014). The Curetis Unyvero pneumonia platform detects 18 bacteria including *S. aureus* and the mecA gene directly from respiratory samples in 4–5 h (Jamal et al. 2014). Direct molecular analysis of clinical samples rather than a colony or suspension after culture allows same day detection of pathogens including MSSA and MRSA and rapid targeting of appropriate therapy.

Diagnostics have also been developed for the specific detection of *S. aureus* and the *mecA* gene including the Cepheid Xpert systems, the LightCycler MRSA Advanced and BD Max MRSA. These PCR tests take about 2 h (Rossney et al. 2008; Peterson et al. 2010; Widen et al. 2014) and have comparable sensitivity and specificity to enrichment and plating on different chromogenic agars (all > 92 %) whilst saving 24–72 h (Lee et al. 2013). Agreement between enriched culture and PCR was 96 % in this study. Although these *S. aureus* specific tests have been predominantly applied to MRSA screening swabs to target infection control interventions, they could also be used on clinical samples such as skin and soft tissue samples (Wolk et al. 2009) and respiratory specimens (Cercenado et al. 2012) to target early appropriate therapy.

Although molecular diagnostics dramatically reduce analysis time and can provide same day identification of MSSA and MRSA, the adoption into routine laboratory service is not straightforward. Molecular technologies are usually more expensive than traditional techniques, require a period of double running during evaluation and are then often used alongside rather than completely replacing the routine culture bench, so fixed costs remain. The time taken to transport specimens to the laboratory, particularly when the laboratory is off-site, can make a same-day test into a next-day test for a significant proportion of specimens, particularly if batch processing rather than random access platforms is used (Jeyaratnam et al. 2008). Samples submitted in the afternoon may provide results in the middle of the night when specialists who advise on result interpretation and patient management may not be available; hence, decisions may be postponed until the following day, when results would have become available using culture-based techniques: this may be particularly relevant if the advice is to narrow antibiotic spectrum. Molecular diagnostics are therefore disruptive for the laboratory and clinical teams, and adoption will require evidence of clinical benefit and cost effectiveness, and education of staff across the clinical pathway to realize the anticipated benefits of rapid diagnostics (Wassenberg et al. 2011; Van Der Zee et al. 2013).

1.4 Point-of-Care Technologies

An even more radical advance for clinical microbiology is the movement of diagnostics out of the laboratory to the ward or bedside. Laboratory platforms or new point-of-care (POC) devices that can rapidly identify pathogens including MSSA and MRSA are being evaluated on the wards. Unlike laboratory-based testing, sample analysis at the bedside can influence initial empiric treatment and infection control decisions. Some studies have assessed laboratory platforms such as the Cepheid Xpert system to detect MRSA on the ICU, in general ward and in outpatient clinics (Leone et al. 2013; Parcell and Phillips 2014). Many companies are also developing small bench top devices that are specifically designed for use by non-laboratory personnel on the wards; for example, Cobas and Alere-I, Atlas Diagnostics, Enigma Diagnostics, BioCartis Idylla, Orion Diagnostica and GNA Biosolutions and clinical utility studies are being performed for some pathogens (Binnicker et al. 2015; Goldenberg and Edgeworth 2015; van den Kieboom et al. 2015). Even closer to the patient from a ward-based to bedside-based system, a hand-held device is being developed that can identify MSSA or MRSA within 30 min (www.quantumdx.com). This field is in its infancy, and the technology advancing so rapidly, it is unclear what, where and when rapid POC infectious diseases diagnostics will enter into routine clinical practice. There will be many factors to consider including training front-line staff, quality control, accreditation, regulatory and legal constraints, linking results to hospital health records, resolving discrepancies between POC- and laboratory-generated results, and having mechanisms to alert specialist teams for advice and follow-up. At an organizational level, there will need to be strong governance processes to ensure POC devices are introduced safely and consistently, recognizing that the clinical environment is more complex than the laboratory, where there is a tradition of high-quality process control. Health economic evaluations that incorporate all the costs and benefits of laboratory versus POC-based testing will be needed to support decision-making of clinicians and managers.

2 *S. aureus* Carriage

Staphylococcus aureus is part of the commensal flora of human skin and mucosal surfaces, in addition to being a pathogen capable of causing both superficial infections and invasive disease with considerable associated morbidity and mortality. The anterior nares are the main reservoir of *S. aureus* carriage in humans. Other carriage sites include the skin, perineum, pharynx, gastrointestinal tract, vagina and axillae (Wertheim et al. 2005). About one-third of the population carry *S. aureus* on skin and mucosal sites at any one time (Kluytmans et al. 1997). Some individuals harbour the same strain over an extended period of time, whereas others carry different strains. *S. aureus* may also be present at different anatomical sites with varying frequency in different populations (Wertheim et al. 2005).

The variability in the detection of *S. aureus* at carriage sites has led to the description of distinct states, with potentially distinct underlying mechanisms. In the early 1960s, carriage was designated into four groups, persistent, intermittent, occasional and non-carriage (Williams 1963), but most studies now recognize three states: persistent, intermittent and non-carriage (Nouwen et al. 2004). It has been reported that approximately 60 % of the population are intermittent carriers, whilst 20 % each are either persistent carriers or non-carriers (Kluytmans et al. 1997). A large longitudinal survey published in 1997 analysing nasal swabs from staff at a university hospital found the same strain of *S. aureus* (confirmed by PGFE) in the same individuals on two occasions eight years apart in 3 out of 17 (18 %) staff members, suggesting that persistence reflects a stable host strain relationship (VandenBergh et al. 1999). However, a longitudinal study of 109 healthy individuals over a period of up to three years found persistent carriers having a resident persistent strain for most of the time but with additional distinct strains at other times (Muthukrishnan et al. 2013).

The prevalence of transient and persistent *S. aureus* nasal carriage varies by geographical location, age, gender and ethnicity. Studies have shown carriage ranges from 9 % in Indonesia to 37 % in Mexico (Lestari et al. 2008; Hamdan-Partida et al. 2010). Carriage is highest amongst newborns (up to 70 %) but steadily decreases in childhood. It has been posited, but not proven, that this may be due to pneumococcal competition or interference by other bacteria present in the nasopharynx in childhood (Lebon et al. 2008). There is another peak at adolescence followed by a decrease in early adulthood. Persistent carriage is seen more frequently in children than adults, and a conversion from persistent to transient or non-carriage most commonly occurs in adolescence (Williams 1963; Kluytmans et al. 1997; Wertheim et al. 2005). Rates of carriage have also been found to be higher in patients with Type 1 Diabetes Mellitus, intravenous drug users, haemodialysis patients, surgical patients, AIDS patients and patients with qualitative or quantitative defects in leucocyte function (Lowy 1998).

The fact that *S. aureus* is found at multiple body sites, that many studies have only looked for nasal carriage, and that detection methodologies are of variable sensitivity complicates our understanding of the significance of carriage states. Evidence from longitudinal studies does imply that persistent carriers and persistent non-carriers are distinct and likely therefore to have an underlying biological explanation, but the significance of transient carriage is less clear. Defining the host and bacterial factors involved in carriage should help resolve this issue. A feature of persistent carriers identified in a number of studies is that they carry a higher bacterial load than intermittent carriers (Nouwen et al. 2004; Van Belkum et al. 2009). This higher bacterial load may mean that persistent carriers are also more likely to be implicated in transmission of *S. aureus*. This also has implications for autoinfection—with persistent carriers at significantly higher risk of this than transient and non-carriers (Von Eiff et al. 2001; Wertheim et al. 2004b, 2005).

Studies have also specifically investigated carriage of MRSA in hospitalized patients, to determine the optimal sites for screening programmes. Screening is often performed at the anterior nares alone but this can miss up to a third of

MRSA-colonized patients (Meurman et al. 2005), particularly those with throat or rectal carriage, and the latter may be particularly important for hospital transmission (Boyce et al. 2007). Screening programmes in high-risk areas often take swabs from multiple carriage sites to ensure colonized patients are detected (Batra et al. 2008). It is, however, unclear whether MRSA has a differential propensity for carriage at particular sites compared with MSSA.

A number of studies have attempted to identify human genetic factors associated with carriage. A study in 2007, conducted as part of the Rotterdam study (a prospective, population-based study of the incidence and risk factors of disease in an elderly population), sought to identify polymorphisms in host inflammatory response genes associated with susceptibility to *S. aureus* carriage and infection. They found the Interleukin 4 (IL4)–524 C/C host genotype was associated with increased risk of *S. aureus* carriage, irrespective of organism genotype. They also found that individuals with the C-reactive protein (CRP) haplotype 1184C; 2042C; 2911C were less likely to be colonized, and that individuals with boils were more likely to be carriers of the *CFH* Tyr402 variant and the *CRP* 2911 C/C genotype (Emonts et al. 2008). A study carried out in 2006 and 2008 compared the genetics of *S. aureus* strains, epidemiological risk factors, antibiotic exposure and allelic polymorphisms of human genes posited to be involved in carriage of persistent carriers as compared to those of volunteers in an isolated population of adult Wayampi Amerindians living in an village in the Amazonian forest. The authors concluded that a specific set of host genetic polymorphisms were the main determinants of *S. aureus* persistent nasal carriage, namely single nucleotide polymorphisms (SNPs) for CRP genes (C2042T and C1184T) and *IL4* genes (*IL4 C524T*) (Ruimy et al. 2010). A further study published in 2006, also as part of the Rotterdam study, examined the role of host polymorphisms in the glucocorticoid receptor gene in persistent *S. aureus* carriage. They found GG homozygotes of the exon 9β polymorphism had a 68 % reduced risk of persistent carriage, whereas carriers of the codon 23 lysine allele had 80 % increased risk (Van den Akker et al. 2006).

3 *S. aureus* Transmission

The high prevalence of transient or persistent carriage with genetically diverse *S. aureus* strains in all human populations makes the epidemiology of *S. aureus* complex. Most attention has focused on transmission during outbreaks, particularly with MRSA or clones that are associated with more frequent and severe disease; however, it is important to also focus on transmission of endemic MSSA clones not least to define the mechanistic basis for successful and outbreak strains. Our understanding of *S. aureus* transmission has advanced dramatically with recent developments in whole genome sequencing (WGS) supported by advances in bioinformatics, mathematical modelling and social network analysis. Sequencing and interpreting hundreds of bacterial genomes is now feasible in some centres

within a reasonable time frame, initially months but now weeks and even days, and at ever-decreasing cost (Price et al. 2013). Traditional phenotypic and genotypic typing techniques such as phage typing, pulsed field gel electrophoresis (PFGE), spa typing and multi-locus sequence typing (MLST) lacked the necessary discriminatory ability to infer possible chains of transmission. This was a particular issue for MRSA, given the limited number of dominant clones in any geographical area (Enright et al. 2002). Consequently, the clinical benefit of molecular typing to support infection control practice was limited, apart from outbreaks with newly introduced clones that were distinct from endemic clones (Edgeworth et al. 2007). In contrast, WGS allows analysis of the entire core genome sequence to identify SNP differences between isolates. The range seen is from complete identity, isolates with perhaps a few tens of SNPs differences, to those that have hundreds or thousands of SNP differences (Harris et al. 2010). With knowledge of the mutation rate, which for *S. aureus* is about 2–5 SNPs per megabase per year (Young et al. 2012; Golubchik et al. 2013), it is theoretically possible to link related isolates to a recent transmission event in a healthcare setting that would indicate a lapse in infection control practice and an opportunity to target training and other interventions (Harris et al. 2013). Sequences of both epidemiologically linked isolates and those with no prior suspected linkage can be compared, allowing both exclusion of an epidemiologically suspected transmission event and inclusion of other cases in a potential chain of transmission that were epidemiologically unsuspected (Harris et al. 2013). However, individuals do not just have one core genome sequence type, but more commonly carry multiple related isolates that can vary up to 20 or even more SNPs. Indeed, in one study a long-stay patient admitted with MRSA to an ICU in Thailand had 99 ST239 MRSA isolates sequenced over a 64-day stay on ICU which revealed 147 SNP differences between sequenced isolates (Tong et al. 2015). There is also evidence that SNP accumulation can occur faster in invasive disease (Young et al. 2012). These observations complicate the linkage of cases based solely on SNP analysis. Nevertheless, the potential of WGS to identify transmission events and therefore target education and infection control interventions in real time justifies the considerable efforts being made to translate this technology from the research setting into clinical practice. The application of WGS to *S. aureus* transmission research and then on to routine clinical practice is a fast-moving field and beyond the scope of further discussion here.

3.1 MRSA Transmission in the Hospital

MRSA was first identified in the UK in 1961, following which a number of distinct dominant clones emerged to spread worldwide. During the 1960s and 1970s, prevalence of methicillin resistance was often reported as being up to about 20 % of all *S. aureus* isolates, and there were many reports of outbreaks (Brumfitt and Hamilton-Miller 1989). There was a general consensus that eradicating MRSA once it had become endemic was almost impossible (Thompson et al. 1982), and some

proposed that attempting control caused more problems than it solved (Barrett et al. 1998). Nevertheless, there were encouraging reports of successful control of endemic MRSA; for example, Denmark had levels of 15 % between 1967 and 1971 that fell to 0.2 % in the 1980s in response to a national control programme (Rosdahl and Knudsen 1991). The Netherlands and Scandinavia implemented an effective national "search and destroy" policy before MRSA became endemic that has been associated with low rates of healthcare-associated (HA) MRSA to this day (Vandenbroucke-Grauls 1996; Wertheim et al. 2004a). During the 1980s and 1990s, prevalence increased further in many countries to between 30 and 50 %, often linked with emergence of a few highly successful geographically restricted HA-clones (e.g. ST5, ST8, ST22, ST36, ST239 and ST247). It is unclear whether this increase in prevalence was due to dominant clones becoming progressively better adapted to spread in the hospital environment (Holden et al. 2010, 2004), or a failure to implement and sustain effective infection control programmes during the first few decades.

3.2 Preventing MRSA Transmission

Colonized patients are the main reservoir of MRSA in hospitals with transmission predominantly occurring from colonized to non-colonized patients via healthcare worker hands that become transiently colonized during delivery of routine care (Thompson et al. 1982; Pittet et al. 2006). Patients are also thought to acquire MRSA from the environment that has become contaminated by shedding of MRSA by colonized patients (Bernard et al. 1999; Bhalla et al. 2004; Sexton et al. 2006; Otter et al. 2011) or from staff carriers, but these are generally considered minor routes in most settings.

Comprehensive guidelines are available providing evidence and recommendations for preventing transmission of multi-drug-resistant bacteria particularly MRSA (Coia et al. 2006; Yokoe et al. 2008). They comprise non-targeted interventions that have an effect on transmission of all pathogens and targeted interventions that are directed specifically against MRSA-colonized patients. Non-targeted interventions include universal hand hygiene, environmental cleaning and reduction in antimicrobial use. Targeted interventions comprise contact precautions with gloves and aprons whilst delivering care, isolation or cohorting of MRSA patients in a side-room, bay or ward with use of dedicated equipment and facilities (e.g. stethoscopes, commodes) and decolonization using surface acting or systemic agents to suppress MRSA. Decolonization is also been used as a non-targeted intervention in the ICU and can be effective against MRSA transmission (Huang et al. 2013). Targeted methods are dependent on identification of MRSA-colonized patients, either through identification in routine clinical specimens or from a risk factor based or universal screening programme.

Infection control interventions are generally implemented as part of a bundle and although there is debate about the relative importance of each intervention, their

heightened implementation at national and institutional over the past 10 years has been associated with a dramatic reduction in endemic levels of MRSA in many countries (Jarlier et al. 2010; Johnson et al. 2012). The national MRSA control programme in England was particularly effective and has led to a greater than 80 % reduction in MRSA in many hospitals. Interestingly, a WGS study performed in an ICU in Brighton in 2012 that had implemented hand hygiene campaign, barrier nursing and decolonization did not find evidence of significant transmission of MRSA or MSSA over a 6-month period (Price et al. 2014b). This contrasts with a study performed in a Thai ICU, where adherence to infection control interventions including hand hygiene was poor, and there was significant number of MRSA transmissions linked to a small number of long-stay patients with prolonged MRSA colonization (Tong et al. 2015).

There are a number of risks to sustainable control of MRSA both in organizations and in countries that have seen impressive recent reductions and those that have maintained low levels for a long period. New HA-MRSA strains may emerge to spread despite current infection control interventions. For example, some strains can acquire clinically significant resistance to antiseptics such as chlorhexidine (Batra et al. 2010), which have become a major component of infection control practice in many countries (Edgeworth 2011; Huang et al. 2013). Some HA-MRSA strains may be intrinsically more transmissible (Cooper et al. 2012), and outbreak still occurs in settings where endemic transmission has been controlled (Harris et al. 2013).

Alternatively, new MRSA strains may emerge from the community and become imported into hospitals. Livestock-associated MRSA (LA-MRSA) clones such as ST-398 have emerged in a number of countries, including Denmark and the Netherlands that hitherto had low rates of MRSA (Verkade and Kluytmans 2014). LA-MRSA have been imported into hospitals, although there is evidence these strains are less transmissible in hospitals than HA-MRSA strains (Hetem et al. 2013; Verkade and Kluytmans 2014). Of perhaps more concern is that successful human CA-MRSA clones known to spread well in the community are transported into hospitals to become endemic and a common cause of nosocomial infection (Seybold et al. 2006; Otter and French 2011). The recent success with control of HA-MRSA was dependent on the hospital being the main reservoir. If the community were to become the main reservoir, sustained control of nosocomial MRSA infections would be much more challenging (Tosas Auguet et al. 2016).

3.3 MRSA Transmission in the Community

Although HA-MRSA-colonized patients return to the community where infection control practice is minimal, there has been little evidence that such strains undergo

sustained transmission outside a healthcare facility (Tosas Auguet et al. 2016). Since the 1980s, MRSA outbreaks have been increasingly described in the community with individuals and groups that had no epidemiological exposure with hospitals (Fridkin et al. 2005; David and Daum 2010). These strains were also genotypically distinct from the known HA-MRSA strains in that area. They were frequently found to carry the panton valentine leucocydin (PVL) gene and were characterized by clusters of severe skin and soft tissues infection and sporadic severe necrotizing pneumonia with concomitant influenza infection in children and young adults that had a high mortality (Gillet et al. 2002). In the USA, a dominant CA-MRSA strain, USA300, has spread rapidly to become a leading cause of skin and soft tissue disease in the community (King et al. 2006) and as a cause of abscesses presenting to emergency departments across the USA (Moran et al. 2006). USA300 is the dominant clone in some other countries (Reyes et al. 2009; Deleo et al. 2010), but in most countries different clones have emerged often with no one clone dominating (e.g. ST80/81, SWP, ST22) (Deleo et al. 2010; Otter and French 2010; Chuang and Huang 2013). Outbreaks of skin and soft tissue infection have common risk factors of overcrowding, frequent skin abrasion or limited personal hygiene, such as with contact sports, in prisons, amongst intravenous drug users and indigenous communities (Campbell et al. 2004; Kazakova et al. 2005).

Studies have identified the home as the main setting for amplification of successful clones in a community, with links from there to schools, the work place, sports clubs and other places where there is frequent human contact (Davis et al. 2012; Knox et al. 2015). Therefore, although the literature is dominated by reports of outbreaks in community facilities (Campbell et al. 2004; Kazakova et al. 2005), it is proposed that most transmission actually takes place in the home (Macal et al. 2014; Knox et al. 2015). WGS analysis is being applied to CA-MRSA transmission studies in the community and households where environmental contamination is thought to play an important role in transmission and infections (Knox et al. 2012; Eells et al. 2014). Attempts have been made to apply infection control interventions to prevent transmission and infection in homes, but decolonization and household cleaning has had only limited success (Fritz et al. 2012; Miller et al. 2012).

It is unclear what underpins the dominance of clones such as USA300 or of successful international HA-MRSA clones such as ST239 and ST22. Recent evidence that MRSA clones can differ in their transmissibility implies there is a bacterial genetic basis for emergence of dominant clones (Cooper et al. 2012; Hetem et al. 2013). Use of WGS for surveillance and analysis of emerging endemic and outbreak clones (Holden et al. 2010; Harris et al. 2013; Miller et al. 2014) may help identify genetic markers of increased transmissibility that can help rapidly target interventions. Indeed, although recent control of endemic HA-MRSA was achieved largely without molecular diagnostics and WGS, such technologies may prove vital in the future for identifying new clones that have overcome current preventative strategies and help us keep ahead of this highly versatile and virulent pathogen.

4 Summary

This chapter has provided an overview of where *S. aureus* is carried on the human body, traditional and emerging molecular technologies for identification and genetic analysis of sampled isolates, and how that information is used to prevent and treat infection due to particularly MRSA but also MSSA strains. A particular priority focus of infection prevention and control teams on MRSA over the last 10 years drove development and introduction of rapid molecular techniques. However, in many countries MRSA prevalence has now fallen dramatically and other emerging nosocomial bacteria, particularly multi-drug resistant GNB, are gaining more attention. MRSA molecular diagnostics introduced at the height of the epidemic have often been de-commissioned, returning to slower but usually cheaper methods, such as chromogenic agar sometimes supported by culture automation platforms to reduce laboratory costs. Similarly, landmark WGS MRSA transmission studies that pointed towards their imminent introduction into routine service, now seems a more distant proposition in most settings. These developments illustrate the pragmatic nature of service laboratories that constantly adapt to changing clinical need and laboratory cost pressures. Indeed, looking ahead to when new potentially more virulent and transmissible MRSA or MSSA clones emerge, experience already gained with rapid molecular and WGS techniques will facilitate rapid re-deployment to play an important role in guiding infection control and treatment decisions.

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Worldwide Epidemiology and Antibiotic Resistance of *Staphylococcus aureus*

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Abstract *Staphylococcus aureus* is an important human pathogen, responsible for infections in the community and the healthcare setting. Although much of the attention is focused on the methicillin-resistant "variant" MRSA, the methicillin-susceptible counterpart (MSSA) remains a prime species in infections. The epidemiology of *S. aureus*, especially of MRSA, showed a rapid evolution in the last years. After representing a typical nosocomial multidrug-resistant pathogen, MRSA has recently emerged in the community and among farmed animals thanks to its ability to evolve and adapt to different settings. Global surveillance has shown that MRSA represents a problem in all continents and countries where studies have been carried out, determining an increase in mortality and the need to use last-resource expensive antibiotics. *S. aureus* can easily acquire resistance to antibiotics and MRSA is characteristically multidrug resistant. Resistance to vancomycin, the principal anti-MRSA antibiotic is rare, although isolates with decreased susceptibility are recovered in many areas. Resistance to the more recently introduced antibiotics, linezolid and daptomycin, has emerged; however, they remain substantially active against the large majority of MSSA and MRSA. Newer antistaphylococcal drugs have been developed, but since their clinical use has been very limited so far, little is known about the emergence of resistance. Molecular typing techniques have allowed to identify the major successful clones and lineages of MSSA and MRSA, including high-risk clones, and to trace their diffusion. In the face of a continuously evolving scenario, this review depicts the most common clones circulating in different geographical areas and in different settings at present. Since the evolution of *S. aureus* will continue, it is important to maintain the attention on the epidemiology of *S. aureus* in the future with a global view.

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Abbreviations

ACME	Arginine catabolic mobile element
CA-MRSA	Community-associated MRSA
CC	Clonal complex
ccr	Cassette chromosome recombinase
CLSI	Clinical and Laboratory Standard Institute
ECDC	European Centre for Disease Prevention and Control
EARS-Net	European Antibiotic Resistance Surveillance Network
EUCAST	European Commission for Antimicrobial Susceptibility Testing
GISA	Glycopeptide-intermediate *Staphylococcus aureus*
GRSA	Glycopeptide-resistant *Staphylococcus aureus*
HA-MRSA	Healthcare-associated MRSA
HGT	Horizontal gene transfer
h-VISA	Heterogeneous vancomycin-intermediate *Staphylococcus aureus*
LA-MRSA	Livestock-associated MRSA
mec	Methicillin-resistant gene
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
PBP	Penicillin-binding protein
PFGE	Pulsed-field gel electrophoresis
PVL	Panton–Valentine leukocidin
SCC*mec*	Staphylococcal chromosome cassette *mec*
spa	Staphylococcal protein A
ST	Sequence type
VISA	Vancomycin-intermediate *Staphylococcus aureus*
VRE	Vancomycin-resistant enterococci
VRSA	Vancomycin-resistant *Staphylococcus aureus*
WHO	World Health Organization
WGS	Whole genome sequencing

Contents

1 Introduction

Staphylococcus aureus is a common colonizer of the skin and mucosa surfaces of humans and approximately 30 % of the individuals carry *S. aureus* in the anterior nares (Wertheim et al. 2005). Since the beginning of the microbiological era, *S. aureus* has been recognized as an important pathogen responsible for infections in the healthcare setting and in the community. *S. aureus* infections are initiated by the entrance of the microorganism through a breach of the skin or mucosa and can involve local structures or spread to distant organs to generate life-threatening invasive infections such as bacteremia, pneumonia, and osteomyelitis.

The success of *Staphylococcus aureus* as both a colonizer and a pathogen is largely due to its ability to adapt to different environments thanks to the acquisition of new DNA by horizontal gene transfer (HGT) and to spread clonally. Through HGT *S. aureus* can use an ample and flexible repertoire of colonization determinants, immune evasion factors, and toxins (enterotoxins, exfoliative toxins, leukocidins, etc.) (Lindsay 2014) and can evolve rapidly in response to the greatest challenge to the microbial world in the last 70 years: the introduction of antibiotics. Although *S. aureus* is a species naturally susceptible to antibiotics, over the years it has become resistant to virtually every antibiotic that has entered clinical use. In the span of 10 years after penicillin was available at the middle of the last century, a large proportion of nosocomial *S. aureus* strains became resistant to penicillin by acquisition of a plasmid carrying the penicillinase gene (*penZ*, now *blaZ*) complex (Novick and Bouanchaud 1971; Pantosti et al. 2007) and two decades later 80 % of *S. aureus* isolates were resistant to penicillin (Chambers 2001).

The penicillinase-resistant antistaphylococcal penicillins, whose prototype is methicillin, appeared an adequate response to penicillin-resistant *S. aureus*. However, resistance emerged soon and in 1960 the first MRSA was identified in a London hospital (Jevons 1961). Methicillin resistance is due to the acquisition of a new gene, *mecA,* that codes for a novel penicillin-binding protein (PBP), designated PBP2a that makes the strain resistant to all beta-lactam antibiotics, including antistaphylococcal penicillins, cephalosporins, and carbapenems (Pantosti et al. 2007). The *mecA* gene is contained in a mobile genetic element designated Staphylococcal chromosome cassette (SCC) *mec* that is chromosomally integrated (Katayama et al. 2000).

Acquisition of *mecA* initiated the successful spread of methicillin-resistant *S. aureus* (MRSA), one of the most important multidrug-resistant (MDR) nosocomial pathogens.

2 General Epidemiology of *S. aureus*

The recent epidemiology of *S. aureus* is especially focused on the increase and spread of MRSA in healthcare setting and the community. However, in the past century methicillin-susceptible *S. aureus* (MSSA) was a prominent cause of outbreaks and global spread in healthcare settings and today remains one of the principal pathogens in hospital infection. An example from the past is represented by the MSSA strain called phage type 80/81 that was rampant at the middle of the last century in hospitals causing infections and death in newborn units, in patients and hospital staff in the UK, USA, and Canada (Uhlemann et al. 2014), becoming the first pandemic *S. aureus* clone to be identified (Chambers and Deleo 2009). This strain was resistant to penicillin, highly transmissible, and hypervirulent. Interestingly, it contained the genes for the Panton–Valentine leukocidin (PVL), a leukotoxin that later became a marker for community-associated (CA)-MRSA.

Although this clone disappeared in hospitals with the introduction of methicillin (Chambers and Deleo 2009), the success of *S. aureus* continued into the last decades of the nineteenth century, when *S. aureus* prevalence in healthcare-associated infections, especially bacteremia, increased. This event was ascribed to the increase in the number of immunocompromised individuals, in the use of intravascular devices and, finally, in the multidrug resistance of a portion of the isolates that were MRSA (Lowy 1998). MRSA increased importantly in the mid-1970s in Europe, in the next decade in the USA and later at a global level (Chambers and Deleo 2009). Indeed, MRSA did not replace MSSA infections but actually added to them (Johnson et al. 2005).

At present, *S. aureus* maintains a leading role as a nosocomial pathogen in different countries. In the USA, *S. aureus* was number one among the pathogens isolated from infections according to the National Healthcare Safety Network that collected data from approximately 2.000 hospitals. In particular, *S. aureus* was the most prevalent pathogen in ventilator-associated pneumonia and in surgical site infections. A variable portion of the isolates, from 43 to 58 % according to the type of infections or the hospital ward, was MRSA (Sievert et al. 2013).

In Europe, a recent point-prevalence survey, carried out in acute care hospitals of 33 countries and coordinated by the European Center for Disease Control and Prevention (ECDC), revealed that *S. aureus* is the second most commonly isolated microorganism after *E. coli*, and it remains the first cause of surgical site infections, while MRSA proportion greatly varies according to the country (ECDC 2013). Data collected by the European Antibiotic Resistance Surveillance Network (EARS-Net) has clearly shown important differences among countries in the proportions of MRSA from bacteremia, showing a distinct North–South trend. In 2013, in the face of a European population-weighted mean percentage of 18 %, Iceland, the Scandinavian countries, and the Netherlands reported an MRSA proportion below 2 %, while some East European and South European countries reported a proportion from 32 to 64 % (ECDC 2014). Interestingly, EARS-Net documented a downward trend for MRSA in France, UK, Germany, and Ireland likely due to the

implementation of strategies to control the spread and transmission of MRSA in the healthcare settings (Pearson et al. 2009; Jarlier et al. 2010).

A worldwide picture of MRSA spread is shown in the global report on antimicrobial resistance surveillance, issued by the World Health Organization in 2014 (WHO 2014). Although comprehensive antibiotic resistance data were available only for Europe, America, and Australia, MRSA was reported in all the continents. Most countries reported a proportion of MRSA exceeding 20 % and, occasionally, up to 80 %. This implies that second-line (or "reserve") antibiotics are required for the treatment or the prophylaxis of *S. aureus* infections in most countries worldwide. Noteworthy, MRSA infections are associated with an increase in mortality and in length of hospital stay, leading to a high economic burden with respect to MSSA infections (WHO 2014).

3 Molecular Epidemiology

3.1 Molecular Typing Methods

The ability of *S. aureus* to cause a wide range of infections, to spread in both hospital and the community and to cause outbreaks, has required the development of tools able to distinguish isolates and to outline *S. aureus* epidemiology. Phenotypic methods, including phage typing (Blair and Williams 1961), have been commonly used since the 1960s but in the last decades they have been replaced by molecular typing methods (Deurenberg and Stobberingh 2008). Today, sequence-based methods are the most used to monitor the spread and circulation of the diverse *S. aureus* lineages and to study evolutionary events (Nubel et al. 2011).

A description of the main molecular methods currently used to characterize *S. aureus* is given below.

3.1.1 Pulsed-Field Gel Electrophoresis (PFGE)

Before the introduction of the sequence-based methods, PFGE was considered the gold standard for typing many bacterial species, including *S. aureus*. PFGE is a fingerprinting method based on macrorestriction of genomic DNA by using rare-cutting restriction enzymes, such as SmaI for *S. aureus* (Bannerman et al. 1995). The resulting banding patterns can be resolved in an electric field applying an alternative voltage gradient and analyzed by visual inspection (Tenover et al. 1995) or by using specialized software (Reed et al. 2007). PFGE is a useful tool to study the local epidemiology, such as in the occurrence of an outbreak (Tenover et al. 1995) showing a higher discriminatory power than other typing methods. In USA, the major MRSA clones are defined based on the national PFGE database (e.g., USA100 and USA 300) (McDougal et al. 2003). PFGE limitations include

cost, a rather labor-intensive procedure and the need for technical expertise (Deurenberg and Stobberingh 2008). In addition, protocols and nomenclature are scarcely harmonized (Stefani et al. 2012).

3.1.2 Multilocus Sequence Typing (MLST)

MLST represents the most widely used method to classify *S. aureus* isolates into clones. The method is based on sequencing the internal fragments of 7 housekeeping genes; sequences are then analyzed with the help of the software and the database at the MLST Web site (http://saureus.mlst.net) to obtain an allele number for each gene. The succession of the alleles of the seven genes originates an allelic profile defined sequence type (ST) (Enright et al. 2002). Using the algorithm eBURST (www.eburst.mlst.net), related STs can be grouped into clusters designated clonal complexes (CC)s. Advantages of MLST are its reproducibility, portability, and its universal nomenclature, so that ST data can be easily compared (Deurenberg and Stobberingh 2008). MLST can also provide basic insights of the *S. aureus* population structure in terms of clonal relatedness (Nubel et al. 2011).

3.1.3 Staphylococcal Protein A (*spa*) Typing

This technique is based on the sequence of a single gene, the staphylococcal protein A gene, and in particular of the highly polymorphic X-region which contains different short tandem repeats whose combination originates different *spa* types (Harmsen et al. 2003). The method is supported by a central *spa* server (http://www.seqnet.org/) that at the moment hosts more than 15,000 *spa* types. The discriminative power of *spa* typing is lower than that of PFGE but higher than that of MLST with which it is mostly concordant in terms of CC definition (Cookson et al. 2007; Strommenger et al. 2008). However, the high mutation rate of the *spa* locus may lead to an evolutionary convergence (homoplasy) and, in turn, to problems with the distinction of clones (Nubel et al. 2011). Nevertheless, *spa* typing represents a rapid and easy tool to investigate the epidemiology of *S. aureus* infections, especially at the local level.

3.1.4 SCC*mec* Typing

This method is based on the identification of the structurally different SCC*mec* elements; thus, it can be used to classify MRSA only. SCC*mec* typing is performed by targeting its key elements, the *mec* complex class and the cassette chromosome recombinase (*ccr*) complex type (Kondo et al. 2007). The Web site of the International Working Group on the Staphylococcal Cassette Chromosome elements (IWG-SCC) (http://www.sccmec.org/Pages/SCC_HomeEN.html; accessed on June 26, 2015) currently reports 11 SCC*mec* types differing in size from 20 to

60 kb. The larger SCC*mec* elements (types I–III), which are characteristic of the "classical" nosocomial lineages, can also contain transposons and integrated plasmids that carry resistance to other antibiotics and heavy-metal resistance operons (Pantosti et al. 2007). SCC*mec* type IV encodes methicillin resistance only and being smaller than other SCC*mec* is probably more easily transferable (Ma et al. 2002). It can be further distinguished into 8 subtypes (named from a to h) on the basis of differences in the J1 (accessory or junkyard) region (de Lencastre et al. 2007; Milheirico et al. 2007). SCC*mec* types IV and V are typically found in community-associated MRSA (CA-MRSA) and in livestock-associated (LA)-MRSA. The type of SCC*mec* can be useful to trace the evolutionary origin of MRSA clones and, therefore, it is often part of the designation of a specific clone.

3.1.5 Whole Genome Sequencing (WGS)

Whole genome sequencing (WGS) has the potential to become a primary typing technique in microbiology laboratories, replacing all the other typing methods (Price et al. 2013) also due to the decreased costs of equipment and materials. It offers the best possible resolution for measuring inter-strain similarity and for phylogenetic analysis and can produce information on antigenic array, virulence and antibiotic resistance, predicting phenotypes of interest (Sabat et al. 2013). Several platforms for next-generation sequencing (NGS) are now available (Price et al. 2013; Metzker 2010). Sequencing results consist of thousands of reads corresponding to genomic DNA fragments, generally smaller than 400 base pair (Price et al. 2013). In order to rebuild the genome sequence, the reads have to be assembled. Two methods can be used: A method called *mapping-based assembly* consisting in the comparison of the sequences with those of a reference strain or by de novo *assembly* where the reads are assembled in larger regions named contigs that need to be further assembled (Schatz et al. 2010) but that often do not generate a complete coverage (Nielsen et al. 2011). The typing strategies that can be obtained by WGS are based on allelic variations of genes that are part of the core genome (extended MLST or cgMLST) (Maiden et al. 2013) or on the analysis of the single-nucleotide mutations (SNPs) in the genome as compared to a reference sequence. These types of analyses might be the most readily implementable for typing, although other types (e.g., K-mer) have been proposed (Maiden et al. 2013; Koser et al. 2012a).

The major open problems with WGS rely on the reproducibility of the results obtained with different platforms, the availability of rapid and easy bioinformatics tools, the harmonization of bioinformatic analyses, and the development of a common nomenclature and an open-access database (Sabat et al. 2013). Regarding *S. aureus*, WGS can show differences among strains that are indistinguishable by the PFGE, the most discriminative method used so far (Salipante et al. 2015). WGS has been successfully used to investigate hospital outbreaks, such as MRSA outbreaks in neonatal intensive care units where SNP analysis allowed to clearly discriminate outbreak from non-outbreak strains (Koser et al. 2012b; Harris et al. 2013).

3.2 *Worldwide Distribution of the Principal Clones and Lineages*

The development and extensive use of molecular typing techniques has allowed the identification of different MSSA and MRSA clones and their worldwide distribution. The vast majority of *S. aureus* isolates collected during 1960–2004 have been found to belong to 11 CCs, the most abundant being CC30 (Chambers and Deleo 2009). With respect to MSSA, the MRSA lineages are less numerous since introduction of SCC*mec* must occur into MSSA lineages that are "permissive" for this element, that has to be acquired and maintained (Enright et al. 2002; Robinson and Enright 2003). This event has occurred a limited number of times, although according to recent findings MRSA emergence is probably more common than previously thought (Nubel et al. 2008).

Most of the recent molecular epidemiology data concerning *S. aureus* are focused on MRSA, while the molecular epidemiology of MSSA is quite scarce. Therefore, the following paragraphs deal with the distribution of MRSA clones in different settings and geographical areas and only a short part is dedicated to MSSA.

Recent studies have demonstrated that clones are in continuous evolution: old clones wane and sometimes re-emerge (Chambers and Deleo 2009); exchange and spreading of clones and lineages between different settings and countries are occurring at a rapid rate due to globalization. Therefore, the following description must be intended as an epidemiological snapshot that is due to change with time.

3.2.1 Healthcare-Associated MRSA

For a couple of decades after the emergence of MRSA, these strains were confined to the healthcare setting in Europe and later also in the USA (Chambers and Deleo 2009). The majority of MRSA infections were caused by *S. aureus* phage type 83A (now classified as ST250, CC8) designated as the "archaic clone," to which also the very first MRSA isolate belonged.

The archaic clone gradually disappeared in the 1980s to be replaced by new pandemic clones (Enright et al. 2002; Chambers and Deleo 2009). One successful lineage was ST239-SCC*mec* III also designated the Brazilian/Hungarian clone. ST239 is a hybrid clone originating by the introduction of a large chromosomal fragment from ST30 (CC30) into the CC8 background (Deurenberg and Stobberingh 2008; Smyth et al. 2010). ST239 became prevalent in UK, Australia, and USA between the 1970s and the early 1980s, in Europe and South America in the following decade (1980–1990) and subsequently in Asia and Middle East (1990–2000).

The original nomenclature of the HA-MRSA clones included the geographical area where the clones were first recovered or more widespread (e.g., the New York clone, the Brazilian clone) (Murchan et al. 2003) and their classification was based on phage typing and other phenotypic traits (Kerr et al. 1990) and later on PFGE (Oliveira et al. 2002).

Although for the major clones the original nomenclature or the local designation are often maintained (e.g., EMRSA-15 for ST22 in UK or USA100 for ST5 in USA) (Chambers and Deleo 2009), today, the most accepted nomenclature of the circulating clones is based on the ST-SCC*mec* type and additionally the corresponding CC. Indeed, a single CC can include MRSA clones with different geographical distribution that can be associated with different SCC*mec* elements and possibly other characteristics such as antibiotic resistance determinants or virulence factors (Monecke et al. 2011; Nubel et al. 2011). CC5, for example, encompasses clones belonging to ST5-SCC*mec* II (USA100), which is the most common HA-MRSA in USA (Tenover and Goering 2009) as well as ST5-SCC*mec* IV (USA800), also known as the "Pediatric clone" (Monecke et al. 2011).

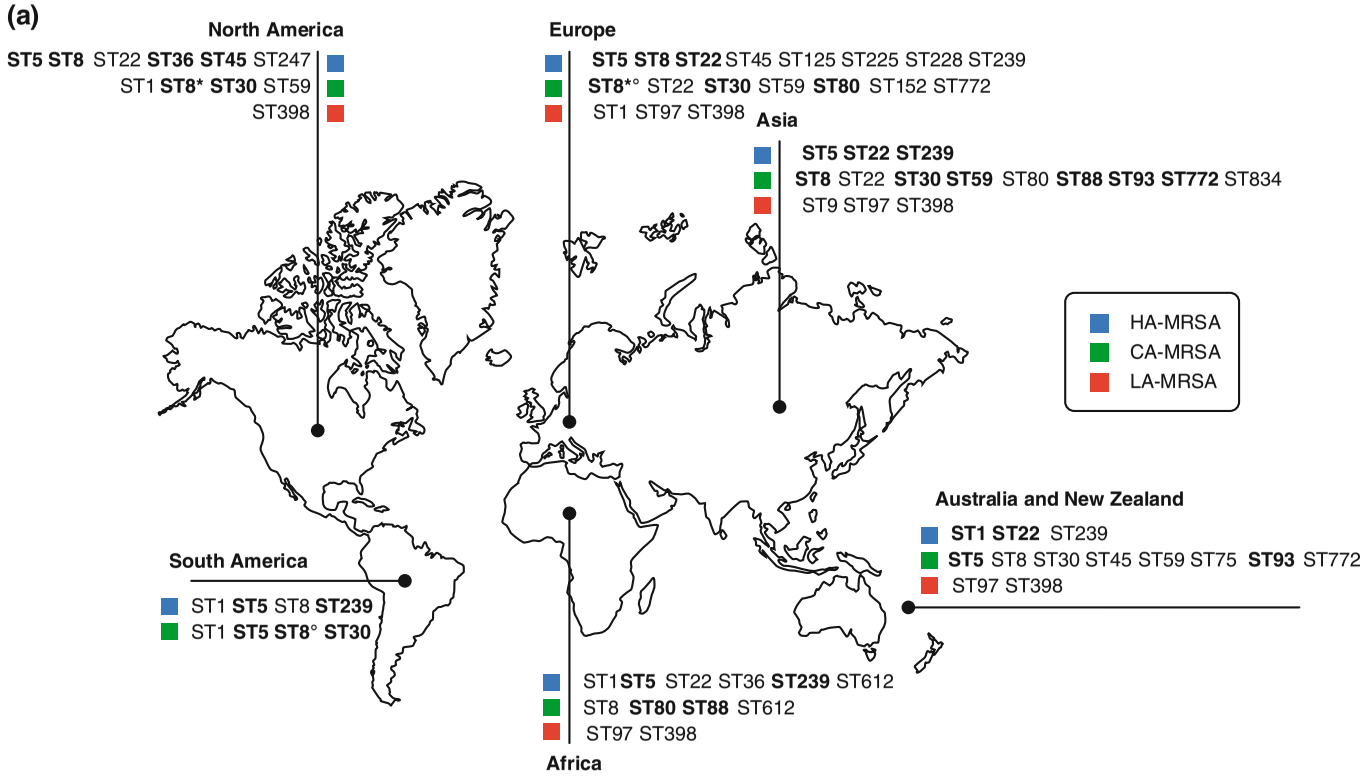
The geographical distribution of the most common HA-MRSA clones is shown in Fig. 1.

In North America, ST5-SCC*mec* II (USA100), ST8-SCC*mec* IVh (USA500), ST36-SCC*mec* IV (USA200), and ST45-SCC*mec* IV are the most common HA-MRSA clones. Other clones, such as ST22-SCC*mec* IV and ST247-SCC*mec* I, are also present, although at lower frequency (Chatterjee and Otto 2013; Stefani et al. 2012; Nichol et al. 2013).

In South America, MRSA belonging to ST5 and ST239 are the most frequent HA-MRSA isolates. In particular, in Brazil the predominant nosocomial lineages are ST239-SCC*mec* III (the Brazilian clone), ST5, and ST1 (Silva-Carvalho et al. 2009; Caboclo et al. 2013), while in Argentina the majority of HA-MRSA isolates are related to ST5-SCC*mec* I, which is also locally called the Cordobes–Chilean clone (Becker et al. 2012; Egea et al. 2014). The latter clone is also present in Colombia together with ST8-SCC*mec* IVc representing the most prevalent lineages; the Chilean clone has recently displaced the Pediatric clone that was disseminated in Colombia at the end of 1990s (Jimenez et al. 2012).

In Europe, ST5 (CC5), ST8 (CC8), and ST22 (CC22) are predominant in most countries. In addition, specific clones display a preferential geographical distribution at country level; for instance, the northern Balcan-Adriatic clone, ST228-SCC*mec* I, is typically detected in Italy, Germany, Austria, Croatia, Hungary and Slovenia (Grundmann et al. 2010a; Monaco et al. 2010); ST125-SCC*mec* IV has been identified in Spain with *spa* type t067 (Perez-Vazquez et al. 2009); the Berlin epidemic clone, ST45-SCC*mec* IV is common in Germany and Belgium, but it also circulates in the Netherlands, Switzerland, and Croatia; in France, ST8-SCC*mec* IV, named "Lyon clone," is the most abundant HA-MRSA followed by ST5-SCC*mec* I, which is also known as the "Geraldine clone" (Dauwalder et al. 2008). In the recent years, ST22-SCC*mec* IV (EMRSA-15), the most common clone in the UK since the 1990s, has spread into several countries including Germany, Hungary, Portugal, and Italy becoming the major European HA-MRSA clone (Grundmann et al. 2014; Holden et al. 2013). The rapid evolution of the HA-MRSA clones and the expansion of ST22-SCC*mec* IV in Europe have been documented by two surveys involving isolates from surveys involving isolates from bacteremia from 25 European countries (Grundmann et al. 2010a, 2014) (Fig. 2).

In Africa, genotyping data of HA-MRSA isolates are still limited; nevertheless, recent studies suggest the predominance of the clones ST5 carrying different



* USA300
° USA300-LV
The most common clones are indicated in bold

Fig. 1 (continued)

(b)

MRSA	CC	MLST	SCC*mec*	Geographic distribution	References
HA	CC1	ST1	IV, V	South America, Africa, Australia and New Zealand	Monecke et al. 2011; Silva-Carvalho et al. 2009; Caboclo et al. 2012; Ben JomaaJemili et al. 2013; Raji et al. 2013; Ritchie et al. 2014; Williamson et al. 2014
	CC5	ST5	I, II, IV	North America, South America, Europe, Africa, Asia	Nichol et al. 2013; Egea et al. 2014; Chatterjee et al. 2013; Stefani et al. 2012; Abdulgader et al. 2015; Schaumburg et al. 2014; Chen et al. 2014
		ST228	I	Europe	Grundmann et al. 2010; Monacoet al. 2010
	CC8	ST8	IV	North America, Europe, Australia, New Zealand	Nichol et al. 2013; Nimmo et al. 2014; Dauwalderet al. 2008; Grundmann et al. 2010; Williamson et al. 2014
		ST239	III	South America, Europe, Africa, Australia, New Zealand	Egea et al. 2014; Smyth et al. 2010; Grundmann et al. 2010; Schaumburg et al. 2014; Abdulgader et al. 2015; Chenet al. 2014; Williamson et al. 2014
		ST247	I	North America, Europe	Nichol et al. 2013; Grundmann et al. 2010
		ST612	IV	Africa, Australia, New Zealand	Abdulgader et al. 2015; Schaumburg et al. 2014; Williamson et al. 2014
	CC22	ST22	IV	North America, Europe, Africa, Asia, Australia, New Zealand	Holden et al. 2013; Nicholet al. 2013; Grundmann et al. 2010; Abdulgader et al. 2015; Schaumburg et al. 2014; Chenet al. 2014; Williamson et al. 2014
	CC30	ST36	II, IV	North America, Europe, Africa	Nichol et al. 2013; Grundmann et al. 2010; Abdulgader et al. 2015
	CC45	ST45	IV	North America, Europe	Nichol et al. 2013; Stefani et al. 2012
CA	CC1	ST1	IV	North America, South America, Australia, New Zealand	Nichol et al. 2013; Egeaet al. 2014; Gelatti et al. 2013; Williamson et al. 2014
		ST772	IV, V	Asia, Europe	Chen et al. 2014; Williamson et al. 2014; Ellington et al. 2010; Sanchini et al. 2011
	CC5	ST5	IV	South America, Europe, Africa, Australia, New Zealand	Egea et al. 2014; Gelattiet al. 2013; Grundmann et al. 2010; Abdulgader et al. 2015; Schaumburg et al. 2014; Williamson et al. 2014
	CC8	ST8	IV	North America, South America, Europe, Africa, Asia, Australia, New Zealand	Nichol et al. 2013; Nimmo et al. 2014; David et al. 2010; Egeaet al. 2014; Jimenezet al. 2012; Reyes et al. 2009; Abdulgader et al. 2015; Schaumburg et al. 2014; Sanchini et al. 2011; Williamson et al. 2014
	CC22	ST22	IV	Europe, Asia	Ellington et al. 2010; Moneckeet al. 2011
	CC30	ST30	IV, V	North America, South America, Europe, Asia, Australia, New Zealand	Nichol et al. 2013; Egeaet al. 2014; Gelattiet al. 2013; David et al. 2010; Mediavilla et al. 2012; Chen et al. 2014; Williamson et al. 2014
	CC45	ST45	IV, V, VI	North America, Australia, New Zealand	Monecke et al. 2011; Williamson et al. 2014
	CC59	ST59	IV, V	North America, Europe, Asia, Australia, New Zealand	David et al. 2010; Mediavilla et al. 2012; Sowashet al. 2014; Monecke et al. 2011; Chenet al. 2014; Williamson et al. 2014
	CC80	ST80	IV	Europe, Africa	David et al. 2010; Moneckeet al. 2011; Grundmann et al. 2010; Sanchiniet al. 2011; Abdulgader et al. 2015; Schaumburg et al. 2014
	CC88	ST88	IV	Europe, Africa, Australia	Monecke et al. 2011; Sanchiniet al. 2011; Abdulgader et al. 2015; Schaumburg et al. 2014
	Singleton	ST93	IV	Asia, Australia, New Zealand	Williamson et al. 2014; Moneckeet al. 2011; Chenet al. 2014
	CC152	ST152	V	Europe, Africa	Monecke et al. 2011; Mediavilla et al. 2012; Abdulgader et al. 2015; Schaumburg et al. 2014
LA	CC1	ST 1	IV	Europe	Cuny et al. 2013; Franco et al. 2011
	CC9	ST 9	IV, V	Europe, Asia	Monecke et al. 2011; Kocket al. 2013; Chenet al. 2014
	CC97	ST97	IV, V	Europe, Africa, Australia	Pantosti et al. 2012; Kock et al. 2013; Monecke et al. 2011
	CC398	ST 398	IV, V	North America, South America, Europe, Africa, Asia, Australia, New Zealand	Casey et al. 2014; Verkadeet al. 2014; Cunyet al. 2013; Van Cleefet al. 2011; Monacoet al. 2013; Ehlani et al. 2015; Moneckeet al. 2011; Williamson et al. 2014

CC: clonal complex; MLST: multilocus sequence typing; SCC*mec*: Staphylococcal chromosome cassette *mec*

Fig. 1 Geographical distribution of the prevalent MRSA clones

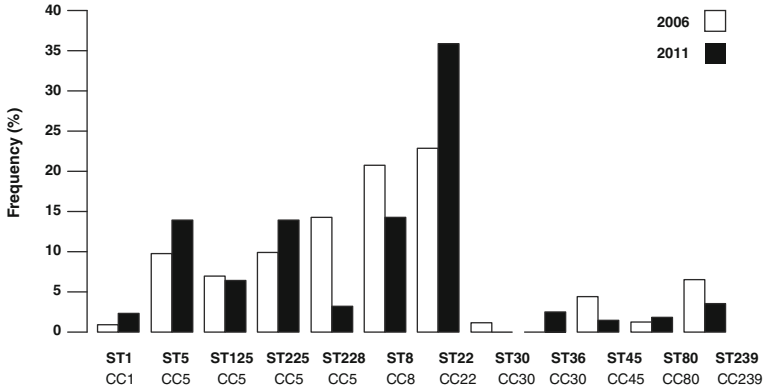


Fig. 2 Evolution of HA-MRSA clones in Europe (2006 and 2011) according sequence type (ST) and clonal complex (CC) distribution

SCC*mec* types (I, II or IV) and ST239-SCC*mec* III. Other lineages, such as ST22-SCC*mec* IV, ST36-SCC*mec* II, and ST612-SCC*mec* IV, a double-locus variant of ST8, are reported mainly from South Africa (Abdulgader et al. 2015) while ST1 has been reported from hospitals in Tunisia and Nigeria (Raji et al. 2013; Mariem et al. 2013).

In Asia, about 90 % of hospital infections are due to ST239-SCC*mec* III (Smyth et al. 2010). This clone is the predominant clone in all countries where molecular typing of HA-MRSA has been carried out including China, Korea (Moon et al. 2010), Indonesia, Philippines, Thailand, Vietnam, India, and Pakistan (Shabir et al. 2010; Chen and Huang 2014). The only exception is represented by Japan where ST5-SCC*mec* II is the predominant clone since 1990s (Chen and Huang 2014). ST5-SCC*mec* II is also largely disseminated in China, Korea, and Taiwan (Tsao et al. 2014). In Southeast Asian countries, ST241, a single-locus variant of ST239, is also present (Chen and Huang 2014).

In Australia, the major circulating HA-MRSA clone was ST239-SCC*mec* III till the end of 1990s when ST22-SCC*mec* IV became the most common clone (Williamson et al. 2014). In New Zealand, ST1 is the prevalent clone detected among strains isolated from patients with bacteremia (Ritchie et al. 2014); other clones belonging to CC1, CC30, CC59, and CC101 are also found in hospitals in the Southwest Pacific area (Williamson et al. 2014).

3.2.2 CA-MRSA

In the 1990s, MRSA epidemiology dramatically changed due to the emergence of new MRSA lineages in the community, later called CA-MRSA.

CA-MRSA infections were first identified in remote areas of Western Australia in the late 1980s; the causative strain was subsequently identified as ST8-SCC*mec* IV (Nimmo and Coombs 2008). At the beginning of the 1990s, in the USA, MRSA

infections started to emerge in the community among children without predisposing risk factors (Herold et al. 1998). During 1997–99, in the mid-western region of USA, an MRSA strain, designated as USA400 by PFGE, was responsible for a small outbreak of sepsis and necrotizing pneumonia among healthy children (DeLeo et al. 2010; David et al. 2015). USA400 was the prevalent CA-MRSA clone in USA until 2001 when the new unrelated clone USA300 replaced it to become one of the most successful clone ever. CA-MRSA were responsible for skin and soft tissues infections (SSTIs) in young healthy individuals without predisposing risk factors for MRSA acquisition and with no association with the healthcare system (Stryjewski and Chambers 2008), causing outbreaks among prisoners, athletes, military population, homosexual men, and newborns. Sporadically, CA-MRSA were responsible for serious infections, such as necrotizing pneumonia, necrotizing fasciitis, sepsis, and osteomyelitis (Crum et al. 2006; Bukharie 2010).

Molecular typing of CA-MRSA revealed that these isolates generally carry SCC*mec* elements type IV or V and display resistance to fewer non-beta-lactam antibiotics with respect to "classical" HA-MRSA that harbor SCC*mec* type I, II, or III and are usually multiresistant (Benoit et al. 2008; David and Daum 2010). In addition, CA-MRSA carry genes for PVL, a prophage-encoded, bicomponent pore-forming cytotoxin that specifically targets human neutrophils, causing their destruction and the consequent tissue damage (Boyle-Vavra and Daum 2007; Spaan et al. 2015). SCC*mec* IV and PVL genes have represented molecular markers used to trace the emergence of CA-MRSA worldwide (Vandenesch et al. 2003). Sixty to 100 % of CA-MRSA strains carry PVL genes (Rossney et al. 2007; Shallcross et al. 2013); the prevalence of PVL-positive isolates is dependent on lineages and geographical areas (Munckhof et al. 2003). According to recent studies, PVL is an important contributor, in association with other factors, to the virulence of CA-MRSA and to its ability to disseminate (Chambers and Deleo 2009; Zhang et al. 2008). Recently, the role of PVL in the pathogenesis of CA-MRSA necrotizing pneumonia has been clearly established using a rabbit model (Diep et al. 2010; Chi et al. 2014).

In recent years, the epidemiological distinction between CA-MRSA and HA-MRSA infections has become blurred due to the introduction of CA-MRSA into the healthcare system, especially in USA (David and Daum 2010; Pantosti and Venditti 2009). Therefore, the distinction between CA-MRSA and HA-MRSA requires more than one criteria including molecular typing (David and Daum 2010; Otter and French 2012).

The burden of CA-MRSA infections is geographically diversified. Although data from many areas are sparse and difficult to compare, evidence shows that the prevalence of CA-MRSA infections is higher in the USA than in other areas (Mediavilla et al. 2012; Witte 2009). PVL-positive CA-MRSA were reported as responsible for the majority of acute SSTIs in patients presenting at the US emergency departments (Moran et al. 2006). In Europe, PVL-positive CA-MRSA infections were relatively rare in England and Ireland but common in Greece (Shallcross et al. 2013). In Australia, CA-MRSA represented 7.8 % of the isolates obtained from outpatient infections (Nimmo and Coombs 2008).

At present, CA-MRSA are associated with more than 20 distinct world-wide-spread genetic lineages. The most common CA-MRSA lineages are displayed in Fig. 1.

In USA, the major CA-MRSA clone is USA300 (ST8-SCC*mec* IVa), which contains the PVL genes and the arginine catabolic mobile element (ACME) that has been shown to enhance the ability of the strain to colonize the skin (David and Daum 2010). Besides USA 300, USA400 (ST1-SCC*mec* IV), ST30-SCC*mec* IV, and ST59-SCC*mec* IV represent other less common CA-MRSA clones that can be found throughout the country (Mediavilla et al. 2012; Monecke et al. 2011). In Canada, USA300 and USA400 are the most common CA-MRSA clones (Nichol et al. 2013).

In South America, one of the most frequent clones is represented by the USA300 variant named USA300-LV (ST8-SCC*mec* IVc) that contains PVL but lacks ACME. USA300-LV, first reported in Colombia in 2006 (Reyes et al. 2009), is now present in several other Latin American countries, including Argentina, Venezuela, Peru, Ecuador, and Brazil. More recently, USA300-LV has also been described as cause of HA infections (Jimenez et al. 2012; Egea et al. 2014). In Argentina, the most common CA-MRSA clones are ST5-SCC*mec* IV and ST30-SCC*mec* IV, while in Uruguay and Brazil ST30-SCC*mec* IV is prevalent (Egea et al. 2014; Gelatti et al. 2013; Jimenez et al. 2012).

In Europe, circulating CA-MRSA strains belong to a variety of clones; the majority of the infections are due to the European clone ST80-SCC*mec* IV (Vandenesch et al. 2003), which typically shows resistance to fusidic acid (Monecke et al. 2011). USA300 was reported from Denmark in 2000 (AR Larsen et al. 2007) and subsequently from other countries (Austria, England, France, Ireland, Netherlands, Spain, and Italy), as cause of sporadic infections or small outbreaks (Nimmo 2012; Sanchini et al. 2013). USA300-LV has also been reported from Spain and Italy (Cercenado and Ruiz de Gopegui 2008; Sanchini et al. 2011; David and Daum 2010). ST30-SCC*mec* IV is also largely spread in different geographical areas (David and Daum 2010; Sanchini et al. 2011), while a variety of other CA-MRSA clones have been identified with variable frequency including ST22, ST59, ST152, and ST772 (Monecke et al. 2011; Ellington et al. 2010; Mediavilla et al. 2012).

In Africa, few predominant CA-MRSA clones are spread in different regions. In North Africa, the European clone ST80-SCC*mec* IV predominates, likely due to the geographical proximity to Europe. ST88-SCC*mec* IV is predominant in West, Central, and East Africa, while ST8-SCC*mec* IV, including isolates closely related to USA300, has been recently reported in Gabon and Ghana (Schaumburg et al. 2014). ST612-SCC*mec* IV, PVL-positive, typically circulates in South Africa (Abdulgader et al. 2015).

In Asia, there is great heterogeneity in the circulating CA-MRSA clones and their prevalence varies considerably among countries. ST59, carrying SCC*mec* type IV, V, or a variant named V_T (V_{Taiwan}), is spread in Taiwan, China, Vietnam, and Japan (David and Daum 2010; Sowash and Uhlemann 2014; Chen and Huang 2014); ST30-SCC*mec* IV is ubiquitously detected but prevails in Singapore, Hong Kong, Philippines, and Japan. ST772-SCC*mec* V, named the Bengal Bay clone, is

predominant in India, where ST22-SCC*mec* IV is also present. ST8, ST88, and ST93 are spread in Japan, while ST834, belonging to CC9, is characteristically present in Cambodia (Chen and Huang 2014; David and Daum 2010; Monecke et al. 2011; Sowash and Uhlemann 2014; Williamson et al. 2014).

In Australia, the most common CA-MRSA clone is ST93-SCC*mec* IV (the Queensland clone). However, a large diversity of CA-MRSA clones has also been documented in this geographical area, including PVL-negative clones (Nimmo and Coombs 2008), such as ST75, which is a genetically divergent *S. aureus* strain and should probably be allocated to a separate species (Williamson et al. 2014). In New Zealand, ST30-SCC*mec* IV has been the prevalent clone up to 2005 when ST5-SCC*mec* IV, an emerging clone resistant to fusidic acid, has displaced it. Other CA-MRSA lineages, typical of other geographical areas, are present both in Australia and New Zealand, such as USA300, ST59-SCC*mec* V_T, and ST772-SCC*mec* V (Williamson et al. 2014).

3.2.3 LA-MRSA

It has been recently established that livestock represents a reservoir of MRSA (Pantosti 2012; van Cleef et al. 2011). Several cases of human colonization and infections caused by MRSA of animal origin (designated LA-MRSA), mainly from pigs or cattle, have been reported (Smith and Pearson 2011). The first LA-MRSA was identified in Europe in 2005 (Voss 2005). It belonged to a new MRSA lineage (ST398, CC398) and showed features different from those of other MRSA clones such as non-typeability by PFGE (Bens et al. 2006), presence of SCC*mec* type IV or V, and resistance to tetracycline and trimethoprim–sulfamethoxazole, antibiotics commonly used in animal production (Argudin et al. 2011). PVL and enterotoxins genes were generally not present (Hallin et al. 2011). Colonization and infection with ST398 have been documented mainly in countries where animal production is intensive, occurring primarily in farm workers, veterinarians, and other people exposed to livestock (Monaco et al. 2013; Van Cleef et al. 2010; Casey et al. 2014). However, cases have also been reported in subjects with no known contact with animals and in hospitalized patients (Wulf et al. 2008; Kock et al. 2009).

Although LA-MRSA ST398 was found to be globally spread, other MRSA non-CC398 have emerged in farm animals in different geographical areas: ST9 (CC9) detected in pigs in Asia and Europe; ST97 (CC97) isolated from bovine mastitis and chickens in different areas of the Americas and Europe; ST1 (CC1) frequently found in bovine mastitis and in pigs in Europe (Franco et al. 2011); ST22 (CC22) and ST5 (CC5) isolated from pigs in Ireland and Canada, respectively (Cuny et al. 2013). Another clone, ST130 (CC130), recovered in cattle, horses, and sheep, was found to harbor *mecC*, a novel *mec* homologue that is not detectable by conventional diagnostic assays (Garcia-Alvarez et al. 2011; Ito et al. 2012). All these non-CC398 LA-MRSA lineages were found to colonize or infect humans with different prevalence, being ST9 and ST97 the less frequently recovered (Kock et al. 2013).

3.2.4 Molecular Epidemiology of MSSA

Although MSSA is a leading cause of infections, both in the community and in the healthcare setting, only few studies have been published describing the molecular epidemiology of MSSA. Since MSSA represents the reservoir for the emergence of MRSA through SCC*mec* introduction, it is important to recognize MSSA clones endowed with capacity to cause serious infections and to spread globally. For instance, the PVL-positive MSSA clone phage 80/81 that was spread in hospitals in the middle of the past century has been recognized by modern typing techniques as belonging to CC30. After acquiring *mecA,* the clone has disseminated globally as MRSA CC30, one of the principal CA-MRSA clones (Robinson et al. 2005).

In general, all the studies have highlighted that the MSSA population is more heterogeneous than the MRSA population, since the MSSA isolates belong to a larger number of different clones and lineages. This depends, at least in part, on the fact that MSSA are carried by approximately one-third of the human population and that their circulation is much antecedent to MRSA emergence (Deurenberg and Stobberingh 2008; Grundmann et al. 2010b).

Approximately 40–50 % of MSSA isolates in different geographical areas have a genetic background shared with the major MRSA CCs, namely CC5, CC8, CC22, CC30, and CC45, while the rest belongs to lineages that contain predominantly MSSA, such as CC7, CC9, CC12, CC15, CC25, CC51, and CC101 (Deurenberg and Stobberingh 2008). Each of these lineages includes different *spa* types and/or PFGE types (Deurenberg and Stobberingh 2008; Goering et al. 2008). The presence of successful MSSA lineages with a wide geographical distribution suggests that they possess factors favoring the ability to cause and to transmit disease among humans.

In a recent study, MSSA isolates from uncomplicated SSTIs in the community setting obtained in global clinical trials were characterized by using PFGE and other molecular typing techniques. The most common clones, accounting for approximately 36 % of the isolates, were ST30, ST45, ST1 (USA400), and ST8 (USA300) that were recovered in USA, South America, South Africa, and Europe (Goering et al. 2008).

In a large study performed in USA, MSSA collected from a variety of sources, including blood, urine, the respiratory tract, and the skin, representing both community- and healthcare-acquired infections were typed by *spa* typing (Miko et al. 2013): 274 *spa* types were identified among 708 isolates, obtaining 15 genetic clusters. The most common genetic clusters corresponded to USA100, USA800 (CC5) and to USA300 (CC8), the same lineages found among MRSA in the healthcare setting or in the community.

In the previously cited European survey on *S. aureus* from invasive infections conducted in 2006–2007, the diversity index based on *spa* typing was higher (0.985) for MSSA than for MRSA (0.940). In this study, the most frequent MSSA clones, isolated from bacteremia in hospitalized patients, were (in ranking order): ST7, ST15, ST5, ST45, ST8, ST30, ST1, and ST22. Moreover, MSSA showed a lower degree of geographical clustering than MRSA (Grundmann et al. 2010).

Although MSSA is more rarely associated with PVL than MRSA, based on the epidemiology in USA and some European countries (Shallcross et al. 2013) the global scenario is very diversified. Several MSSA lineages are found to carry PVL genes; ST1, ST5, ST25, and ST152 have a pandemic spread (Rasigade et al. 2010), while other lineages appear to be more restricted to some geographical areas; for instance, ST8 MSSA, related to MRSA USA300, is frequent in USA, while ST80 is present in Europe and Africa as the MRSA counterparts, and ST188 is found in France, New Caledonia, and Polynesia. In Europe, in the community setting, the most prevalent PVL-positive MSSA lineages are CC30 and CC121 (Rasigade et al. 2010; Sanchini et al. 2014).

Several studies found a high rate of carriage and infections due to PVL-positive MSSA in Africa, with isolates belonging to a variety of different clones including ST15, ST30, ST121, and ST152 (Schaumburg et al. 2011). ST152 is a divergent MSSA clone that was first identified in Mali. The reason for this occurrence is unknown, although the humid environment of tropical Africa and host factors, such as altered C5a receptor, which has been identified as PVL target (Spaan et al. 2013), could contribute to this peculiar epidemiological picture (Schaumburg et al. 2014).

Another MSSA clone has emerged recently becoming a source of concern: MSSA ST398 (CC398) mainly with *spa* type 571. This strain that contains the phage-encoded immune evasion cluster genes (Chroboczek et al. 2013) and is characteristically resistant to erythromycin, due to presence of *ermT,* and susceptible to tetracycline, seems to represent the basal human clade from which the animal-adapted ST398 MRSA clone emerged (Valentin-Domelier et al. 2011). MSSA ST398 is responsible for serious human infections in different geographical regions including North America, Europe, China, and the Caribbean (Verkade and Kluytmans 2014). In France, MSSA ST398 accounts for 7.5 % of all MSSA endocarditis cases (Chroboczek et al. 2013).

The molecular epidemiology of MSSA shows a large clonal heterogeneity across geographical areas. The prevalence of MSSA clones with the same genetic background of pandemic MRSA clones suggests that factors, other than methicillin resistance, contribute to the success of a specific clone.

4 *S. aureus* and Antibiotic Resistance

As already mentioned, *S. aureus* has a unique ability to rapidly acquire antibiotic resistance to virtually any antimicrobial molecules that has been developed. Resistance is often acquired by HGT from other species or genera, although chromosomal mutations also contribute to resistance to some antibiotics. HGT allows acquisition of preconstituted clusters of genes that concur to a resistance trait (e.g., the *mec* complex or the *vanA* complex for methicillin or vancomycin resistance, respectively), while mutations can provide resistance to novel or synthetic antibiotics that do not have natural analogues and for which resistance determinants are not available in nature (e.g., for linezolid).

The evolution of the different MRSA lineages has involved the acquisition of antibiotic resistance determinants. Therefore, certain MRSA clones can be associated with characteristic resistance traits or patterns. For instance, CA-MRSA lineages retain susceptibility to most non-beta-lactam antibiotics, but USA300 is characteristically resistant to erythromycin and ciprofloxacin (David and Daum 2010) and the European CA-MRSA ST80 clone is resistant to fusidic acid and tetracycline (Monecke et al. 2011). LA-MRSA is commonly resistant to tetracycline, the most used antibiotic in the farming industry (Pantosti 2012). HA-MRSA lineages tend to be resistant to a broad range of antibiotic agents including the aminoglycosides although the most recent emerging clones are resistant to a narrower spectrum of antibiotics. ST22 (EMRSA-15) is characteristically resistant to fluoroquinolones and macrolides, but it is susceptible to gentamycin (Ellington et al. 2010; Johnson et al. 2005); the Lyon clone (ST8) is resistant to fluoroquinolones, susceptible to gentamycin and variably susceptible to other aminoglycosides and macrolides (Dauwalder et al. 2008).

The mechanisms and the genetic determinants leading to resistance to the most common agents used to treat staphylococcal infections have been extensively reviewed (Lowy 2003; Pantosti et al. 2007). Today, there are a number of newly developed antibiotics that display good anti-MRSA activity, such as lipoglycopeptides (derivatives of vancomycin or teicoplanin such as telavancin and dalbavancin) and new antistaphylococcal cephalosporins, such as ceftobiprole and ceftaroline (Morata et al. 2015). These two last molecules, as all beta-lactam antibiotics, are substrate analogues of PBPs resulting in their block, impaired cell wall synthesis and cell death. But, unlike other beta-lactams, both ceftobiprole and ceftaroline have high affinity also for PBP2a, that mediates methicillin resistance in *S. aureus*, thus are active also against MRSA (Moisan et al. 2010; Davies et al. 2007). Little is known about resistance development with these molecules since their clinical use has been very limited so far. Here, we will briefly summarize resistance to the last-line antibiotics for MRSA treatment: vancomycin, linezolid, and daptomycin.

4.1 Vancomycin

Vancomycin and the other glycopeptide antibiotic teicoplanin have been the mainstay of MRSA treatment for 30 years (Srinivasan et al. 2002). Isolates with decreased susceptibility to vancomycin were described for the first time in Japan in 1997 (Hiramatsu et al. 1997b) and thereafter in several other countries. These isolates, mostly MRSA, showed a spectrum of vancomycin minimal inhibitory concentrations (MICs) ranging from borderline susceptibility to full resistance (Gardete et al. 2012). In between these extremes, isolates with intermediate susceptibility to vancomycin (VISA) and those still susceptible but containing a minority population with intermediate susceptibility (heterogeneous VISA or hVISA) were present (Liu and Chambers 2003).

The recognition of VISA and hVISA is complicated by problems with laboratory methods and with different breakpoints; the reference method is MIC determination by broth microdilution (CLSI 2014; EUCAST 2015), and a labor-intensive test, such as the population analysis profile, is required to detect hVISA (Howden et al. 2010). Both the Clinical and Laboratory Standard Institute (CLSI) and the European Commission for Antimicrobial Susceptibility Testing (EUCAST) have established the vancomycin breakpoint for susceptibility at MIC <=1 ug/ml (CLSI 2014; EUCAST 2015), thus indicating that vancomycin is poorly or not effective against isolates with higher MIC. However, CLSI has retained the intermediate category (MIC 4-8 ug/ml) to define VISA, clearly differentiating them from vancomycin-resistant *Staphylococcus aureus* (VRSA) (MIC => 16 mg/ml) since completely different resistance mechanisms are implicated in these strains. This difference is recognized in terms of nomenclature also by EUCAST that has designated glycopeptide-intermediate *S. aureus* (GISA) and glycopeptide-resistant *S. aureus* (GRSA) isolates with low-level and high-level resistance, respectively (EUCAST 2015).

VISA is associated with a thickened cell wall that traps vancomycin before it reaches the molecular target that is the nascent peptidoglycan at the inner side of the cell wall. Different mutations or expression of genes that are related to cell wall synthesis, is associated with the emergence of VISA from susceptible parental strains in vitro or in vivo (Howden et al. 2010). In particular, type I and type II polymorphisms of the accessory gene regulator locus (*agr*) or alterations of its function have been associated with the development of VISA and hVISA (Howden et al. 2010; Sakoulas et al. 2002). True homogeneous VISA isolates remain a small number in the published reports (Gardete et al. 2012) and have not been found in susceptibility studies in large series of isolates (Mendes et al. 2014b, c). On the contrary, hVISA have been detected in most institutions where they have been searched for; thus, their prevalence may be underestimated (Howden et al. 2010). In 2003, the hVISA prevalence for was reported to be 2 % in MRSA and 0.05 % in MSSA (Liu and Chambers 2003), although strong inter-institutional differences were noted (Hiramatsu et al. 1997a). Previous vancomycin treatment and the genetic background of *S. aureus* are predisposing factors for VISA or hVISA development (Howden et al. 2014). Although VISA and hVISA have emerged in every principal MRSA lineage, including CA-MRSA USA300 (Gardete et al. 2012) and also in MSSA (Pillai et al. 2009), they have been found especially in CC5 and CC8 background (Howe et al. 2004; Monaco et al. 2010).

The occurrence of VRSA is quite rare but has always raised fear of bleak scenarios (Conly and Johnston 2002). Only 17 VRSA that have been confirmed by molecular methods are reported in the indexed literature so far (Table 1).The first VRSA was isolated in Michigan in 2002 (Weigel et al. 2003) and the vast majority of the other VRSA strains (13 out of 17) were reported from the USA, in particular from Michigan. The isolates from other countries originated 1 each from India, Iran, Brazil, and Portugal, this last being to date the only VRSA from Europe (Finks et al. 2009; Friaes et al. 2015; Limbago et al. 2014; Rossi et al. 2014; Saha et al. 2008; Sievert et al. 2008; Azimian et al. 2012).

Table 1 VRSA isolated from 2002 to 2013 reported in indexed journals

Country or state or city	Date	Source	Vancomycin MIC (μg/ml)	SCC*mec*	*spa* type	MLST	CC	References
USA/MI	2002	Plantar ulcers	1024	II	t062	ST371	CC5	Weigel et al. 2003, Limbago et al. 2014
USA/PA	2002	Plantar ulcers	32	II	t002	ST5	CC5	Tenover et al. 2004, Sievert et al. 2008
USA/NY	2004	Urine	64	IV	t002	ST5	CC5	Sievert et al. 2008
USA/MI	2005	Toe wound	256	II	t002	ST5	CC5	Zhu et al. 2008; Sievert et al. 2008
USA/MI	2005	Surgical site wound	512	II	t002	ST231	CC5	Zhu et al. 2008; Sievert et al. 2008
USA/MI	2005	Plantar ulcers	1024	NT	t002	ST85	CC5	Zhu et al. 2008, Sievert et al. 2008
USA/MI	2006	Triceps wound	512	II	t062	ST231	CC5	Zhu et al. 2008, Sievert et al. 2008
USA/MI	2007	Toe wound	1024	ND	t002	ST5	CC5	Finks et al. 2009
USA/MI	2007	Surgical wound	1024	ND	t002	ST5	CC5	Finks et al. 2009
India/West Bengal	2007	Pus	64	ND	-	-	-	Saha et al. 2008
USA/MI	2009	Plantar wound	NR	ND	t002	ST5	CC5	Limbago et al. 2014
USA/DE	2010	Wound drainage	NR	ND	t002	ST5	CC5	Limbago et al. 2014
USA/DE	2010	Vaginal swab	NR	ND	t045	ST5	CC5	Limbago et al. 2014
USA/DE	2012	Foot wound	256	ND	t019	-	CC30	Limbago et al. 2014
Iran/Mashhad	2011	Bronchial aspirate	512	III	t037	ST239[a]	CC8	Azimian et al. 2012
Brazil/Sao Paolo	2012	Blood culture	32	IV	t292	ST85	CC8	Rossi et al. 2014
Portugal/Lisbon	2013	Toe wound	1024	II	t002	ST105	CC5	Friaes et al. 2015

MIC: Minimal inhibitory concentration; SCC*mec* Staphylococcal chromosome cassette *mec*; *spa:* Staphylococcal protein A; MLST: multilocus sequence typing; CC: clonal complex

[a]ST updated according to Larsen J et al. 2012

Vancomycin resistance in *S. aureus* is a clear example of HGT from another bacterial species, as resistance is conferred by the acquisition of the *vanA* cluster, an operon consisting of 5 genes, carried by the transposon Tn1546, the resistance hallmark of vancomycin-resistant enterococci (VRE) (Courvalin 2006). The genes composing the *vanA* cluster act synergistically to modify the cell wall peptidoglycan making it resistant to the vancomycin action. Tn1546 acquisition by *S. aureus* generally occurs from a VRE species (more commonly *Enterococcus faecalis* or *Enterococcus faecium*) by means of a plasmid, such as a promiscuous plasmid of the Inc18 family (Zhu et al. 2010). This may explain why VRSA generally emerge in chronic infections with mixed flora, such as ulcers of the extremities, where MRSA and VRE may coexist, in chronically ill patients under long-term vancomycin therapy (Sievert et al. 2008). Most VRSA belong to CC5, suggesting that only some genetic backgrounds, are permissive to the introduction of the *van*A cluster. The burden carried by this group of resistance genes on the overall fitness of *S. aureus* could also explain the apparent low propensity of VRSA to transmit to other patients or cause outbreaks (Howden et al. 2010). It is noteworthy that the VRSA from Brazil is related to USA300 and PVL-positive (Rossi et al. 2014); this emergence is worrisome due to the intrinsic high transmissibility of the USA300 clone.

Sources different from the peer-reviewed journals suggest that VRSA may indeed be more common. For instance, the MLST Web site (http://saureus.mlst.net/ accessed on June 4, 2015) includes 23 VRSA although the molecular evidence for the presence of Tn1546 is not provided. This list includes isolates from the USA (other than those already published) and isolates from Pakistan, Japan, South Chorea, Iraq, India, China, and Brazil. A number of isolates from Pakistan appears to be genetically heterogeneous, belonging to at least 5 different lineages. An apparent high number of VRSA is also reported from India (Askari Ea et al. 2013). The VRSA status of these isolates has never been confirmed by independent investigators; therefore, some skepticism about the circulation of VRSA in these countries must be maintained.

4.2 Linezolid

Linezolid belongs to a new antibiotic class, the oxazolidinones, introduced into medical practice in 2000. Linezolid exerts its antibacterial action by binding to the 23S subunit of the bacteria ribosome at domain V, thus inhibiting protein synthesis (Leach et al. 2007). Although linezolid is a synthetic drug and no natural reservoir of resistance genes would be expected, in 2001 the first linezolid-resistant *S. aureus* was reported in the USA in a patient who had received 1-month linezolid treatment (Tsiodras et al. 2001).

Two different mechanisms are known to confer linezolid resistance to *S. aureus*. The first is due to mutations occurring in the linezolid binding site (23S rRNA), the most common being the G2576T mutation, or in the ribosomal proteins L3 and L4

(Mendes et al. 2014a). The second mechanism is due to the presence of the plasmid-born chloramphenicol-florfenicol resistance (*cfr*) gene that encodes a 23S rRNA methyl transferase (Schwarz et al. 2000), conferring resistance to different antibiotics, including linezolid.

cfr carrying MRSA have caused intra- and inter-hospital outbreaks; a large outbreak that occurred in an ICU in Madrid, was due both to clonal expansion of the linezolid-resistant MRSA as well as to transmission of the *cfr* plasmid to other MRSA clones (Bonilla et al. 2010; Ikeda-Dantsuji et al. 2011; Morales et al. 2010; Sanchez Garcia et al. 2010). The prevalence of linezolid resistance among clinical *S. aureus* isolates remains very low: a study including isolates only from the USA reported resistance rates below 0.2 % from 2004 to 2012 (Mendes et al. 2014b). In addition, a surveillance program conducted across the same period on 25,000 *S. aureus* isolates mostly from blood, wound and lower respiratory tract, the percentage of the linezolid-resistant strains remained below 0.1 % in countries from 5 continents (Mendes et al. 2014c). Linezolid resistance generally develops in patients who had been receiving long linezolid treatments; therefore, it can be higher in selected groups of patients, such as cystic fibrosis patients (Endimiani et al. 2011).

4.3 Daptomycin

Daptomycin is a natural lipopeptide antibiotic introduced in 2003 in the USA and in 2005 in Europe (Sakoulas 2009) for treatment of skin and soft tissue infections and bacteremia (Bayer et al. 2013). Its mechanism of action is probably multifaceted and not completely understood. Daptomycin is an anionic molecule that requires the presence of calcium ions to be active (Straus and Hancock 2006): the daptomycin–calcium complex inserts itself in the bacterial cell membrane causing depolymerization and permeabilization with leakage of small ions and cell death (Humphries et al. 2013). Cell wall probably represents another target of daptomycin, since resistant isolates exhibit a thickened cell wall (Bertsche et al. 2011). Also the genetic determinants of daptomycin resistance have not been fully identified. Resistance seems to be associated with a progressive accumulation of mutations in a few *S. aureus* genes. The most common mutations occur in *mpr*F, coding for a bifunctional enzyme involved in the metabolism of the cell membrane. The *mpr*F mutations are associated with gain-in-function determining increase in the relative positive charge of the cell membrane leading to a decreased insertion of the calcium–daptomycin complex (Jones et al. 2008). Resistance has been associated also with mutations in the *yyc* cluster and in *rpoB* and *rpoC* (Bayer et al. 2013) and with enhanced expression of the regulatory systems *vra*SR (Mehta et al. 2012).

Soon after the introduction of daptomycin in clinical use, treatment failures due to the emergence of daptomycin-resistant *S. aureus* were reported, especially in patients treated for endocarditis where the bacterial load is presumably high (Hayden et al. 2005; Julian et al. 2007). However, in spite of the increasing

daptomycin use in clinical practice in recent years, reports about daptomycin resistance emergence remain sporadic. Data collected in a surveillance study including over 97,000 *S. aureus* from the years 2005–2012 obtained from 400 clinical centers in the Americas, Europe, and the Asia-Pacific Region, showed that the prevalence of daptomycin-resistant *S. aureus* was extremely low (0.05 %) in all geographical regions with no trend toward increased resistance over the years. Daptomycin MICs were similar between MRSA and MSSA isolates, yielding a similar prevalence of resistance in both groups (Sader et al. 2014).

Several studies documented that *S. aureus* isolates from patients, who previously received vancomycin therapy, were more prone to develop daptomycin resistance (Julian et al. 2007) and showed a relationship between decreased susceptibility to vancomycin and resistance to daptomycin (Bayer et al. 2013). In one of the first study, out of 70 VISA isolates, 80 % were found resistant to daptomycin (Patel et al. 2006). The link between VISA and daptomycin-resistant phenotypes consists probably in the thickened cell wall that may influence the penetration of both vancomycin and daptomycin, thus preventing the interaction with their respective targets (Cui et al. 2006).

5 Conclusions

The recent evolution in *S. aureus* epidemiology has led to the emergence of lineages that are endowed with characteristics of adaptation to different environments or hosts, and with antibiotic resistance traits. In this scenario, the characterization of *S. aureus* isolates by molecular typing is of utmost importance to better understand *S. aureus* micro and macroevolution. The dynamic evolution of *S. aureus* facilitated by travel, migration, and globalization has enabled geographically restricted clones to spread and become pandemic. The early recognition of high-risk clones that are particularly able to adapt and spread in the clinical environment is important for their control, since effective antibiotics are limited. Antimicrobial agents such as glycopeptides, linezolid, daptomycin, and other newer antibiotics are still active against the majority of isolates, but their efficacy will be jeopardized by increased use.

Given the affordable costs, the advent of WGS in routine use will represent a major breakthrough in the study of *S. aureus* epidemiology. Molecular typing should be applied on a larger scale to improve the understanding of *S. aureus* circulation, especially in low-income countries where the burden of *S. aureus* infections is probably higher than in industrialized countries, but it is not well acknowledged so far.

Economic and political efforts should aim at strengthening surveillance systems based on molecular typing at local and global level and at introducing appropriate control measures both in healthcare and in community settings.

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