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Methicillin-resistant *Staphylococcus aureus*: related infections and antibiotic resistance

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SUMMARY

Staphylococcus aureus is a well adapted human pathogen, capable of living freely in the inanimate environment and spreading from person to person, existing as a colonizer or commensal, hiding in intracellular compartments and, most importantly, inducing various forms of human disease. Infections caused by *S. aureus*, above all by antibiotic-resistant strains, have reached epidemic proportions globally. The overall burden of staphylococcal disease caused by antibiotic-resistant *S. aureus*, particularly by the methicillin-resistant strains, is increasing in many countries, including Italy, in both healthcare and community settings. The widespread use of antibiotics has undoubtedly accelerated the evolution of *S. aureus*, which, acquiring multiple resistance genes, has become able to survive almost all antibiotic families; this evolution versus more resistant phenotypes has continued among the newer agents, including linezolid and daptomycin. The diminished clinical usefulness of vancomycin is seen as one of the most worrisome problems in many clinical settings and in many countries. In fact, the increasing spread of heteroresistant vancomycin-intermediate *S. aureus* (hVISA) and vancomycin intermediate (VISA) strains adds new problems, not only in terms of the treatment of severe infections sustained by these microorganisms, but also in the microbiological definition of susceptibility.

1. Introduction

Staphylococcus aureus is a well adapted human pathogen and infections caused by this microorganism, above all by antibiotic-resistant strains, have reached epidemic proportions globally. The rising prevalence of nosocomial methicillin-resistant *S. aureus* (MRSA) and the recent emergence of community-acquired MRSA (CA-MRSA) are major clinical, public health, and economic challenges.

MRSA is a multidrug-resistant pathogen, but the recent evidence that glycopeptides are losing their potency, is particularly worrisome. Serious infections due to MRSA, defined as susceptible using standard routine tests, are not responding well to glycopeptides. The emergence of heteroresistant vancomycin-intermediate *S. aureus* (hVISA) adds new problems, not only in terms of the treatment of severe infections sustained by these microorganisms, but also in the microbiological definition of susceptibility.

2. Pathogenesis and epidemiology

S. aureus is a well adapted human pathogen, capable of living freely in the inanimate environment and spreading from person to person, existing as a colonizer or commensal, hiding in intracellular compartments and, most importantly, inducing various forms of human disease. Additionally, staphylococci may form biofilms in any of their different states of existence.

A fundamental biological property of *S. aureus* is its ability to asymptomatically colonize healthy individuals. Approximately 30% of humans are nasal carriers,[1] such that, in these individuals, the microorganism is part of the normal flora. *S. aureus* carriers are at higher risk of infections and they are presumed to be an important source of the *S. aureus* strains that spread among individuals. Nasal colonization rates by *S. aureus* among hospitalized patients are comparable to those of the general population. However, MRSA carriage among the general population is less than 1%,[2] whereas *S. aureus* (MRSA) prevalence in nosocomial bacteremia is over 50% in certain countries.[3] The primary mode of transmission of this organism is by direct contact, usually skin-to-skin contact, and various host factors can predispose individuals to infection, including the loss of the normal skin barrier, the

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presence of underlying diseases such as diabetes or AIDS, and defects in neutrophil function. To these factors, antibiotic pressure, length of stay in an intensive care unit (ICU), colonization pressure, professional healthcare workload, hand hygiene levels, isolation treatment measures, and environmental contamination have been proposed as important factors in the acquisition and transmission of *S. aureus* in ICUs.[4]

Infections caused by *S. aureus*, above all by antibiotic-resistant strains, have reached epidemic proportions globally.[5] The capacity of this microorganism to cause a spectrum of human disease reflects an incredible ability to adapt to microenvironments in the human body and suggests that the pathogenesis of *S. aureus* is a complex, regulated process, as summarized below, involving a diverse array of determinants coordinately expressed at different stages of infection.[6] As described before, staphylococcal pathogenesis is multifactorial, involving three classes of factors accounting for approximately 100: secreted proteins, including superantigens, cytotoxin and tissue degrading enzymes; cell-surface bound proteins (MSCRAMMs, i.e., microbial surface components recognizing adhesive matrix molecules), including fibrinogen binding protein, other adhesins and anti-opsonins; and cell surface proteins, including the polysaccharide capsule and components of the cell wall peptidoglycan.[7]

The classic staphylococcal infection is an abscess: organisms entering the tissue of a host produce a series of extracellular proteins and other factors, such as cell wall and capsular components, which enable them to coagulate fibrinogen, adhere to the intercellular matrix, degrade tissue components, and lyse local cellular elements. This evokes a potent innate immune response that includes interleukins, opsonins, complement, and phagocytes. Additionally, humans have circulating antibodies to most staphylococcal antigens, and these will obviously participate in the initial response. These antibodies, the innate immune response, and fibrin generated by the organism, wall off the lesion creating a pocket within which a battle between the organism and phagocytes is waged, generating pus.

Although everyone gets superficial skin infections, staphylococcal infections can be initiated occasionally in deep tissue sites and these result in deep tissue infections that often require surgery. *S. aureus* can also alight on the heart valves, more often in intravenous drug abusers or the elderly. Heart valve lesions, known as vegetations, consist largely of platelets, fibrin organisms, and neutrophils and their structure is considered akin to biofilm formed on inanimate surfaces. A particularly troublesome, but non-fatal, condition is osteomyelitis, which again can occasionally occur spontaneously, but much more often follows an open fracture.

A special set of pathological conditions, toxinoses, are caused by single toxins. In many cases, the purified toxin can generate all the symptoms; in some cases, the living organism must be present, contributing, for example, to the ability to adhere to the extracellular matrix or to resist eradication by the host. Examples are toxic shock syndrome (TSS), scalded skin syndrome, and necrotizing pneumonia (Panton–Valentine leukocidin (PVL)).

Such a complex and integrated set of weapons of virulence genes suggests that distinct networks of virulence genes are likely activated in response to host signals; in vitro results have demonstrated regulation in a population density manner under the control of global regulators such as the *agr* system.[8] Knowledge of which genes are turned on and the circumstances under which they are activated is critical for understanding their role in pathogenesis. Much has been learned from the in vitro analyses and from animal model studies, but recent data on the applicability of these models to human disease are opening a new era of confirmation of what is known to date and will probably bring about new strategies to treat serious staphylococcal infections.[9]

The overall burden of staphylococcal disease caused by antibiotic-resistant *S. aureus*, above all by the methicillin-resistant strains, is increasing in many countries, including Italy, in both healthcare and community settings.[10,11] In a recent American update by the National Healthcare Safety Network (NHNS)[12] performed on antimicrobial-resistant organisms responsible for healthcare-associated infections, *S. aureus* was the most prevalent isolate in skin and soft tissue infections (SSTIs), in blood stream infections (BSI), and in ventilator-associated pneumonia (VAP); in the case of surgical site infections its prevalence changed as follows: neurologic < orthopedic < cardiac < vascular < obstetric/gynecological. The authors found an overall MRSA prevalence of 56.2% and their data were comparable to data from other surveillance systems in the USA and Europe.[3,12,13]

It is clear that MRSA is still the number one cause of hospital-associated infections. The mortality rate associated with invasive infections is approximately 20%[14] and in the USA, but also in some countries in Europe, these infections are probably the leading cause of death by any single agent; fatalities resulting from these infections are estimated to surpass those caused by HIV/AIDS.[14]

The recent appearance of CA-MRSA, i.e., infections that occur outside of healthcare facilities in otherwise healthy people, is one of the most surprising events in recent years and adds to our concerns.[11,15] In general CA-MRSA is particularly virulent compared to HA-MRSA due to the presence of many virulence factors.[16] It primarily causes SSTIs, but invasive, fatal infections such as bacteremia and necrotizing pneumonia have also been reported. These strains are also well characterized by genetic markers such as the presence of SCC*mec* type IV, V or VII and the presence of the PVL. It still remains a mystery why these antibiotic-resistant bacteria have emerged in a niche such as the community, not under the high selective pressure exerted by antibiotics, but the success of these clones is demonstrated by their rapid spread, and, in recent years, by the possible permeation into hospitals of these new more virulent CA-MRSA strains.[10,14]

3. History of MRSA and evolution of resistance

The history of the epidemic behavior of *S. aureus* starts in the 'antibiotic era' immediately following the introduction of penicillin in the 1940s; during the 1950s, the notorious penicillin-resistant clone of *S. aureus,* known as phage type 80/81, PVL producer, emerged and caused serious hospital-acquired (HA) and community-acquired (CA) infections worldwide (now known as ST30-CA-MRSA-IV, formerly Southwest Pacific clone).[17,18] Then suddenly, in 1959, penicillin-resistant *S. aureus* strains spread in hospitals and in the community, accounting for around 80% of the total *S. aureus* population. From this point on, the development of antimicrobial resistance in this microorganism was rapid. Among the numerous resistance determinants acquired by *S. aureus*, the most manful one was that to methicillin, synonymous now with multi-drug resistance. Methicillin resistance emerged and diffused widely all around the world by a clonal spreading, and the 'age of MRSA' commenced: we have faced 40 years of MRSA and it is here to stay!

As already mentioned, since its appearance, MRSA prevalence has constantly increased over time, especially in severe infections in hospitalized patients, currently contributing to a high mortality rate, above all in intensive care patients with BSIs. The first methicillin-resistant strain (MRSA strain COL), was isolated in a patient in Colindale (London, UK) in 1961; this was a major global defeat as, since their appearance, MRSA strains have been able to evolve rapidly and create new clinical problems.[19]

The MRSA clones evolved from the MRSA COL strain (formerly archaic clones) shared archaic molecular features and were members of the same genetic lineage (CC8), including the major penicillin-resistant methicillin-susceptible *S. aureus* (MSSA)

strains (ST250), HA- and CA-MRSA strains. The archaic clone circulated in the UK and in Europe from the 1970s to the 1980s, but did not establish itself in the USA. The 1980s were characterized by the appearance of gentamicin-resistant MRSA strains in several countries, including the USA and Europe. In the following 20 years, various international multidrug-resistant (MDR) MRSA lineages acquired new SCC*mec* allotypes, homogeneously disseminated in hospitals and in the community, due to their ability to cause various infections, to persist and disseminate among diverse geographic areas, including continents, thus making them pandemic microorganisms.[20]

Methicillin resistance is associated with the acquisition of a particular resistance island called SCC*mec*, an exogenous piece of DNA, variable in size, that is absent from the methicillin-susceptible strains. At least eight types of SCC*mec* have been discriminated on the basis of the structure of their *ccr*A-B and *mec*A complexes.[21] SCC*mec* types I, II, III and VIII have been shown to belong to hospital clones; while types IV, V, VI, and VII have been identified as being typical of CA-MRSA. The latter group of cassettes is much smaller than their hospital-related congeners, do not carry multiple antibiotic resistance genes, and may be easier to mobilize. These cassettes are associated, in the community strains, to pathogenicity islands carrying multiple staphylococcal exotoxin genes, including the virus-encoded PVL toxin. Together, these elements may make the organisms particularly 'fit' and virulent.

MRSA strains are characterized by a multiple-resistance phenotype as a consequence of accumulation and organization in the SCC*mec* region of many resistance determinants over time; this region can act as a hot-spot of integration for plasmids, transposons, and insertion sequences. SCC*mec*-types and insertion/deletion of other mobile genetic elements may be involved in modulating the epidemic behavior of MRSA strains of similar genetic background, independently of antibiotic usage. The dynamics of these genotypic changes are strongly linked to shifts in the levels of susceptibility to many classes of antibiotics, and the percentage of antibiotic resistance is associated with the epidemiologic change and replacement of clones with specific markers of resistance. Some examples could be the decrease in resistance to tetracycline, rifampin, co-trimoxazole, and spectinomycin, due to the replacement of the MDR-MRSA clones ST247-MRSA-IA, ST239-MRSA-IIIA, and ST247-MRSA-I/IA with the tetracycline-susceptible ST228-MRSA-I clone, or the increased level of susceptibility to gentamicin found in MRSA isolated in Europe during 2000–2007, mainly due to the appearance of a gentamicin-susceptible clone, belonging to ST22.

The widespread use of antibiotics has undoubtedly accelerated the evolution of *S. aureus*, which, acquiring multiple resistance genes, has become able to survive almost all antibiotic families, i.e., beta-lactams, macrolides, tetracyclines, fluoroquinolones, and aminoglycosides: this evolution versus more resistant phenotypes has continued among the newer agents, including linezolid and daptomycin. No antibiotics have been able to escape this correlation between the use of antibiotics and the development of resistance: no sooner has an antibiotic been introduced into clinical practice, than some resistant strains have arisen. This is true for all classes of antibiotics with the exception of glycopeptides (considered the gold standard for MRSA therapy): in fact, resistance to these agents among *S. aureus* strains took almost 40 years to be recognized.[22]

These recent increasing reports of reduced susceptibility to glycopeptides due to their selective pressure from years of use, has resulted in two different phenotypes: hVISA and vancomycin-intermediate (VISA) strains. In contrast, high-level resistance mediated by the *van*A gene complex acquired by vancomycin-resistant enterococci (VRE) has emerged in specific areas of the world, above all in the USA in the area of Detroit. These resistant strains have variable expression of resistance.[17]

The diminished clinical usefulness of vancomycin is seen as one of the most worrisome problems in many clinical settings and in many countries.[22] Although isolates with homogeneous resistance to vancomycin (VISA; minimum inhibitory concentrations (MICs) >4 mg/l) continue to be rare, there are increasing reports describing the possible existence of heteroresistance sub-populations (hVISA), hidden often in strains with vancomycin MICs in the 1–2 mg/l range.[23] Despite the fact that microbiological resistance to vancomycin in *S. aureus* remains very rare, failure of vancomycin therapy is already a clinical problem in hospitals, in ICUs, especially in BSIs and infective endocarditis.[22,24] In an Italian study, an increase in vancomycin and teicoplanin MIC levels over time has been described in HA-MRSA strains isolated from 1999 to 2007.[10] More recently, a study (presented at the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) Meeting, 2009) has demonstrated a high percentage of hVISA strains among HA-MRSA isolated in Italy, possessing MICs to vancomycin between 1 and 2 mg/l; these strains came from documented bloodstream, pneumonia and skin-structure infections, during 2005–2006. They all belonged to the major nosocomial clones, and some of them possessed characteristics that resembled the classic VISA strain (Mu50), inducing us to hypothesize that VISA behavior can be hidden with a MIC <4 mg/l.[10,20] It should be noted that not all studies have documented a high percentage of hVISA in their MRSA isolates; but it is well documented that infections by hVISA are highly predictive of vancomycin treatment failure.[25]

In vitro detection of hVISA is insurmountable by current standard methods, which employ inoculum levels that may be too low; sophisticated and cumbersome tests, i.e., the population analysis profiling/area under the curve test (PAP/AUC), not routinely used in clinical microbiology laboratories, is required. Recent attempts to identify hVISA by macro-Etest have shown it to be useful, but with the problem of sensitivity, and confirmation is still required using the PAP/AUC ratio test.[20,22,23]

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Conflict of interest

The authors have no conflict of interest to report.

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