MRSA: the first half century

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Fifty years ago methicillin-resistant Staphylococcus aureus (MRSA) first revealed themselves to the medical community, having been described in a landmark article published in the British Medical Journal. Among other things, their discovery set off a major response from the scientific and medical professions to control or even eliminate them as major human pathogens. Despite these efforts, however, MRSA have spread throughout the world and a half century after they burst upon the scene they continue to pose major challenges to research scientists and clinicians alike. In a very real sense, this year marks the ‘birthday’ of a remarkably successful pathogen. The major reasons for the ability of MRSA to prosper and cause disease in settings inimical to its survival form the basis of this article.

Keywords: methicillin-resistant S. aureus, antibiotic resistance, vancomycin, glycopeptides

Introduction

Fifty years have elapsed since Patricia Jevons described the first isolates of methicillin-resistant Staphylococcus aureus (MRSA), only 2 years after the initial clinical use of methicillin. In the ensuing half century these organisms have spread throughout the world and although we have learned a great deal about them we have been totally unable to eradicate them or to consistently prevent the serious infections they continue to cause. S. aureus has many characteristics that help to account for its remarkable success as a human pathogen. Among these are its virulence and quorum sensing mechanisms, which enable it to cause a broad variety of serious infections in man—perhaps more so than any other bacterial species. Its genetic diversity and ability to acquire new exogenous genes allow it to adapt to a variety of changing environmental conditions and to modulate its pathogenicity. It has the ability to establish asymptomatic carriage, which promotes widespread dissemination among human hosts. Finally, it has shown a remarkable propensity to acquire resistance to multiple antimicrobial agents. These factors and a number of other characteristics help to explain why this organism has thrived and spread throughout the world in the 50 years since its first identification, despite the efforts to defeat it by some of the best and most brilliant medical minds in the world.

In this review I will attempt to highlight a number of the issues that are germane to our understanding of this remarkable and challenging pathogens, emphasizing where possible new information that has been generated concerning the origins, epidemiology, pathogenesis, antibiotic resistance and possible control or eradication of MRSA as we observe the 50th anniversary of their discovery.

Fossil evidence suggests that staphylococci have existed on earth for more than a billion years, although it was not until the 19th century that they were actually identified as bacterial pathogens. Nonetheless, it is clear that they have undoubtedly caused serious wound and other infections throughout recorded human history. When subjected to formal testing, many agents used by ancient civilizations to treat wounds, including copper salts such as malachite and chrysocolla, and honey and myrrh, have been shown to have definite activity against staphylococci in vitro. However, it was not until the discovery of penicillin by Alexander Fleming in 1928 that truly effective therapy became possible for staphylococcal infections. Unfortunately, S. aureus quickly developed penicillin resistance due to acquisition of genes producing β-lactamase, leading to a search for β-lactamase-resistant agents. This search resulted in the synthesis of the semisynthetic antistaphylococcal penicillins, beginning with methicillin and including other derivatives such as oxacillin, cloxacillin, dicloxacillin, flucloxacillin and nafcillin. Vancomycin was also discovered in the 1950s, but was not widely used as the penicillins were considered safer and possibly more effective.

Methicillin was first used clinically in 1959 and only 2 years later the first MRSA emerged. Unlike the penicillin resistance in S. aureus, which is largely due to β-lactamase production, methicillin resistance is due to the acquisition of genes encoding a unique penicillin-binding protein, designated 2′ or 2a, that has decreased affinity for β-lactams and catalyses effective cell wall synthesis even in the presence of penicillins, including anti-staphylococcal penicillins, as well as cephalosporins and carbapenems. It is encoded by the mecA gene. Unlike the
penicillin-binding proteins in pneumococci, which are mosaic genes consisting of native DNA and DNA from naturally penicillin-resistant streptococci acquired through transformation, the mecA genes have been acquired intact, often along with a variety of other genetic elements.6

MRSA are generated when methicillin-susceptible S. aureus (MSSA) acquire the mecA gene, which is carried on a mobile element known as the staphylococcal cassette chromosome mecA, also referred to as staphylococcal cassette chromosome mecA (SCCmecA). The origin of mecA and the other genes on these cassettes has been the subject of a good deal of enquiry since the original discovery of MRSA. Earlier work suggested considerable homology with mec genes found in the coagulase-negative Staphylococcus sciuri group, which are not frequently found in humans, but are isolated from animals and food products.7,8 Interestingly, although S. sciuri contains the mecA gene it remains susceptible to methicillin because the gene is not expressed.7 More recently, Hiramatsu and colleagues6 have noted that three of the four S. sciuri species groups (S. sciuri, Staphylococcus vitulinus and Staphylococcus fleurettii, but not Staphylococcus lentus) contain the mecA gene with varying degrees of homology with mecA genes in the contemporary MRSA strain N315. Of the three species groups, only S. fleurettii exhibits in vitro methicillin resistance and it has the highest homology (99.8% nucleotide identity) with N315, thus pointing the finger squarely at S. fleurettii, rather than S. sciuri, as the source of mecA. Interestingly, S. fleurettii mecA is found on the chromosome where is it linked with genes essential to growth, but is not associated with the SCC cassette. Hiramatsu goes on to speculate that the species of S. fleurettii developed the mecA5 gene in an environment where β-lactam antibiotics frequently served as selective pressure during the speciation process.9 Although this still leaves in doubt the ultimate source of the mecA gene found in S. fleurettii, it raises some other important considerations. Hiramatsu speculates that, under conditions where β-lactam antibiotics have been used in humans and animals, the mecA gene from S. fleurettii likely combined with the SCCmec element in a coexistent species of MSSA, from which it could be easily transferred to other human strains, reversing an evolutionary trend in which many of the genes may have been lost from S. fleurettii because they were of no advantage to the organism in an environment devoid of β-lactam pressure.

Epidemiology of MRSA

Since their initial description in 1961, a number of clones of MRSA have spread widely throughout the world. It is not clear whether this represents differentiation from a single clone or introduction of SCCmecA into multiple clones, some of which are more capable of dissemination than others. Enright et al.5 speculate, on the basis of multilocus sequence typing with application of the BURST algorithm to an international collection of 912 MRSA and MSSA isolates, that there are 11 major MRSA clones from five groups of related genotypes. Moreover, their data suggest that methicillin resistance first appeared in sequence type (ST) 250, which likely evolved from an ST8 isolate of MRSA that acquired the mecA gene. A minor variant of ST250 (ST247-MRSA-I), known as the Iberian clone, is one of the major strains circulating the world today.5

Until recently the majority of these clones of MRSA have caused hospital- or healthcare-associated infections [hospital- or healthcare-associated MRSA (HA-MRSA)]. The prevalence of infections caused by HA-MRSA shows considerable geographical variation, which, at least in part, has been related to efforts to decrease the colonization and spread of these organisms. In Europe, for example, the prevalence of MRSA has historically increased from north to south (the UK being an exception). The low prevalence of MRSA infections in Finland, Denmark, Norway, Iceland, Sweden and the Netherlands has been thought to be due to major ‘search and destroy’ operations in these countries. Nonetheless, MRSA continue to cause significant problems in Europe.10 Data from the European Antimicrobial Resistance Surveillance System (EARSS), now known as the European Antimicrobial Resistance Surveillance Network (EARSnet), documented that more than 25% of bacteraemias in central and southern European countries were due to MRSA in 1999. By 2008 efforts to decrease these infections had shown some success, especially in the UK, where the percentage of MRSA in bloodstream infections decreased from 31% in 2007 to 19.3% in 2009, perhaps related to government action making the reporting of MRSA bacteraemia mandatory and setting a target of decreasing rates of infection by 50%.10,11 Nonetheless, MRSA continued to account for more than 25% of bloodstream infections in one-third of the European countries studied.12

Community-acquired or community-associated MRSA (CA-MRSA)

Prior to the 1990s most MRSA were associated with hospitals or other healthcare units, but, beginning in the early 90s, infections due to MRSA in patients without previous healthcare exposure were reported from six continents, including Australia, where several outbreaks had been previously noted in Western Australia and the Northern Territory.13,14 More frequent infections were noted in Taiwan, Canada and especially the USA, where the epidemic of CA-MRSA infections took off with a vengeance. Initial infections in the USA were due to strains of ST1 lineage (also known as USA400 based on PFGE typing) and these were shown to contain an SCCmecA element (SCCmec IV) distinct from the elements I–III seen in most hospital-associated strains.15 These organisms also contain genes encoding the Panton–Valentine leucocidin (PVL), which targets and damages the membranes of polymorphonuclear leucocytes. USA400 was rapidly replaced by another clone, ST8 (or USA300), which now accounts for >85% of the CA-MRSA isolates in the USA.16 Although the reasons for the success of USA300 are not altogether clear, the fact that it contains an SCCmec element that is smaller than SCCmec I–III and carries fewer resistance genes probably accounts, at least in part, for its fitness advantage over its counterparts that contain larger SCCmec elements.17,18 Moreover, the linkage of the arginine catabolic element with SCCmec IV in USA300 appears to confer increased fitness.16 There is also evidence that the arginine catabolic element may aid in colonization of integumentary surfaces.15

Recent data confirm that the prevalence of USA300 in the USA has tripled since 2004. Using data from Eurofins Medinet and the Nationwide Inpatient Sample, researchers identified three clonal groups of USA300 (two MRSA and one MSSA),
which accounted for 89% of all the isolates that led to a tripling of the rate of hospitalizations due to USA300 between 2004 and 2008 in the USA.16

The origin of the SCCmec IV variants found in USA300, USA400 and other CA-MRSA strains has been the subject of a recent study by Barbier et al.,19 who have found SCCmec-type IVa (and other subtypes) in methicillin-resistant coagulase-negative staphylococci and suggest that these organisms may be the reservoir for the transfer of SCCmec IV elements into MSSA.

As these organisms have spread throughout North America (especially the USA) and Australia, not surprisingly they have been introduced into Europe and other countries and they are now causing community-associated infections along with other STs, such as ST80 and ST5,20,21 albeit at lower frequencies than in North America and Australia.15 In addition to the problems caused by the infections they produce, these MRSA are also posing challenges in infection control, especially in the Scandinavian countries, where guidelines are tailored to prevent inpatient rather than outpatient infections.22

Classically, it has been held that humans are the primary reservoir of S. aureus, with asymptomatic nasal and/or nasopharyngeal carriage serving as the major areas from which these organisms are spread and cause infection.23,24 However, nasopharyngeal colonization rates for CA-MRSA are considerably lower than for MSSA13 and colonization at other sites (especially the groin) may be more important for MRSA than MSSA.25 Although it has been accepted as dogma that heavy nasopharyngeal colonization is a risk factor for increased infection,15,25 a recent study from London could find no evidence that nasal carriers of MSSA were more likely to become colonized with MRSA on admission to hospital,26 thus providing further evidence that MRSA do not have a selective advantage over MSSA in colonizing the nasopharynx. Nonetheless, it is clear that CA-MRSA are easily spread by direct contact and via contaminated fomites.27

Non-human reservoirs of MRSA

It has been previously noted that the genes for methicillin resistance almost certainly originated in strains of S. fleurettii that are primarily found in animals, not humans, and that these genes have spread to human pathogens from that source. Another recent example of this phenomenon is the report of the discovery of phenotypically MRSA from bulk milk in which the mec element (type XI SCCmec) exhibited only 70% homology with S. aureus mecA homologues and was not initially detected by PCR for that reason. Human and bovine isolates of these strains have been shown to be widely disseminated throughout the UK and Denmark.28 In this instance, besides producing resistance to methicillin, the staphylococcus has once again scored a victory in the battle with modern medicine as it has developed a mechanism of methicillin resistance that cannot be detected by the molecular diagnostic tests currently available in the clinical microbiology laboratory.

Pigs have also been implicated as a possible reservoir of MRSA in Europe, where MRSA of ST398 have been isolated from pigs in more than 10 countries.29 Of greater concern are reports of nasal carriage and infection due to ST398 among farm workers and others associated with pigs or pig farming.10 There are also anecdotal reports of the spread of CA-MRSA from horses, dogs, cats and guinea pigs to humans, but such occurrences are rare and not nearly as important as direct human-to-human spread in the dissemination of these organisms. Moreover, it is difficult to determine from the information available whether there is a greater likelihood that these animals serve as a true reservoir for the spread of CA-MRSA or whether they are merely sophisticated ‘fomites’.

Pathogenicity of CA-MRSA

The continued evolution of MRSA is illustrated by the infections caused by CA-MRSA. While the majority of these infections are non-life-threatening infections of the skin and soft tissues, these organisms are also capable of producing devastating disease in certain patients.30 Among these infections are necrotizing fasciitis, septic thrombophlebitis of the extremities, a ‘pelvic syndrome’ (septic arthritis of the hips, pelvic osteomyelitis, pelvic abscesses and pelvic septic thrombophlebitis), Waterhouse–Frederickson syndrome and rapidly progressive pneumonia.31

At this point there are tantalizing clues as to the basis for the apparent increased virulence of USA300 in certain circumstances, but no consensus as to the exact mechanism by which this occurs. Studies of production of PVL in animals have yielded conflicting results, in part because the polymorphonuclear leucocytes of rats, mice, rabbits and humans differ in susceptibility to lysis by PVL.32 Several other toxins, including α-toxin (or α-haemolysin) and phenol-soluble modulins, have also been implicated in the pathogenesis of serious infections due to these organisms.33,34 The fact that the latter are chromosomally mediated suggests that differential expression of the genes may play a significant role, perhaps in response to host factors, but this remains to be definitively proven.35 This is an important area of active investigation, and in addition to providing important data on pathogenesis it is possible that targeting one or more of these toxins may lead to key therapeutic advances. The fact that such an approach may have merit is reflected in a recent study in which targeting of α-haemolysin by active or passive immunization has been shown to decrease the severity of USA300 skin infections in a mouse model.37 However, as is so often the case, initial enthusiasm for a new therapeutic approach to MRSA must be modified on the basis of clinical evidence. In this instance it should be noted that there have been attempts to treat human staphylococcal infections with antibodies directed at α-toxin, but these have not been successful.38

MRSA—evolving antimicrobial resistance

That new approaches, including immunotherapy, are warranted is almost self-evident. When first discovered in the late 1990s, USA300 strains were susceptible to almost all antimicrobial agents except the β-lactams.13 However, as the epidemic has progressed in the USA and elsewhere they have become increasingly resistant to a number of antimicrobials, including erythromycin and the fluoroquinolones.39 A recent CDC study confirms this and notes resistance to tetracycline and clindamycin in 9% and 6.2% of isolates respectively among 823 recently isolated
invasive strains. Of even greater concern is that a few of these organisms contain transferable resistance plasmids apparently acquired from the hospital strain USA100.

Throughout the past 50 years, therapy of infections due to MRSA has relied on the glycopeptides vancomycin and teicoplanin, although other agents, including co-trimoxazole, the tetracyclines, clindamycin and fusidic acid, have been employed, as have several more recently released antibiotics, including linezolid, daptomycin, tigecycline, telavancin and ceftaroline. Historically, glycopeptides (especially vancomycin) have been considered the gold standards for treatment of serious MRSA infections and so it has been with considerable chagrin that we have noted the occurrence of resistance to these agents in enterococci and subsequently in S. aureus. In enterococci, glycopeptide resistance is due to the acquisition of transferable aperon containing genes that enable the organism to synthesize cell walls from precursors in which lactate replaces the terminal alanine, rendering vancomycin incapable of binding to its D-Ala-D-Ala target. Although these genes have been transferred into a roughly a dozen documented strains of vancomycin-resistant MRSA isolated from patients, there have to date been no secondary cases and thus far the genetic elements appear to have been unstable in staphylococci. Of greater concern are the so-called VISA (vancomycin-intermediate S. aureus) or GISA (glycopeptide- intermediate S. aureus) strains, in which MICs of vancomycin are ≥4 mg/L, as well as strains with vancomycin MICs ≤2 mg/L that exhibit heteroresistance (so-called hVISA strains). The frequency of such strains shows considerable geographical variation, but they are frequently associated with therapeutic failure of glycopeptides. Moreover, in a recent systematic review and meta-analysis of the significance of hVISA, van Hal and Paterson noted that the failure rate in patients infected with hVISA is 2.3 times higher than that in patients infected with vancomycin-susceptible S. aureus. We have documented prolonged bacteremia despite vancomycin therapy (up to 42 days) in patients whose isolates had vancomycin MICs of 4 mg/L. Interestingly, the patients did not succumb to their bacteremia. A potential explanation for this is that a number of these strains have mutations in the agr system, which results in decreased production of a number of staphylococcal toxins possibly accounting for the fact that these organisms exhibit decreased virulence in worms (Caenorhabditis elegans), caterpillars (Galleria mellonella) and mice. Moreover, we have recently discovered a mutation in stpI, a gene that encodes a serine/threonine phosphatase and is associated with an increased vancomycin MIC as well as decreased virulence. Despite these observations, the relationship of clinical outcomes in known infections to vancomycin MICs and even to agr dysfunction remains unclear, as a recent paper suggests that agr dysfunction (as measured surrogately by α-lysin production) is associated with a worse outcome. It is likely that factors in addition to vancomycin susceptibility may play a role in allowing persistent infection despite adequate dosage of vancomycin for patients infected with hVISA (and VISA) strains.

A variety of mutations involving vraSR and graSR have been associated with conversion of susceptible strains of MRSA to hVISA and of hVISA to VISA. More recently Hiramatsu and colleagues have shown that rpoB mutations selected by rifampicin confer dual heteroresistance to vancomycin and daptomycin (presumably by leading to increased cell wall thickness) and they have demonstrated that these mutations are frequently found in VISA strains (71%). Moreover, 95.6% of their laboratory-derived rifampicin-resistant mutants showed decreased vancomycin susceptibility. They conclude that ‘the rpoB mutation, although not exclusive, is one of the major contributors to vancomycin resistance in S. aureus. The use of rifampicin in the treatment of MRSA infections would be better if reevaluated to prevent further increase of hvISA and VISA in clinical settings.

The potential clinical implications of these observations are fascinating. They may well serve to explain the delay in clearing of S. aureus bacteremia when rifampicin is added to vancomycin and for the poor outcomes seen in several recent observational studies of patients with MRSA bacteremia and endocarditis when treated with rifampicin in combination with vancomycin. The above notwithstanding, MRSA always presents difficult challenges. In this case it revolves around the use of rifampicin in combination with other agents to treat osteomyelitis, prosthetic joint infections and other biofilm-related infections. Animal studies and limited clinical data document the effectiveness of rifampicin in this setting. So it may turn out that rifampicin combinations should be avoided for bacteremia and endocarditis, but not for biofilm-related infections. Further studies are clearly warranted!

One of the more interesting aspects related to daptomycin is that resistance to this drug can be selected by prior vancomycin (and, as noted above, rifampicin) exposure. We have recently completed whole-genome sequencing on 11 isogenic pairs of MRSA that developed resistance to vancomycin via laboratory exposure or during treatment of clinical infections. All developed non-susceptibility to daptomycin even though they had not been exposed to the drug (R. C. Moeller Jr, unpublished data). As with resistance to vancomycin, the mechanisms by which MRSA develop resistance to daptomycin are complex and not fully understood. They include increases in positive surface charge (as seen in those with mprF and dltABCD mutations), increased cell wall thickness (as seen with rpoB mutations) and mutations in clp and pgaA that alter membrane lipids and may be associated with decreased daptomycin binding and/or alterations in surface charge. In some instances, resistance is associated with a combination of these mutations.

In an interesting report, Dhand et al. showed that the addition of oxacillin to daptomycin was effective in the treatment of seven patients with MRSA bacteremia unresponsive to vancomycin and daptomycin. The authors showed increased binding of daptomycin to the cell membrane of MRSA in the presence of oxacillin. Although not studied, this may have been due to a decrease in the positive surface charge induced by oxacillin and raises the possibility that combining daptomycin with another antimicrobial that alters bacterial surface charge may be useful in overcoming resistance, at least in those with mprF and dlt mutations. But the fact that MRSA employ multiple mechanisms to develop resistance to the lipoglycopeptides makes it more difficult to define simple methods to overcome it. Once again the staphylococcus presents medical science with a difficult challenge.

In addition to the glycopeptides and daptomycin, linezolid has proven highly effective against MRSA and until recently resistance to linezolid has not been a significant problem. Most resistance is due to mutations at the binding site of linezolid on the 23S rRNA of the ribosome. Much less common are mutations
in genes encoding ribosomal proteins. Because Gram-positive bacteria (including MRSA) have multiple (usually four to six) copies of the 23S rRNA genes, it requires multiple mutations to produce resistance. Moreover, strains with multiple mutations exhibit decreased fitness. Taken together, these two factors likely account for the fact that linezolid-resistant MRSA have not been a problem so far despite more than a decade of clinical use. However, the recently described cfr gene found on a transferable plasmid and initially isolated from coagulase-negative staphylococci found in farm animals in Germany has found its way into MRSA. The ribosomal site methylated by the cfr gene product confers resistance to oxazolidinones, chloramphenicol, pleuromutilins, lincosamides and streptogramin A. Thus far there have only been sporadic reports of MRSA containing this gene in various parts of the world, but two recent outbreaks of infection due to cfr-containing staphylococci in Madrid are cause for real concern.

Vaccines

Based on what we know about MRSA to date, a vaccine strategy would seem the most effective way to control the human infections they cause. However, this organism once again poses a daunting challenge to investigators attempting to produce effective vaccines. One such vaccine that targeted the two most common surface antigens of S. aureus looked promising in an initial clinical trial in dialysis patients, but failed in a follow-up Phase III trial. Several other vaccines are in early stages of development, but none appears close to approval at this point.

New therapeutic approaches

From the above it should be clear that, despite 50 years of vigorous attempts to find vaccines or antimicrobial agents that can eradicate MRSA, we have not been able to do this. The battle goes on and a number of new agents, some with unique mechanisms of action, are in the pipeline. However, even here the staphylococcus shows its ingenuity. Several recent examples illustrate this fact. One attractive approach to finding new antimicrobials is to search for essential genes in bacteria that are not found in mammalian cells. Bacteria initiate protein synthesis by first incorporating N-formyl-methionine as the initial amino acid of the subsequently synthesized polypeptide chain. After completion of the polypeptide, the N-formyl-methionine must be cleaved off (by an enzyme known as deformylase) to allow function of the resulting protein. This step is not found in mammalian cells and so appears to be an ideal antimicrobial target. A number of deformylase inhibitors have been synthesized and some have made it to early phase clinical trials. Unfortunately, however, unlike the effects in vitro, when studied in a rat endocarditis model, there was rapid and complete development of resistance in S. aureus despite the deformylase inhibition (R. C. Moellering Jr, unpublished data).

The pleuromutilins represent a new class of antimicrobials with a unique mechanism of action that inhibit ribosomal protein synthesis in a manner that overcomes mechanisms of resistance to other protein synthesis inhibitors that bind to the 50S ribosome, such as macrolides, chloramphenicol and lincosamides. They have been used in animal husbandry in Europe, but, except for a recently approved topical formulation, the pleuromutilins have not been employed in human therapy. Despite this, as noted above, the recently described cfr methylases selected by oxazolidinones and other antibiotics could mediate cross-resistance to the pleuromutilins before they have even been in widespread clinical use. Our success in controlling the spread of MRSA containing the cfr methylase may determine the ultimate success of the pleuromutins against MRSA. So far, as noted, there has not been widespread dissemination of these MRSA.

Conclusions

Given the hundreds of millions of years of their potential exposure to antibiotics in nature, it is not surprising that staphylococci have tricks up their sleeves to overcome most true antibiotics. Our efforts in infection control have yielded only partial success in dealing with staphylococci and even here the organism seems to show a remarkable ability to thwart our efforts. The recent emergence of CA-MRSA means that staphylococci now have a way to get in through the back door as they circumvent infection control interventions largely directed to hospital-acquired, not community-acquired infections.

To date, immunization has been the only way to eradicate infectious diseases such as smallpox and possibly polio, but this remains an elusive goal for S. aureus, despite the remarkable amount of effort expended to find an effective vaccine. The failure of a potentially effective vaccine in a recent Phase III trial after an apparently successful initial trial clearly illustrates this fact.

The past 50 years could have been called the half century of MRSA, as these organisms, with a genome of fewer than 4,000 open reading frames, have spread throughout the world and defied our most vigorous efforts to contain them. I am a scientist, not a gambler, but if I were a betting man, short of the development of an effective antistaphylococcal vaccine, I would bet on the staphylococcus to successfully complete the second half of the century of MRSA. This is a situation in which I would be happy to be wrong!

Transparency declarations

R. C. M. has served as a consultant to Cubist, Forest, Pfizer, Theravance, Astellas, Merck and Novartis pharmaceutical companies, and serves on the Board of Directors of Nabriva Therapeutics AG.

References


