Staphylococcus Aureus: an overview for Methicillin-resistant

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ABSTRACT

 Methicillin-resistant Staphylococcus aureus (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. *MRSA is any strain of Staphylococcus aureus that has developed, through the process of natural selection, resistance to betalactam antibiotics .The appearance of (MRSA) strains has created serious therapeutical problems. In this article, we present an overview of the biochemical and genetic mechanisms of pathogenicity of S. aureus strains. Virulence factors, organization of the genome and regulation of expression of genes involved in virulence, and mechanisms leading to methicilin resistance are presented. This review describe the management of severe healthcare-associated infections due to methicillin-resistant Staphylococcus aureus (MRSA), including the limitations of current therapy, potential alternative agents, new therapeutic options.*

Keywords: *MRSA, molecular genetics, diagnosis, prevention*.

INTRODUCTION

S. aureus is a commensal and a pathogen. The anterior nares are the major site of colonization in humans. About 20–30% of individuals are persistent carriers of *S. aureus*, which means they are always colonized by this bacterium, and 30% are intermittent carriers (colonized transiently)   [1]. Colonization significantly increases the risk of infections since it provides a reservoir of the pathogen from which bacteria are introduced when host defense is compromised [2]. Patients with *S. aureus* infections are usually infected with the same strain that they carry as a commensal [28]. *S. aureus* is one of the main causes of hospital- and community-acquired infections which can result in serious consequences [3]. Nosocomial *S. aureus* infections affect the bloodstream, skin, soft tissues and lower respiratory tracts. *S. aureus* can be a cause of central venous catheter-associated bacteremia and ventilator- assisted pneumonia. It also causes serious deep-seated infections, such as endocarditis and osteomyelitis) [27]. In addition to the infections listed above, *S. aureus* is often responsible for toxin-mediated diseases, such as toxic shock syndrome, scalded skin syndrome and staphylococcal food borne diseases (SFD). Hospitalized patients are particularly exposed to *S. aureus* infections due to their compromised immune system and frequent catheter insertions and injections [29]. The SENTRY Surveillance Program investigating worldwide *S. aureus* infections during a two-year period has revealed that this pathogen is the leading cause of bloodstream, lower respiratory tract and skin/soft tissues infections in all regions surveyed [3].The importance of this human pathogen, apart from its ability to cause life-threatening infections, is its remarkable potential to develop antimicrobial resistance. *Staphylococcus aureus* is a species of bacterium commonly found on the skin and/or in the noses of healthy people. Although it is usually harmless at these sites, it may occasionally get into the body (eg through breaks in the skin such as abrasions, cuts, wounds, surgical incisions or indwelling catheters) and cause infections. These infections may be mild (e.g. pimples or boils) or serious (eg. infection of the bloodstream, bones or joints) [4]. *Staphylococcus aureus* (SA) has been a

plague of mankind since the dawn of history. In fact, an outbreak of staphylococcal skin disease may even be mentioned in the Bible. The modern recognition of *S. aureus* dates back to the late 19th century. Sir Alexander Ogston, a Scottish surgeon and early advocate of antisepsis, first described the organism in 1881 as a bacterial cause of "acute suppuration." He named the organism, "Staphylococcus pyogenes aureus, taking clues from its microscopic morphology, purulent nature, and tendency to form golden colonies on plated media. For much of the next half-century, this bacterium remained a notable cause of severe morbidity and death among patients. During World War I, post-influenza staphylococcal pneumonia occurred in young, healthy military personnel producing "dirty salmon-pink anchovy sauce" colored sputum, ultimately leading to "cherry-red indigo-blue cyanosis" and rapid progression to death [30]. Skin and soft tissue infections also frequently progressed to sepsis without effective antibiotic therapy to halt their spread. In 1941, 82% mortality was documented for patients who were treated for *Staphylococcus aureus* septicemia in the pre-antibiotic era [31]. The survival rate in the over-fifty population in their sample was only 2% [32]. Staphylococci are notorious for rapidly evolving resistance to many antibiotics. Penicillin and other β-lactam antibiotics kill bacterial cells by interfering with cell wall synthesis. Not long after penicillin was first used to treat human infections, *S. aureus* strains producing penicillinase (an enzyme that degrades penicillin) were detected and it is estimated that now >80% of *S. aureus* produce penicillinase. Methicillin (meticillin), a β-lactam antibiotic that is not inactivated by penicillinase, was introduced in the late 1950s. But by 1961, there were reports of methicillin resistant staphylococci in a hospital in the United Kingdom [33]. Although epidemiology of MRSA (methicillin-resistant *S. aureus*) is currently being intensely studied, it should be noted that in most hospitals and geographic areas MSSA (methicillinsusceptible *S. aureus*) are responsible for a greater number of infections and are often also resistant to multiple classes of antibiotics) [34]. MRSA stands for methicillin-resistant Staphylococcus aureus, which is a type of *Staphylococcus aureus* that is resistant to the antibacterial activity of methicillin and other related antibiotics of the penicillin class. The treatment of infections due to *Staphylococcus aureus* was revolutionised in the 1940s by the introduction of the antibiotic penicillin. However, most strains of *Staphylococcus aureus* are now resistant to penicillin. This is because *Staphylococcus aureus* can make a substance called ß-lactamase (pronounced beta-lactamase), that degrades penicillin, destroying its antibacterial activity. In the early 1960s, a new type of penicillin antibiotic called methicillin was developed. Methicillin was not degraded by ß-lactamase and so could be used to treat infections due to ß-lactamase-producing strains of Staphylococcus aureus. Subsequently, methicillin was replaced by newer and better penicillin-type antibiotics (such as flucloxacillin) that were also not affected by ß-lactamase [4].Unfortunately, shortly after the introduction of methicillin, certain strains of *Staphylococcus aureus* emerged that were resistant to methicillin (and also to the newer drugs such as flucloxacillin) These methicillin-resistant *Staphylococcus aureus* became known as 'MRSA' for short, and although methicillin is no longer prescribed, having been replaced by flucloxacillin, the term MRSA continues to be used. Although other types of antibiotics can still be used to treat infections caused by MRSA, these alternative drugs are mostly not available in tablet form and must be administered through a drip inserted into a vein or by injection. Methicillin-resistant *Staphylococcus aureus* was discovered in 1961 in the United Kingdom. It made its first major appearance in the United States in 1981 among intravenous drug users. MRSA is often referred to in the press as a "superbug". The number of MRSA infections in the United States has been increasing significantly. A 2007 report in Emerging Infectious Diseases, a publication of the Centers for Disease Control and Prevention (CDC) [100], estimated the number of MRSA infections in hospitals doubled nationwide, from approximately 127,000 in 1999 to 278,000 in 2005, while at the same time annual deaths increased from 11,000 to more than 17, 000 [5]. It has been argued that the observed increased mortality among MRSA-infected patients may be the result of the increased underlying morbidity of these patients. Several studies, however, including one by Blot and colleagues that have been adjusted for underlying disease still found MRSA bacteremia to have a higher attributable mortality than methicillin-susceptible *Staphylococcus aureus* (MRSA) bacterrmia [6]. MRSA is sometimes sub-categorized as community-acquired MRSA (CA-MRSA) or healthcare associated MRSA (HA-MRSA), although the distinction is complex. Some researchers have defined CA-MRSA by the characteristics of patients whom it infects, while others define it by the genetic characteristics of the bacteria themselves. The first reported cases of CA-MRSA began to appear in the mid-1990s in Australia, New Zealand, the United States, the United Kingdom, France, Finland, Canada and Samoa, and were notable

because they involved people who had not been exposed to a healthcare setting [7]. In 1997, four fatal cases were reported involving children from Minnesota and North Dakota [7]. Over the next several years, it became clear that CA-MRSA infections were caused by strains of MRSA that differed from the older and better studies health care-associated strains [8].

Pathogenesis of CA-MRSA infection

Until the 1990s, MRSA rarely caused infections among community members without exposure to the health care setting (one exception is injection drug users). An outbreak of CAMRSA infections occurred between 1989 and 1991 among indigenous Australians in Western Australia without health care contact [9]. CA-MRSA infections were also reported in people from neighboring regions [10]. In the late 1990s, several cases of aggressive MRSA infection also occurred among individuals in the United States without established risk factors for MRSA. Four children died of CA-MRSA infections in Minnesota and North Dakota from 1997 to 1999. All the cases were rapidly fatal and were associated with necrotizing pneumonia or pulmonary abscesses and sepsis [91]. The strain responsible for these infections was ST1 and PFGE type USA400 (also known as the MW2 strain) [11]. Subsequently, clonal outbreaks of skin and soft-tissue infection caused by CA-MRSA were also reported among, soldiers, and athletes, particularly football players [12]. The strain responsible for these infections was ST8 and PFGE type USA300 [13]. Cases of CA-MRSA skin infection and necrotizing pneumonia were reported internationally as well [14, 15]. In addition to causing necrotizing pneumonia, CA-MRSA has recently been reported to cause infections or infectious complications in situations in which *S. aureus* or MRSA is an unusual pathogen. These have included cases of necrotizing fasciitis caused by PFGE type USA300 [16], as well as cases of pyomyositis [17, 18], purpura fulminans with toxic shock syndrome [19], and Waterhouse-Friderichsen syndrome [20]. The number of CA-MRSA infections appears to be increasing, and the strains responsible for these infections have now entered the health care setting, blurring the line between "community" and "hospital" strains [21].The strains that cause these virulent infections carry *SCCmec*IV (sometimes *SCCmec*V), the smallest of the SCCs that confer methicillin resistance, and are generally susceptible to several non–β-lactam antibiotics. This is in contrast to the multidrug-resistant nosocomial MRSA strains that carry larger *SCCmec* types [22]. CA-MRSA strains may also have a growth advantage over HAMRSA strains [23, 8]. Although *SCCmec*IV has appeared in several different genetic backgrounds [24], PFGE types USA300 (ST8) and USA400 (ST1)—both agr type III—accounted for the vast majority of CA-MRSA infections in individuals without the usual MRSA risk factors or health care contact in the United States [11, 25]. USA300 is now the predominant strain. Worldwide, there are other prevalent CA-MRSA strains, such as ST80 (France-Switzerland), ST30 (SWP clone), and ST93 (Australia Queensland clone) [14, 25] identified additional "community-acquired strains" (CA-MRSA strains defined as containing *SCCmec*IV); however, these were in individuals with MRSA risk factors or health care contact. The basis for the apparent increased virulence of CA-MRSA strains is incompletely understood. Numerous factors have been proposed, such as increased fitness, improved evasion of the host immune system, and unique toxin production. The genes and mechanisms by which CA-MRSA strains may cause aggressive disease are discussed in the sections that follow. Because these strains usually contain Panton-Valentine leukocidin (PVL), which is usually absent in HA-MRSA strains, some researchers postulate that this protein, with leukocytolytic and dermonecrotic activity, is responsible.

MRSA: Methicillin-resistant Staphylococcus aureus

Methicillin-resistant *S. aureus* (MRSA) are resistant to all currently available β-lactam antibiotics, including penicillins, cephalosporins, carbapenems, and their derivatives. Resistance to methicillin is mediated by the *mec*A gene which encodes an altered penicillin binding protein, located in the cell wall that has a low affinity for β-lactam antibiotics. Since β-lactam antibiotics interfere with bacterial cell wall synthesis, this decreased binding of β-lactams renders them ineffective against MRSA. *The mec*A gene resides on a large heterogeneous mobile genetic element called the staphylococcal cassette chromosome (*SCCmec*) [26, 35]. To

date, nine *SCCmec* variations have been described but types I–V are the most common. *SCCmec* types I–III are relatively large and are typically found in strains associated with hospitals and other healthcare facilities. *SCCmec* types IV and V are smaller in size and are usually found in MRSA associated with community-associated infections. Molecular analyses of numerous MRSA strains indicate that resistance genes have been transferred to various methicillin-susceptible *S. aureus* (MSSA) strains on multiple occasions [36]. These resistance genes have also been transferred to other staphylococcal species. Many MRSA are also resistant to other classes of antibiotics, which makes it a challenge to treat serious infections.

Table 1 lists important events in the emergence of methicillin-resistant staphylococci that infect humans.

Year(s)	Event	Reference
1961	1st methicillin-resistant *S. aureus* identified in UK hospital	[33]
1965	1st MRSA cases recorded in Australia	[85]
1968	1st hospital outbreak of MRSA in U.S.	[86]
1981	CA-MRSA in injecting drug users	[87]
1988	CA-MRSA in hospitalized children, Chicago	[88]
1991–1992	1st CA-MRSA detected in Australia	[9]
1992–1993	Foodborne outbreak of HA-MRSA	[90]
1997	CA-MRSA in otherwise healthy children in Minnesota and North Dakota	[91]
1999–2000	Highly virulent USA300 strain first reported in football players (Pennsylvania) and prisoners (Missouri)	[92]
2000	Outbreak caused by USA300; prison, Mississippi	[93]
2001	Foodborne outbreak of CA-MRSA	[94]
2003	LA-MRSA strain ST398 from pigs in Netherlands detected in humans	[95, 99]
2008	Emergence of CA-MRSA strain USA300 in Japan	[96]
2008–2009	Multi-drug-resistant, dog-related strains of methicillin-resistant *S. intermedius/pseudintermedius* (ST71) detected in humans in U.S., Switzerland	[97, 98]

Organization of the S. aureus genome

The first genome sequences of *S. aureus* strains Mu50 and N315 were published in 2001 [37]. At present, complete genomic sequences of ten *S. aureus* strains are available, and the genomes of several others have been partially determined [37, 23, 38, 39]. The genome of *S. aureus* is a circular chromosome that is 2.8–2.9 Mbp in size, with a G+C content of about 33% .The chromosome encodes approximately 2700 CDSs (protein coding sequences) as well as structural and regulatory RNAs. It has been proposed that the *S. aureus* genome is composed of the core genome, accessory component and foreign genes. The core genes are present in more than 95% of isolates, represent 75% of any *S. aureus* genome and determine the backbone of the genome. The organization of the core component is highly conserved and the identity of individual genes between isolates is 98–100%. The majority of core genes are associated with fundamental functional categories of housekeeping functions and central metabolism. The accessory component includes genetic regions present in 1–95% of isolates and accounts for about 25% of any *S. aureus* genome. It typically consists of mobile genetic elements that have or previously had the ability of horizontal transfer between strains. These genetic elements include pathogenicity islands, genomic islands, prophages, chromosomal cassettes and transposons [29].

Insertion sequences and transposons

Insertion sequences (IS) carry at least one gene coding for a transposase which participates in the recombination required for transposition. Most IS elements also contain short inverted terminal repeats acting as transposase binding sites [23]. Insertion elements are randomly scattered throughout the genome of S. aureus, both in coding and non-coding regions. In MRSA, *S. aureus* N315 and Mu50 strains, eight copies of IS1181 have been found [37, 38]. Transposons are larger transposable genetic elements that, in addition to a transposase gene, carry other genes which very often are antibiotic resistance determinants. *S. aureus* is the host to more than ten transposons, the majority of which carry antibiotic resistance genes [23].

Plasmids

Plasmids, defined as extrachromosomal genetic elements bearing only non-essential genes which, may provide a benefit to the host under special environmental conditions, often encode factors determining resistance to antibiotics or heavy metals, virulence factors and proteins facilitating survival in the presence of unusual nutrients [40]. Plasmids of *S. aureus* have been categorized into three classes. Class I plasmids are of the size of 1–5 kb and occur in high copy number (15–50 per cell). They usually carry a single antibiotic resistance determinant. The class II plasmids are of intermediate size and occur in intermediate copy number, and they usually code for β-lactamase and confer resistance to inorganic ions. The last group of staphylococcal plasmids, class III, consists of large conjugative plasmids (40–60 kb). Class III plasmids carry multiple resistance determinants, exemplified by resistance to trimethoprim, gentamycin and ethidium bromide [41]. The plasmids often can serve as means by which antibiotics resistance is transmitted. Moreover, the conjugative plasmids encode their own conjugative horizontal transfer mechanism by tra genes that offer an advantage by which transfer of extrachromosal genetic information to other bacteria occurs [42, 37, 29, 38, 39].

Staphylococcal chromosome cassette (SCC mec)

SCCmec is a 21–67-kb genetic element that is found in the chromosome of methicillin resistant S.aureus at a unique site designated *attBscc*, located near the origin of replication. *attBscc* is found in an open reading frame of unknown function, identified as *orfX*, that is well conserved among *S. aureus* strains. The integration site of *SCCmec*, *attBscc*, contains a 15-bp sequence that, when *SCCmec* is inserted in the chromosome, is found at both chromosome- *SCCmec* junctions (*att*L and *att*R) [43]. However, unlike the direct repeats found in transposons, which are created by target duplication, one of the two repeat sequences is located within *SCCmec* at its right end. Degenerate inverted repeats are also present at both ends of *SCCmec* [44]. These incomplete inverted repeats are thought to be recognized by *SCCmec*-specific recombinase during excision and integration of this element from and to chromosome (Fig. 1) [45, 44]. *SCCmec* is a variable genetic element with certain conserved features. Among the conserved elements, *SCCmec* contains the mec operon composed of mecA and its regulatory genes, as well as the cassette chromosome recombinase complex *ccr* [23, 29, 38,39]. The *ccr* locus is composed of the cassette chromosome recombinase genes, *ccr*A, *ccr*B or *ccr*C that are involved in the integration into the chromosome and in precise excision form the chromosome of the *SCCmec* element [35]. The variable regions of *SCCmec*, called J-region, contain integrated genetic elements such as plasmids (pT181, pUB110 and p1258), transposons (Tn554) and insertion sequences (IS431, IS1272 and IS256) [46]. The hallmark of *SCCmec* is the mec operon that consists of *mec*A and its regulatory genes *mec*I and *mec*R1. The operon is found in several variants as a component of different *SCCmec* elements. The variants of the mec operon are divided into two main categories: those with both *mec*I and *mec*R1 genes intact and those with portions of one or both of these regulatory genes deleted. The first group of the mec complex is known as class A mec operon while the latter have been categorized as classes B, C, D, E. All classes of the mec operon include a copy of IS431 associated with the *mec*A gene and therefore designated IS431mec. Classes B–E contains deletions of *mec*I that may be extended to part of the mecR1 gene. Usually these deletions coincide with insertion of IS elements. Moreover, the *ccr* locus of *SCCmec* elements also exists in several variants.The *ccr* locus contains either *ccr*A and *ccr*B or *ccr*C. Based on differences in nucleotide sequences, the combination *ccr*A and *ccr*B allotypes are divided into eight complexes of the *ccr* gene (types I through VIII, [35]. To date, at least five types of *SCCmec* elements have been defined based on combination of different *mec* complexes they contain.
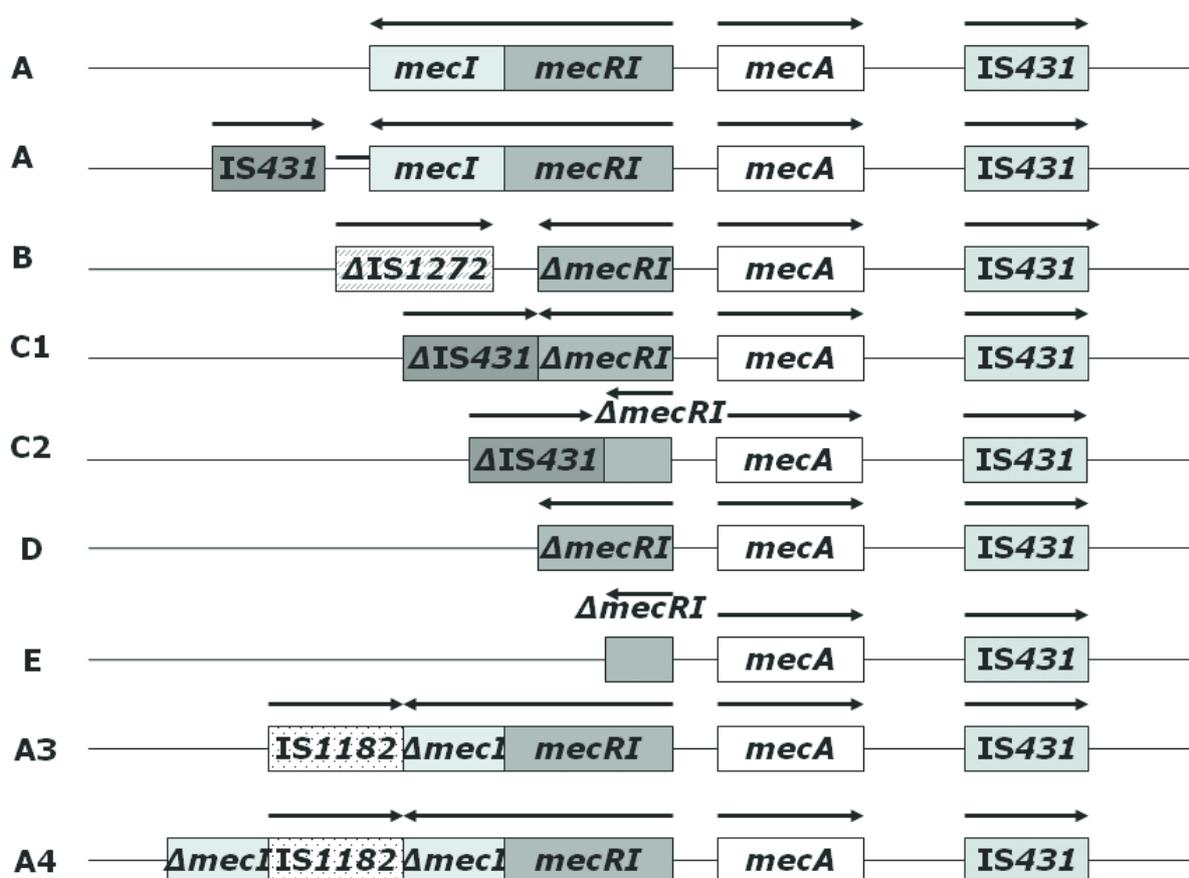
Fig. 1. Structural classes of *mec* operon.
Direction of transcription is indicated by arrows above each element. Designation of each variant is shown on the left [46].

β -Lactams mode of action and mechanisms of resistance

Of the β -lactam antibiotics that are currently available all feature the reactive β -lactam ring system, a highly strained and reactive cyclic amide. There are five relevant ring systems, including the penam, penem, carbapenem, cefem and monobactam ring structure.

Penams

Penams are a large group of β -lactams that include penicillin. Therefore, penicillin possess a basic bicyclic structure, 6-aminopenicillanic acid or 6-APA. This structure is composed of an enclosed dipeptide formed by the condensation of L-cystein and D-valine, resulting in the β -lactam ring and in the thiazolidinic ring [47]. The reactive nature of the β -lactam ring system makes penicillins (penams) and related compounds susceptible to a variety of degradative processes. At acid environments and room temperature, the β -lactam ring is reconfigured: beginning with the protonation of the β -lactam nitrogen, followed by the nucleophilic attack of the remaining lateral chain carbonyl. The intermediate oxazolin ring will originate a new imidazol and, thus, form penillic acid [47] this process has some clinical interest due to stomach acidity. So, in order to be able to administrate orally these compounds have to be protected from acid mediums. The original penicillins were produced by fermentation and were often mixtures of various β -lactams, such as penicillins G and V. The availability of 6-APA has allowed the creation of hundreds of synthetic and semisynthetic penicillins. In addition to chemical degradation, many bacteria produce a group of enzymes specifically designed to degrade and inactivate β -lactams. These enzymes are collectively known as penicillinases. By far the most prevalent type of

penicillinase is the β -lactamase, which directly attacks and disrupts the β -lactam bond, inactivating the antibiotic [48]. The first molecule synthesized was methicillin, which differs from benzylpenicillin in the substitutions at positions 2' and 6' of the benzene ring by methoxy groups, causing steric hindrance around the amide bound [49].

Antimicrobial resistance to β -lactam

Since the discovery of the first antibiotic, penicillin, by Alexander Flemming in 1928, until now enormous changes in this field have occurred. First of all, the use of antibiotic was a medical revolution like no other in the treatment of infectious diseases [48]. Nevertheless, a rapid appearance of a great number of bacteria presenting acquired resistance was observed, thus resulting in therapeutic failures. Six years after the introduction of benzylpenicillin in the market, for example, the frequency of staphylococci resistance in British hospitals increased from less than 10% up to 60% and today is over 90% at world level 50]. β -Lactams are a group of antibiotics that have specificity for bacteria. Bacteria are prokaryotic and, hence, offer numerous structural and metabolic effects that differ from those of the eukaryotic cells such as the animal or human host. There are several possible targets for antibiotics [51, 52]. Generally speaking, we can group the mechanisms of action of antibiotics into five categories (Fig. 2): inhibition of cell wall synthesis; impairment cytoplasmic membrane; inhibition of nucleic acid synthesis; inhibition of protein synthesis; and metabolic antagonist action. In general, there are four basic mechanisms (Fig. 2) by which resistance to drug may occur in bacteria: alteration of the antimicrobial target that can be due to the complete loss of affinity or simple reduction of it; reduction in the amount of the antimicrobial that reaches the target by entrance reduction caused by a decrease permeability due to porin mutation or by an exit increase caused by the pumping out by an efflux transporter; the presence of an enzymatic mechanism that totally or partially destroys the antimicrobial molecules; and the development of an alternative metabolic pathway involving precursors [52, 53].
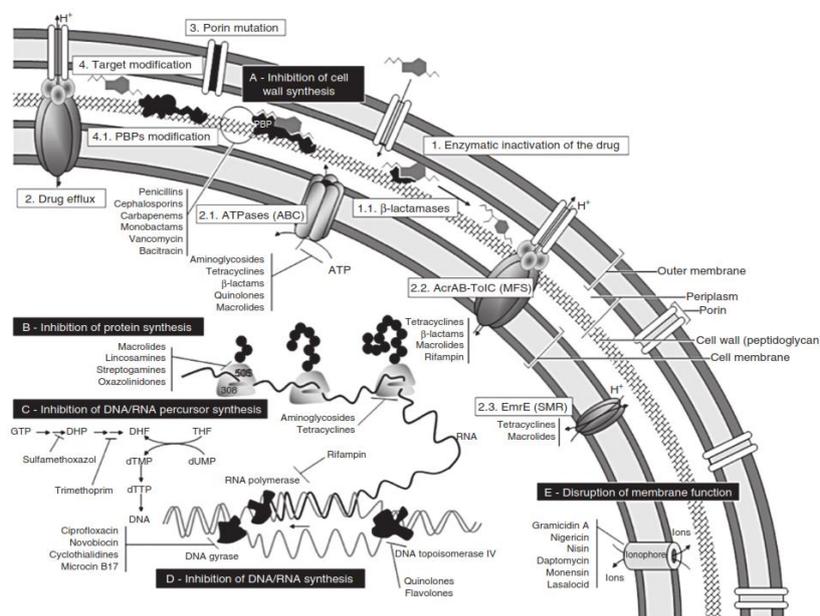

Fig.2. Mechanisms of antimicrobial action and resistance in Gram-negative organisms.
This picture represents a Gram-negative bacteria cell. Black boxes represent mechanisms of drug action and white boxes represent mechanisms of resistance. Below each box there are several examples of drugs presenting those types of mechanisms. The main mechanisms of antimicrobial action can be divided into five major classes. (a) Those who act in the cell wall synthesis; (b) those who act in the protein translation; (c) those who act in metabolic precursor biosynthesis; (d) those who act in the molecular genetics processes (replication, transcription); and (e) those who disrupt membrane function and permeability. Some of the mechanisms of resistance are represented here by numbers. (1) Enzymatic inactivation of the drug by the presence of β -lactamases (1.1); (2) presence of an enhanced efflux pump, whether it is by an active transport system involving ATPases (2.1) or rather if it is driven by proton motive force (2.2, 2. 3); (3) porin mutation obstructing the drug entrance; and (4) target modification of the drug, such as the mutation in the penicillin binding proteins (PBPs).

Heterogeneous and homogeneous methicillin resistance.

The level of methicillin resistance of *S. aureus* varies extremely from one strain to another, spanning the range from several micrograms per milliliter (a value very close to the resistance level of (MSSA) to several milligrams per milliliter. Certain MRSA strains are composed of cells expressing varied levels of resistance to ß-lactams. They are apparently made up of several bacterial sub-populations that significantly differ in their degree of antibiotic resistance. This peculiar non-consistency in the phenotypic expression of antibiotic resistance is called heteroresistance [54]. Heterogeneous expression of methicillin resistance is characterized by a majority of cells expressing low level resistance from which, upon challenge with methicillin, a small proportion of highly,uniformly resistant clones segregate. The frequency of segregation of the highly resistant sub-clones designated homoresistant is a reproducible and straindependent property. The homoresistant phenotype is stable and the highly resistant clones generally maintain their resistance level even in the absence of selective pressure [55]. The ability of MRSA strains to produce PBP2a is essential for their methicillin resistance but there is no correlation between homo- and heteroresistance and the cellular concentration of PBP2a. PBP2a production does not seem to explain the observed variety of resistance levels. Therefore it is thought that additional chromosomal genes are involved in optimal methicillin resistance [56, 57]. It has been proposed that selection of a homoresistant derivative from a heteroresistant population is due to unspecified mutations or genetic rearrangements occurring outside the *SCCmec* element. However, the size of the homoresistant subpopulation selected upon challenge with oxacillin is well above the frequency of spontaneous mutations [58].The same mechanism of selection of a highly homoresistant subpopulation from a heteroresistant population is thought to operate in clinical environments and it could be blamed for the failure of ßlactam treatment against MRSA [59]. Moreover, very low-level resistant MRSA strains are dangerous since they can evade standard phenotypic detection while they appear phenotypically susceptible. These strains still carry the *mec*A determinant and express resistance heterogeneously and upon ß-lactam exposure they are able to segregate highly resistant subpopulations at a frequency well above spontaneous mutation rate [60].

Epidemiology features of s.aureus, MRSA, and risk

MRSA infections appear to occur in patients with decreased susceptibility to infection. Singh et al reported that patients with both cirrhosis and early following liver transplantation are at an increased risk of MRSA infection when colonization is present in the anterior nares. Patients in an ICU, especially a surgical ICU, have wounds, drains, and invasive monitoring devices that breach the skin and increase the risk of developing infections. Additionally, impaired neutrophil function as a result of chronic liver disease, diabetes, or corticosteroid therapy may render these patients more susceptible to MRSA. Specific defects associated with granulocyte function, such as decreased chemotaxis and impaired phagocytosis associated burst activity have been documented with liver disease and diabetes. [61, 62] MRSA in the setting of foreign devices tends to be more virulent because the foreign body appears to facilitate infection by shielding these normally low virulence organisms from being attacked by host defences possibly through (1) alteration in bacterial metabolism, alteration in leucocyte function, or creation of a permeability barrier and (2) attachment, adherence, and slime production are factors which make coagulase negative staphylococci especially adept at surviving on various biomaterials. Several authors have addressed the question of whether MRSA is more virulent than methicillin sensitive S aureus (MSSA). Soriano and colleagues performed a retrospective case control study of 908 (225 MRSA) episodes of bacteraemia and matched 163 pairs. When multiple factors about the patients such as shock, source of bacteraemia, acquisition of the infection in an ICU, and inappropriate empirical therapy were among the factors considered, MRSA was not an independent factor for mortality. However, methicillin was an independent predictor for shock. In a similar study of 504 patients (188 MRSA, 316 MSSA), overall mortality was 22%. Death was significantly greater in the MRSA group (odds ratio 1.68), although these patients were found to be more likely to die due to underlying disease during treatment of bacteraemia, rather than from the MRSA bacteraemia itself [61]. These authors suggest that differences in patient comorbdities in different

centres, true virulence differences, or aggressiveness of treatment may explain the variance in the literature about whether or not MRSA is more virulent than MSSA. With the whole genomic sequencing of MRSA, most of the antibiotic resistant genes are carried on plasmids or by mobile genetic elements including a unique resistance island. Three classes of pathogenecity islands were identified in the genome: a toxic shock syndrome toxin island and clusters of exotoxin and enterotoxin genes were found closely linked with other gene clusters encoding for putative pathogenic factors. These authors also identified 70 candidates for new virulence factors. These newly identified factors may help to explain the biology of staphylococci and the processes of infections caused by *S. aureus* [37, 63].

Current Antibiotic Therapy

Glycopeptides have been the mainstay of treatment for MRSA infections and staphylococcal infections in patients with true penicillin allergy. Glycopeptides are less bactericidal than β -lactam agents, and penetration into tissues is poor. Vancomycin has been reported to clear bacteraemia in patients with endocarditis more slowly than β -lactams, i.e. 7 days vs. 3.4 days in nafcillin-treated patients [64], and has been found to be associated with higher infection-related mortality than β -lactams in treatment of endocarditis caused by MSSA [65]. For these and other reasons, a number of recent reports have called into question the efficacy of this class of antimicrobials in the treatment of severe MRSA infections. Higher rates of relapse, complications, treatment failure and mortality in cases of MRSA bacteraemia and endocarditis have been associated with vancomycin therapy. Increasing the dose of vancomycin may not safely overcome its limited bactericidal activity, and its combination with a second antistaphylococcal agent does not improve its therapeutic efficacy (mortality being the outcome measure). MRSA strains with lower vancomycin MIC values have been associated with increased rates of treatment success with vancomycin as compared with strains that have higher vancomycin MIC values, whereas increased MICs of vancomycin for *S. aureus* may be predictive of increased treatment failure (30-day mortality) and longer duration of bacteraemia in patients receiving vancomycin therapy [66, 67, 89].

Decreasing activity of glycopeptides antimicrobials

Despite more than 50 years of treatment with vancomcyin, fully vancomycin-resistant strains (vancomycin-resistant *S. aureus* (VRSA)) are still an anecdotal phenomenon, with fewer than ten strains having been described, mainly in the USA, but also abroad [68, 69]. These strains have been associated with only limited clinical consequences, because they have not been associated with invasive disease. VRSA and vancomycin intermediate *S. aureus* (VISA) are usually cross-resistant to teicoplanin [70].VISA and heteroresistant VISA strains of *S. aureus* that contain subpopulations of daughter cells displaying intermediate sensitivity to vancomycin, but for which the MICs of vancomycin fall within the susceptible range, can be difficult to detect in the microbiology laboratory, because the phenotypes are unstable and can be lost on subsequent passages. The role of tolerance to vancomycin in *S. aureus* has not been well clarified. It is more frequently associated with MRSA than with MSSA and in isolates from patients with endocarditis [71]. Whether tolerance is a prerequisite for attenuated vancomycin efficacy and the development of glycopeptides resistance warrants further study. Part of the intermediate glycopeptide resistance seen in VISA may be due to tolerance [72]. Several small series and case studies have reported poor clinical response to vancomycin in the treatment of bacteraemia/endocarditis caused by vancomycin-tolerant *S. aureus* and the need for additional agents for a bactericidal effect [73, 74]. In summary, vancomycin is recommended for empirical therapy in healthcare settings with an increased incidence of methicillin-resistant staphylococci or when risk factors for MRSA infections are present, such as MRSA-positive surveillance cultures. Although high-level resistance remains rare, data from some centres suggest an evolutionary change in S. aureus, as evidenced by reduced susceptibility to vancomycin. This, together with the problem of heteroresistance to vancomycin, as well as poor tissue penetration after systemic administration, presents potential obstacles to the successful treatment of *S. aureus* infections with this glycopeptides. Although it has been suggested that these problems may be overcome by

administration of vancomycin in much higher doses by continuous perfusion, the efficacy and safety of this approach remain to be determined.

Classic Alternatives to Standard Therapy

Trimethoprim–sulphamethoxazole.

Trimethoprim–sulphamethoxazole is inexpensive and suitable for sequential therapy. A high proportion of in vitro MRSA isolates susceptible to trimethoprim–sulphamethoxazole have been reported recently [75]. In the animal model, folate antagonist treatment fails when delayed, consistent with the possibility that in vivo thymidine release inhibits folate antagonists. Trimethoprim–sulphamethoxazole can be regarded as a second-line agent for the treatment of severe MRSA infections in patients unable to tolerate other more active drugs, such as glycopeptides or linezolid. In a randomized, prospective trial comparing trimethoprim–sulphamethoxazole with vancomycin in intravenous drug abusers with endovascular infections caused by MSSA and MRSA (47% MRSA), trimethoprim–sulphamethoxazole was inferior to vancomycin .Trimethoprim–sulphamethoxazole may be better suited for infections with a low bacterial burden, as is the case for chronic osteomyelitis and clinical situations with no risk of death in case of clinical failure.

Chloramphenicol

A very high proportion of MRSA isolates in different areas of the world remain susceptible to chloramphenicol, including community-acquired isolates. In a SENTRY study, 82% of the MRSA isolates from cases of pneumonia were chloramphenicol-susceptible [76]. In six sequential multicentre national studies of Staphylococcus performed in Spain from 1986 to 2006, the rates of chloramphenicol susceptibility rose from 92% to 98% [77]. Treatment with chloramphenicol in association with vancomycin has shown an antagonistic effect in vitro [78]. Unfortunately, both the potential myelotoxicity of chloramphenicol and the absence of reported recent clinical experience with its use in the treatment of MRSA infections make it a possibility only as a last resort in situations where no better alternatives are available.

Tetracyclines

The long-acting tetracyclines doxycycline and minocycline are well absorbed by the gastrointestinal tract, have very good tissue penetration, and have better antistaphylococcal activity than tetracycline. In vitro data suggest that minocycline has better antistaphylococcal activity than doxycycline [79], but clinical superiority has not been demonstrated. The data available are insufficient to support their use in serious infections such as bacteraemia or endocarditis.

Antibiotic combinations

The combination of vancomycin and aminoglycosides is synergistic against most infections due to MRSA. The synergism of vancomycin and gentamicin is not predictable for MRSA strains with gentamicin MIC values of 0.5 to >128 mg/L [80]. Combination therapy might confer a small advantage in cases of staphylococcal prosthetic valve endocarditis, in accordance with most animal model data, because, after adjustment for duration of treatment by logistic regression analysis, valves from patients with staphylococcal endocarditis receiving any kind of combination therapy were six times more likely to be culture-negative than those receiving monotherapy [81].

New Therapeutic Options

As stated above, low-dose vancomycin may be inferior to some new comparator agents in the treatment of serious MRSA infections, especially in the presence of increased MIC values. Novel agents with activity against MRSA have become available in Europe in recent years, and others are in the advanced stage of clinical development. In some instances, although most comparative trials with these new agents have important limitations in their design, some indirect evidence of their possible superiority over vancomycin is emerging [82].

Daptomycin.

Daptomycin, a new lipopeptide already present in nature 30 million years ago, has a unique mechanism of action, and is only active against Gram-positive bacteria. It acts at the cytoplasmic membrane, binding but not penetrating the membrane via a calcium-dependent insertion of its lipid tail. Cell death occurs in association with widespread inhibition of the synthesis of DNA, RNA, and protein, but cell lysis and the release of large molecules from the cytoplasm does not occur. Daptomycin heteroresistance is also found among strains that develop vancomycin heteroresistance during treatment with vancomycin, even when the MIC for the organisms remains within the susceptible range. In vitro killing assays demonstrate less rapid killing of these heteroresistant isolates [67]. Daptomycin is approved in the European Union for the treatment of complicated skin and skin structure infections (cSSSIs), right-sided endocarditis due to S. aureus, and *S. aureus* bacteraemia associated with right-sided endocarditis or cSSSIs [83].

Linezolid

Linezolid, the first available agent in the new class of oxazolidinone antibiotics, represents a significant advance in the management options available for combating MRSA infections. Linezolid has a unique mechanism of action whereby it selectively binds to the 50S ribosomal unit and prevents formation of the initiation complex. This action is thought to prevent cross-resistance with other antimicrobial agents. Protein binding is low. This agent is bacteriostatic against staphylococci, and has an MIC of 2 mg/L against MRSA [83]. Linezolid has been used successfully in the treatment of IE caused by resistant Gram-positive cocci. The studies, however, were retrospective, and represent daily clinical practice; in many cases of IE, the patients were those failing with other drugs or those in whom linezolid was introduced sequentially after other primary therapy.

Tigecycline

Glycylcyclines comprise a novel group of antimicrobial agents. These agents retain a central four-ring carbocyclic skeleton of the tetracycline class that is crucial for antimicrobial activity. Tigecycline has a 9-t-butyl-glycylamido side chain on the central skeleton. Active efflux of drugs from inside the bacterial cell, and ribosomal protection, are the two main mechanisms of bacterial resistance to tetracyclines. Tigecycline most likely overcomes these tetracycline resistance mechanisms through steric hindrance by a large substituent at position 9. Tigecycline is bacteriostatic against MRSA (MIC90 0.5 mg/ L) and has in vitro activity against VISA and VRSA (MIC90 ≤0.5 mg/L) [84].

Prevention

Hand washing, avoidance of direct contact with nasal secretions and wounds, barrier precautions when handling Animals with illnesses caused by MRSA, environmental cleaning and other infection control measures are expected to reduce the risk of acquiring MRSA from infected or colonized animals. The best procedure to

follow when a resident animal becomes colonized in a healthcare facility is still uncertain. In one recent outbreak, options presented to the facility included removing the animal until it cleared the bacterium, or allowing it to remain, with or without antibiotic treatment, and with continued monitoring (culture) and the encouragement of good hand hygiene among human contacts. Infection control measures, particularly hand washing, are also important in preventing MRSA transmission from humans. Outpatients with MRSA skin lesions should keep them covered with clean, dry bandages. In some circumstances, such as the inability to adequately cover a MRSA-infected wound, close contact with other people (or susceptible animals) should be avoided. The Netherlands and Scandinavian counties have greatly reduced the incidence of hospital-associated human MRSA by screening and decolonization of hospital staff, and screening of patients on admission. High risk patients, including people who work with animals, are isolated until the screening test de investigated aggressively, and antibiotic use is restricted. Opinions in other countries remain divided on the benefits of screening on admission, compared to universal infection control procedures alone.

Diagnostic Tests

Infection with MRSA, including colonization, can be diagnosed by culture and identification of the organism. MRSA can colonize more than one site, and in many species including dogs and cats, the best sampling site to detect carriers is uncertain. Nasal and rectal sampling should both be done whenever possible. One study reported that nasal swabs detected most colonized pigs, but some animals carried MRSA in both locations, and a few carrier pigs (all weanlings) could only be found using rectal swabs. *S. aureus* grows on a number of media. On blood agar, colonies are usually beta-hemolytic. Enrichment media, as well as selective plates for MRSA, are available. On microscopic examination, S. aurues is a Gram positive, non-spore forming coccus, which may be found singly, in pairs, in short chains or in irregular clusters. Biochemical tests such as the coagulase test are used to differentiate it from other staphylococci. *S. aureus* can also be identified with the API Staph Ident system. If *S. aureus* is isolated from an infection, genetic testing or antibiotic susceptibility testing can identify methicillin resistant strains. The presence of the mecA gene defines MRSA, and tests to detect this gene, such as PCR, are the gold standard for identification. A latex agglutination test can detect PBP2a, the product of mecA. Phenotypic antibiotic susceptibility tests (e.g., disk diffusion or MIC determination) can also be used to identify MRSA, but have some drawbacks compared to detecting mecA or PBP2a. Methicillin-susceptible and resistant subpopulations can coexist in vitro; although the entire colony carries the resistance genes, only a small number of bacteria may express resistance in culture. The expression of resistance in phenotypic tests can also vary with growth conditions such as emperature. In addition, some susceptibility tests can overestimate methicillin resistance; isolates that do not carry mecA (and thus, are not MRSA) can appear to be phenotypically resistant to methicillin. MRSA clones or strains can be identified with molecular tests such as PFGE, MLST, *SCCmec* typing, spa typing and other assays. This is usually done mainly for epidemiological purposes, such as tracing outbreaks. A combination of methods may be needed to identify a strain.

CONCLUSION

S. aureus is a redoubtable pathogen with significant morbidity and mortality .Community-associated MRSA infections are now a common and serious problem. MRSA is a commonly found in the community, and hospital, especially in the ICU. The role of many virulence factors in the pathogenesis of staphylococcal disease is unclear. There is insufficient evidence on other outcomes of universal MRSA screening, including morbidity, mortality, harms, and resource utilization. There is also insufficient evidence to support or refute the effectiveness of MRSA screening on any outcomes in other settings. It is a significant cause of both health care and community-associated infections. Although vancomycin is still commonly used to treat many MRSA infections, concern is rising about vancomycin's role in MRSA therapy due to emergence of less susceptible

strains. Newer therapeutic alternatives, such as daptomycin, linezolid and tigecycline, are available in cases with less susceptible strains, depending on the type of infection. MRSA infections continue to challenge the medical community, and further clinical research continues to provide better guidance on optimal therapy. Increasing knowledge of resistance will help guide the development of new antibiotics.

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