Methicillin-resistant *Staphylococcus aureus*: an ever emerging threat

Among multidrug-resistant bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) is of immediate concern, given its potential for pathogenicity and widespread presence in healthcare settings. Over the last four decades, MRSA has spread throughout the world and the global prevalence of MRSA in hospitals (hospital-associated MRSA) continues to increase with the dissemination of a limited number of MRSA clones, each with a specific genetic background and staphylococcal cassette chromosome mec. Measures to control the hospital spread of MRSA have concentrated principally on transmission-based control policies. European surveillance data suggest that the increasing rate of hospital-associated MRSA is not an inexorable trend, and European countries with variable baseline prevalence and infection control policies were able to reverse the MRSA trend. Recently, MRSA strains have emerged and rapidly spread in the community. These so-called community-associated (CA)-MRSA strains are mainly associated with skin and soft tissue infections in previously healthy and young persons. CA-MRSA isolates from different areas of the world have common characteristics: the production of Panton–Valentine leukocidin and the presence of short staphylococcal cassette chromosome mec elements. In the USA, CA-MRSA became more prevalent than methicillin-susceptible *S. aureus* in community-associated *S. aureus* infections. In Europe, their prevalence remains below 5%, except for in Greece. The recent emergence of virulent CA-MRSA isolates harboring the *tst* gene and of MRSA from animal origin is of major concern. Large efforts are necessary to avoid penicillinase-mediated resistance in *S. aureus* occuring again with MRSA.

KEYWORDS: community ■ emergence epidemiology ■ hospital ■ infection control ■ methicillin resistant ■ *Staphylococcus aureus*

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Staphylococcus aureus is an ubiquitous bacterium that is frequently part of the human microflora, causing disease when the immune system becomes compromized. Although *S. aureus* can be found in different parts of the body, anterior nares are the main ecological reservoir in humans [1]. This versatile pathogen is responsible for a wide variety of diseases, including superficial, systemic and life-threatening infections, and toxinoses [2]. Our capacity to treat *S. aureus* infection, has been increasingly challenged by the emergence and re-emergence of antibiotic-resistant isolates. This microorganism has a great adaptative power to antibiotics, and little by little, it has acquired resistance to multiple antimicrobial agents. Methicillin-resistant *S. aureus* (MRSA) was first described in 1961 in England [3] as the result of the acquisition of an exogenous gene (*mecA*) that probably originated from *Staphylococcus sciuri* [4]. The *mecA* gene encodes an additional penicillin-binding-protein (PBP2a) with low affinity for β-lactam antibiotics. The *mecA* gene is regulated by the repressor MecI and the transducer MecR1. The *mecA* gene, which is 2.1 kb in length, is located on a mobile genomic island, that is called staphylococcal cassette chromosome mec (SCCmec). Until now, seven main types of SCCmec (types I–VII) are recognized. SCCmec type I, IV, V, VI and VII only cause β-lactam antibiotic resistance, while SCCmec type II and III cause resistance to multiple classes of antibiotics due to the additional drug resistance genes integrated into SCCmec.

Over the last four decades, MRSA has spread throughout the world and has become highly endemic in many geographical areas. This pathogen causes severe morbidity and mortality in hospitals worldwide [5,6]. Initially, MRSA nosocomial infections were mainly detected in large tertiary hospitals and in intensive care units, where colonized and infected patients, as well as colonized healthcare workers, were a significant source of cross-contamination. Currently, MRSA is one of the most common pathogens in hospitals of all sizes and of different types (acute, chronic and long-term care facilities) worldwide. Until recently, the MRSA problem was limited to hospitals, and MRSA infections were mostly acquired in hospital units. The emergence and dissemination of community-associated (CA)-MRSA producing the Panton–Valentine

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leukocidin (PVL) in patients with no recognized risk factors for MRSA infection is a source of particular concern. The purpose of this article is to provide up-to-date information on this ever emerging pathogen.

Hospital-associated MRSA

Epidemiology

Although MRSA was identified at the beginning of the 1960s, it was not until the mid-1980s that it became a frequent adversary. The increase in MRSA infections most likely reflects the growing impact of medical interventions, devices, older age and comorbidities of patients [5]. Antibiotic use is also likely to contribute to the spread of MRSA. It is now well established that colonized and infected inpatients are the major reservoir of this pathogen and that the transient carriage of MRSA on the hands of hospital care workers is the most common mechanism of patient–patient transmission. After acquisition, MRSA strains multiply on the contaminated tissue and may then colonize and possibly infect the patient. This progression to symptomatic infection is promoted by the existence of a site of entry, such as a wound or an indwelling venous or urinary catheter.

Many studies have identified individual risk factors for MRSA infection. These factors can be divided into three categories:

- Those reflecting the number of potential reservoirs and the number of opportunities for cross-transmission;
- Those associated with the immunological status of the patients;
- Those related to the antibiotic treatment used to treat the patients [7,8].

The global prevalence of MRSA in hospitals continues to increase worldwide [9]. In the USA, a large surveillance program of nosocomial bloodstream infections demonstrated that among all *S. aureus* isolates, the MRSA rate increased from 22% in 1995 to 57% in 2001 [10]. The SENTRY Antimicrobial Surveillance Program investigated the prevalence of MRSA in hospitals worldwide between 1997 and 1999. It was observed that the MRSA prevalence was 23% in Australia, 67% in Japan, 35% in Latin America, 40% in South America, 32% in the USA and 26% in Europe [11]. The MRSA prevalence between countries in Europe is variable. The European Antimicrobial Resistance Surveillance System (EARSS) reported significant differences in the frequency of MRSA in blood isolates between European countries (Figure 1). The EARSS map of MRSA shows a clear north–south split, which has been well known since the 1990s. The one major anomaly appears to be the UK, which is unique from the Mediterranean countries regarding MRSA rates. In Europe, the frequency of MRSA among *S. aureus* clinical isolates is low (<2%) in Scandinavia, The Netherlands and Iceland, intermediate in central Europe (5–20%) and higher (>25%) in southern Europe as well in the UK and Ireland. However, an unprecedented decrease in MRSA rates has been recently observed in several European countries (UK, France, Slovenia and Turkey), whereas other large countries, such as Germany and Spain, have stabilized their MRSA incidence. This indicates that the increasing emergence rate of MRSA is not an inexorable trend and that a diverse group of European countries with variable baseline prevalence and infection control policies were able to reverse MRSA trend [12].

Worldwide, a limited number of MRSA clones are disseminating, each with a specific genetic background and SCCmec type [13]: Archaic clone (ST250-MRSA-I), Berlin clone (ST45-MRSA-IV), Brazilian/Hungarian clone (ST229-MRSA-III), Iberian clone (ST247-MRSA-I), New York/Japan clone (ST5-MRSA-II), Pediatric clone (ST5-MRSA-IV), UK-MRSA-2 clone (ST8-MRSA-IV) and UK-MRSA-15 (ST22-MRSA-IV) are the most frequently reported clones. Kreiswirth *et al.* suggested the single-clone theory to explain the relationship between the first MRSA and the further various MRSA clones. This theory suggested that the various MRSA clones have a common methicillin-susceptible *S. aureus* ancestor that acquired SCCmec only once [14]. However, several studies support the multiclone theory, and it has now been demonstrated that the SCCmec element was introduced several times into different *S. aureus* lineages [15]. Enright and colleagues demonstrated that major MRSA clones were clustered into five clonal complexes (CC5, -8, -22, -30 and -45) and that the same sequence type carried different SCCmec elements [16].

In addition to the spread of the major hospital-associated (HA)-MRSA clones, shifts over time have been observed of these clones in countries, in small regions within one country or in single hospitals [13,17]. Moreover, coexisting with major clones, minor clones (MRSA strains from single hospitals) and sporadic isolates (MRSA strains from single patients) have also been observed [18].

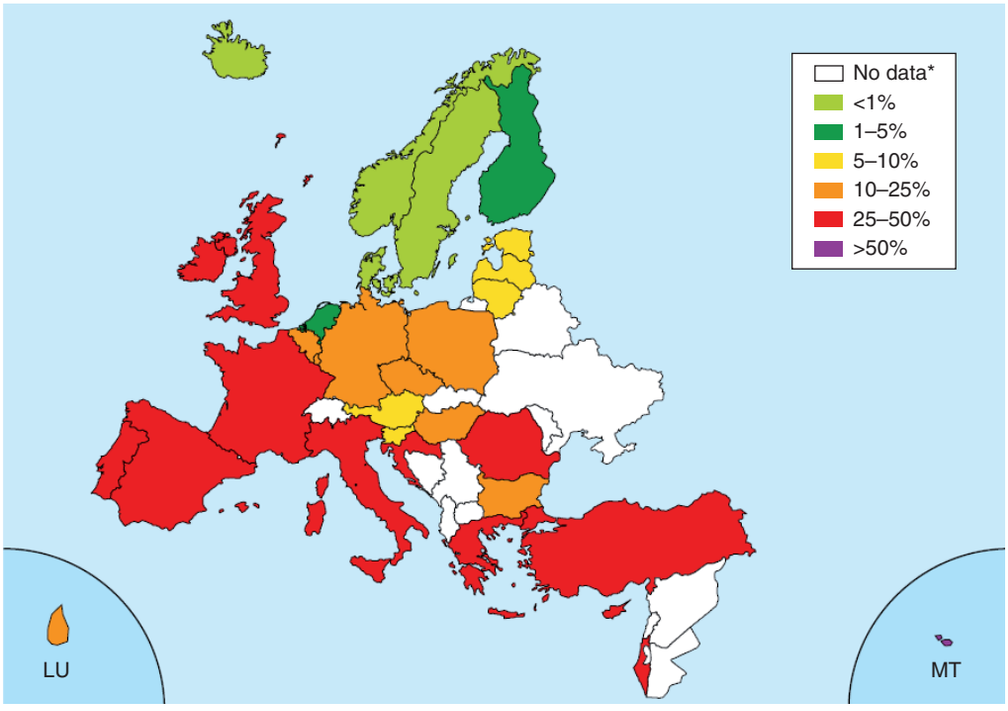

Figure 1. Frequency of methicillin resistance among *Staphylococcus aureus* isolated from blood.
LU: Luxembourg; MT: Malta.
Data taken from [102].

HA-MRSA control measures

Since the first half of the 1990s, most intensive care units, in which the infectious risks associated with MRSA are very high, have implemented prevention measures. These practices have been considered central to most national guidelines and should be applied to all hospital specialties. Adapted measures should also be implemented in long-term care facilities and nursing homes, considering that these settings can no longer be ignored as reservoirs for MRSA [6,19]. Measures to control the spread of MRSA have concentrated principally on transmission-based control policies, such as active surveillance to identify colonized patients (screening by classical culture or PCR-based methods), patient isolation or cohorting, environmental decontamination, MRSA decolonization of patients and healthcare workers, hand hygiene and the use of barrier precautions (i.e., aprons or gowns and gloves) [20]. However, hand hygiene is recognized as the cornerstone of preventing cross-transmission of microorganisms, including MRSA. The hand hygiene procedures recommended are either washing or rubbing with alcohol-based solutions or gels. Nevertheless, rub-in hand disinfection appears to be the best technique; it has a variety of benefits, including better compliance and better tolerance. Furthermore, it has been proven to decrease infection rates significantly as a result of improved compliance [21]. Despite increased awareness, the 'global' incidence of HA-MRSA continues to rise, probably owing to poor adherence to infection-control practices. The Society for Healthcare Epidemiology of America (SHEA) guidelines have reported low adherence rates to hand-hygiene practices in healthcare workers, averaging 40% [20]. There are many reasons, beliefs and attitudes that result in different behaviors, and that influence our knowledge regarding the role of hands in medical care and our understanding of hands as the most important vectors for the transmission of pathogens [22].

If the need for implementing MRSA control strategies is not yet a matter of debate, controversies on the choice of infection-control measures remain. In a broad outline, two approaches are proposed. The first one is active and includes screening of MRSA carriers, isolation or cohorting of positive patients, environmental decontamination and MRSA decolonization of patients and healthcare workers. The second considers the standard precautions to be sufficient to control MRSA. Scientific data are somewhat uncertain and often contradictory. Most scientific publications report quasi-experimental or before/after studies measuring the impact of an intervention.

Consequently, variations in MRSA incidence can be attributed to the intervention, but also to confounding factors [23]. Some epidemiological (use of control group) and statistical methods (time series analysis and linear regression) take these uncertainties into account [24,25]. More complex methods, such as multicenter crossover trials could be proposed but are complicated, expensive and time consuming [26]. Moreover, the epidemiological situation varied in one setting and varied between settings. Generalization of the findings is difficult. As infection control measures are generally applied in a bundle, it is quite impossible to determine the individual impact of a specific measure. Moreover, if a measure is tested individually, it can be efficient by itself, but also modify the behavior of healthcare workers in adherence to other measures. Finally, it can be difficult to evaluate the level of application of recommended infection control measures. It is likely that the success of a strategy depends more on the method of application of the measures than the measures themselves. In summary, infection control measures are essential for the control of MRSA and there is a need to fully adopt international guidelines, such as the WHO's first global patient safety challenge (Figure 2). Hand hygiene should be a global standard for patients in all healthcare settings and other measures should be applied following local guidelines.

In addition, guidelines to control MRSA in hospitals pay much less attention to controlling antibiotic use despite increasing evidence of a relationship between antibiotic use and the spread of MRSA [27–31]. The dissemination of epidemic clones does not necessarily require antimicrobial selective pressure. However, the results of these recent studies suggest that antimicrobials contribute to MRSA spread. Furthermore, hospitals in countries with very low incidences of MRSA, particularly Nordic countries, use the least amount of antimicrobials in Europe [32,33]. Additional research is needed to fully understand the relationship between antimicrobial use and MRSA acquisition. However, there is evidence supporting the implementation of programs to control and improve prescription practices (i.e., antibiotic stewardship) when infection control alone fails to control the spread of MRSA.

Community-associated MRSA

As previously mentioned, MRSA isolates have spread internationally. However, they have traditionally been associated with infections in hospitals, to which they were mostly confined. Recently, MRSA strains have emerged and rapidly spread in the community. In 1993, MRSA isolates with unique genetic elements were reported among infected Australian aborigines, who had no contact with the healthcare system [34]. In the USA, four cases of fatal pneumonia MRSA infections in children were reported [35]. In France, Gillet *et al.* described a series of children with necrotizing pneumonia caused by PVL-positive *S. aureus* [36]. The reasons why various clones of CA-MRSA emerged almost concomitantly in different continents remains unknown. Robinson *et al.* have demonstrated that the Southwest Pacific clone with multilocus sequence typing, type ST30, is closely related to a clone known as phage type 80/81 [37]. The latter is a notorious penicillin-resistant clone of *S. aureus* that has caused serious hospital- and community-associated infections worldwide during the 1950s. This clone, which was largely eliminated in the 1960s, re-emerged after acquisition of SCCmec type IV gene as a CA-MRSA clone and represents a sister lineage to pandemic HA-MRSA. This illustrates the dynamic and complex evolution of bacterial clones (Figure 3).

These so-called CA-MRSA strains are responsible for infections with a particular clinical presentation, unlike infections caused by HA-MRSA. CA-MRSA infections are mostly associated with skin and soft-tissue infections that occur in previously healthy and young persons [38]. Although there is no consensus definition, CA-MRSA isolates from throughout the world have several common characteristics. The most important are the production of PVL, which is infrequent in other *S. aureus* strains, and the presence of short SCCmec elements (of type IV or V) (Table 1). CA-MRSA isolates initially lacked multiple resistance to antibiotics. However, they can be classified as multidrug-resistant organisms since they are resistant to the β-lactam class of antibiotics, which includes major antistaphylococcal agents, and to some other antimicrobials, such as tetracyclines and fusidic acid, when isolated in Europe [39,40]. In the USA, CA-MRSA became more prevalent than methicillin-susceptible *S. aureus* in CA-MRSA infections. In a recent study, including 422 emergency department patients, 59% of *S. aureus* isolates from skin and soft tissue infections requiring drainage were resistant to methicillin with variations from 20 to 72%, depending upon the state [41]. Most infections in the USA are due to an MRSA clone, characterized by sequence type ST8, and called USA300 on the

Figure 2. "My five moments for hand hygiene" from WHO guidelines for hand hygiene in healthcare.
Reproduced from [103].

basis of pulsed-field gel electrophoresis type, initially used to characterize MRSA [42]. This clone emerged within multilocus sequence typing clonal complex 8. Two recent major events in the evolution of the USA300 clone were observed. First, it started to infiltrate hospitals and replace the traditional HA-MRSA strains. From 2000 to 2006, the proportion of MRSA strains isolated from hospital-onset bloodstream infections and displaying a community phenotype of resistance increased from 24 to 49% in a US hospital [43]. The total number of bloodstream infections remained stable, suggesting that the CA-MRSA strains replaced the HA-MRSA strains without causing additional infections. However, the spread of CA-MRSA infections in hospitals is worrying, as it would occur among a more debilitated, older patient population and would provoke more severe infections. In addition, the skin tropism may add to the capacity of CA-MRSA to disseminate, which may eventually lead to an increased global burden of infections. Second, variants of USA300 MRSA that were resistant to clindamycin, ciprofloxacin and mupirocin became common among men who have sex with men [44]. CA-MRSA strains have also been described on other continents. Clones were initially reported as being continent-specific, with the ST80 type spreading in Europe. However, recent data have demonstrated intercontinental exchanges of CA-MRSA clones and the emergence of new PVL-positive clones, resulting in a more complex situation. In Europe, the prevalence remains below 5% [45–47], except for Greece [48]. The prevalence is also high south of Europe, with 72% of MRSA strains containing PVL genes in Algeria [49].

Despite the recent developments, the European ST80 clone seems to have less potential for dissemination than the USA300 clone. The epidemiological success of the USA300 clone (which may be considered as a superbug)

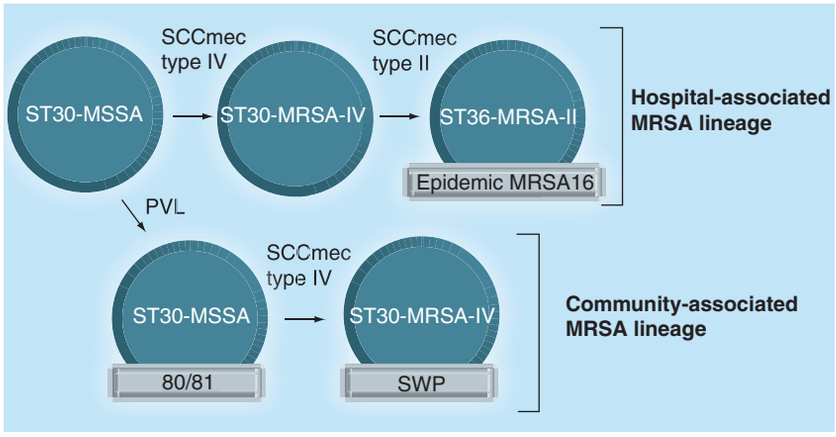

Figure 3. Established community-associated methicillin-susceptible *Staphylococcus aureus* clones that possess genes for the Panton–Valentine leukocidin toxin. One postulated means of evolution of MSSA into MRSA (both hospital-associated and community-associated strains) involves the horizontal transfer of virulence genes, such as PVL genes, and the acquisition of SCCmec allotypes. MRSA: Methicillin-resistant *Staphylococcus aureus*; MSSA: Methicillin-susceptible *Staphylococcus aureus*; PVL: Panton–Valentine leukocidin; SCC: Staphylococcal cassette chromosome; ST: Sequence type; SWP: Southwest pacific clone. Reprinted with permission from [37].

has tentatively been explained by several particular characteristics [50–52]. The possibility that the USA300 clone, which has recently arrived in Europe, may replace ST80 leading to a situation similar to that seen in the USA should be considered.

Toxic shock syndrome toxin-1-producing MRSA

Another MRSA clone, producing toxic shock syndrome toxin (TSST)-1 and ST5, has recently been detected in France and Switzerland, in community-associated infections in some cases [53,54]. In a recent study, Dauwalder *et al.* reported that a TSST-1-positive clone (the Geraldine clone) accounted for 6.3% of invasive MRSA isolates collected in France [45].

Toxic shock syndrome toxin-1, a superantigenic toxin secreted by some *S. aureus* isolates, was first described in 1978 by Todd *et al.* in methicillin-susceptible *S. aureus* [55]. TSST-1, encoded by the *tst* gene, is a major virulence factor implicated in toxic shock syndrome (TSS), staphylococcal scarlet fever and neonatal toxic shock-like exanthematous diseases recently described in Japan and France [53,54,56]. TSS was initially linked to tampon use by young women, but nonmenstruation-associated TSS now predominates, occurring both in the community and in hospitals secondary to local *S. aureus* infection [15].

In a recent French study, Dauwalder *et al.* reported that the TSST-1-positive clone (Geraldine clone) and the PVL-positive clone (European ST80 clone) accounted for 6.3 and 3.6% of invasive MRSA isolates collected in France, respectively [45]. In an, as yet, unpublished large-scale study in 2008, we tested more than 7000 MRSA isolates recovered in French hospitals and found twice as many isolates producing TSST-1 (2.8% of MRSA, mostly belonging to Geraldine clone) as isolates producing PVL (1.5% of MRSA, mostly belonging to the European ST80 clone) [Robert J, Unpublished Data]. These data may represent only a national concern; however, the spread of such clones associated with potentially lethal infections (both PVL and TSST-1), and with potent superantigenic activity (TSST-1) or the ability to cause more serious and recurrent infections (PVL) is of major concern. The recognition of these MRSA clones is essential for the implementation of effective measures to control their spread [39].

Animal reservoir of MRSA

In recent years, MRSA emerged as a veterinary pathogen. Infections have been reported in horses, dogs, cats and pigs among others [57–60], and it has been found that animals can serve as sources of colonization. In 2003, a new MRSA strain, geographically linked to pig farming, emerged in The Netherlands. Molecular typing demonstrated that this MRSA strain belonged to a new sequence type, ST398. This study demonstrates that MRSA from an animal reservoir has recently entered the human population and

Table 1. Main characteristics of community-associated methicillin-resistant *Staphylococcus aureus* strains according to their geographic origin.

	Europe	USA	Oceania
Sequence type†	80	1/59/8	30/93
agr type‡	3	3/1/1	3/3
SCCmec	IV	IV	IV
PVL production	+	+	+
Resistance pattern	Methicillin-R kanamycin-R fusidic acid-R	Methicillin-R	Methicillin-R

†*Determined by using multilocus sequence typing.*
‡*Accessory gene regulator.*
PVL: Panton–Valentine leukocidin; R: Resistance; SCC: Staphylococcal cassette chromosome.

is now responsible for over 20% of all MRSA in The Netherlands [59]. Even meat products may be contaminated [61]. This new clone was identified in numerous countries in Europe and in the USA [60,62–64]. The nasal carriage rates of MRSA among veterinary personnel working with pigs are high [65]. Of the 272 attendees at an international pig conference in Denmark, 34 (12.5%) participants from nine countries were carriers [66]. In regions with a high prevalence of animal MRSA, veterinarians and farmers admitted into hospitals should be considered as potential carriers and warrant MRSA screening and standard measures to prevent intrusion of these strains into the hospital setting [67]. A survey conducted in 2008 by the European Food Safety Authority revealed that 17 Member States detected MRSA in their breeding or production holdings, whereas seven Member States did not detect any MRSA in the surveyed holdings. The EU prevalence of MRSA-positive holdings with breeding pigs, as estimated based on results from the 24 participating Member States, was 22.8%. MRSA ST398 was the predominant MRSA lineage identified in the holdings with breeding pigs in the EU, accounting for 92.5% of the MRSA isolates [101]. These alarming results suggest that ongoing surveillance of MRSA in animals is warranted and investigations are needed to reduce household transmission from animals to humans.

Conclusion

Although much knowledge on the spread of MRSA has been gained in recent decades, there are still a number of issues that must be clarified. Progress has been made in controlling MRSA in hospitals. The rate of MRSA can be reduced substantially through the implementation of intervention strategies, even in settings were MRSA is endemic. To prevent the further dissemination of MRSA-emerging clones worldwide, investigations are needed to establish whether a large 'search-and-destroy' policy should be adapted. Such a policy was implemented 25 years ago in Denmark when the MRSA prevalence was 30%. Since than, the MRSA prevalence has decreased to less than 1%, and this low percentage has been maintained up until now [68]. However, a search-and-destroy policy to prevent CA-MRSA transmission would be more complicated to implement worldwide than when it was implemented in Denmark [69]. Be that as it may, efforts will be inevitably necessary to avoid penicillinase-mediated resistance in *S. aureus* (more than 90% of isolates are actually penicillinase producers) occuring again with MRSA [70].

Future perspective

Staphylococcus aureus is an extraordinarily adaptable pathogen with a proven ability to a acquire antimicrobial resistance and virulence determinants. Forecasting the evolution of MRSA is an hazardous task. In the 1990s, most of the multidrug-resistant specialists thought that emergence and dissemination of vancomycin resistance among MRSA represented a major risk to MRSA evolution. Actually, this emergence remains negligible and unexpected MRSA strains, with different genetic backgrounds from classical HA-MRSA, have emerged, almost concomitantly in different continents, and have rapidly spread in the community. Knowledge regarding MRSA clones that are disseminating

Executive summary

***Hospital-associated methicillin-resistant* Staphylococcus aureus**

- Over recent decades, methicillin-resistant *Staphylococcus aureus* (MRSA) has spread throughout the world and the global prevalence of hospital-associated MRSA continues to increase.
- In Europe, the frequency of MRSA among *S. aureus* clinical isolates is low in northern Europe (<2%), intermediate in central Europe (5–20%) and higher (>25%) in southern Europe, as well in the UK and Ireland. A decrease in MRSA rates has been recently observed in several European countries (UK, France, Slovenia and Turkey), whereas other large countries, such as Germany and Spain, have stabilized their MRSA incidence. This example demonstrates that implementation of infection control policies were able to reverse MRSA trend.

Community-associated MRSA

- The emergence and dissemination of community-associated MRSA producing the Panton–Valentine leukocidin in patients with no recognized risk factors for MRSA infection is a source of particular concern.
- Preventive strategies should be defined and implemented to avoid the globalization of the US situation.

New MRSA threats

- The emergence of virulent community-associated MRSA isolates harboring the *tst* gene and animals as a source of MRSA for subsequent human infections are of major concern.

Conclusion

- The application of a voluntary strategy appears to be essential to avoid repeating a situation where penicillinase-mediated resistance in *S. aureus* occurs with MRSA, and this strategy will be expensive.

is required to implement any strategies to control the transmission of MRSA, either within hospitals, nursing homes or in the community. The increasing emergence of MRSA is not an inexorable trend and a diverse group of European countries with variable baseline prevalence and infection control policies were able to reverse the HA-MRSA trend. However, this requires a great amount of effort and it remains questionable whether these policies would be effective on CA-MRSA. In my opinion, the two major threats regarding MRSA are the intrusion of PVL-producing CA-MRSA within hospitals and the large diffusion of MRSA within the community, leading to the worldwide replacement of penicillinase-producing *S. aureus* with MRSA.

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