The effect of antibiotics on methicillin-resistant Staphylococcus aureus

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Antimicrobial drugs encourage the overgrowth of organisms resistant to the agents used. Acquisition and subsequent overgrowth of methicillin-resistant Staphylococcus aureus (MRSA) are particularly associated with β-lactam antibiotics and quinolones. These drugs allow rapid proliferation of an organism that might have been merely colonizing the skin, leading to clinical infection, treatment difficulties and potential transmission to others. In addition, there is increasing evidence that inappropriate antibiotics not only encourage overgrowth with MRSA but may also enhance pathogenicity. Such virulence is not necessarily due to simple expansion of MRSA across skin and mucosal surfaces; there appear to be molecular changes that facilitate mechanisms such as quorum sensing, adhesion, phage mobilization, exotoxin production, intracellular persistence and biofilm formation, all of which contribute towards more severe infection. This review examines the association between MRSA and certain classes of antibiotics and explores the molecular mechanisms underlying a perceived increase in virulence following inappropriate therapy. It is possible that empirical prescribing has a significant impact on the management of MRSA infections and ultimately patient outcome. It is time to challenge the prescribers’ right to prescribe what they like, when they like, for patients at risk of MRSA.

Keywords: MRSA, antimicrobial chemotherapy, virulence

Introduction

Before the introduction of antibiotics, the mortality rate of staphylococcal bacteraemia was ~70% (Figure 1).1 This rate dropped to ~25% in 1944, presumably due to the widespread availability of penicillin. Within the next 10 years, however, mortality rates rose to reach 45% as Staphylococcus aureus became resistant to penicillin. Soon after, the isoxazolyl group of penicillins, including methicillin and flucloxacillin, was introduced into clinical use and once again, the mortality of staphylococcal bacteraemia dropped to 25%. The capacity for methicillin resistance was documented almost as soon as these drugs became available.2

Methicillin-resistant S. aureus (MRSA) has now exerted its own impact upon the mortality rate. The average mortality rate from a recent meta-analysis of 30 studies was ~36% compared against a mortality rate of ~24% from septicaemia caused by methicillin-susceptible S. aureus (MSSA).3 Seven of the studies in this meta-analysis quoted MRSA bacteraemia mortality rates over 50%, and two of these were over 80%.3–5 Now the clinical consequences of resistance to vancomycin further complicate the management of MRSA infections.6 Mortality was 63% in patients who became infected with vancomycin-intermediate S. aureus (VISA).6 The mortality is even higher (78%) in patients with septicaemia caused by VISA additionally resistant to rifampicin, another staphylococcal agent.7 This progression illustrates the impact of staphylococcal resistance during the last 60 years, and more importantly, what it means for the future.

The overall mortality rate from MRSA bacteraemia will continue to rise as an entire population of coagulase-positive staphylococci becomes inherently resistant to methicillin across the world. New drugs might temper the rate of this rise, but there is no guarantee that they will be as efficacious against MRSA, as flucloxacillin is against MSSA. It has already been shown that MRSA is associated with a worse outcome than MSSA despite appropriate chemotherapy.8,9

Faced with a patient with bacteraemia, clinicians are forced to make an empirical choice of antibiotic for the causative organism, which has not been identified and for which there is no antibiogram. Generic prescribing guidelines and local knowledge help with this choice but increasing resistance has complicated the management of infection.10,11 This is well illustrated by the problems surrounding the management of MRSA. There is evidence to show that less than a quarter of patients with MRSA infections receive correct therapy within 48 h of hospital admission, and only ~40% receive appropriate agents after 48 h.12

It is reasonable to assume that resistance alone is the chief determinant of clinical outcome, in that an infected patient who is prescribed the wrong antibiotic for an infection simply does...
Role of antibiotics in the emergence of MRSA

Antibiotic consumption encourages the overgrowth of organisms on skin and mucosal surfaces. These organisms survive because they are resistant to the drugs prescribed and they proliferate quickly, assisted by the sudden access to nutrients and space. In hospitals, patients acquire resistant organisms from environmental reservoirs and thus low-level colonization may become established carriage with an increased risk of infection. Since MRSA survives well in the hospital environment, acquisition often seems to follow antibiotic therapy. Any antibiotic ineffective against MRSA will encourage acquisition but certain classes, notably the cephalosporins and quinolones, have already been identified as particular suspects. The association between these antibiotic classes and MRSA has been well illustrated by ecological studies. These often use hospital incidence or prevalence and model this against overall antibiotic consumption. Such studies allow measurement of the global effect of antibiotic exposure, encompassing the effects on patient groups as well as indirect effects on transmissibility. Studies examining the effects of antibiotic exposure on both the individual and on a group of patients or unit have demonstrated the added value of multilevel analysis. Muller et al. showed that overall consumption of penicillins was associated with MRSA acquisition at group level whereas fluoroquinolone exposure increased the risk of MRSA acquisition for the individual patient.

Time-series analysis has also been used in the investigation of antimicrobial drug use and MRSA at hospital level. One study showed a quantifiable temporal relationship between use of three classes of drugs (macrolides, cephalosporins and quinolones) and monthly rate of MRSA. The authors suggested that the effects of antibiotic consumption might have an impact upon resistance in future patients, in that there appeared to be a time-lag effect between increased use of an antibiotic class and increasing rates of MRSA.

Given that there appears to be a relationship between some antibiotics and MRSA over time and at different population levels, then reducing antimicrobial consumption might be useful in controlling MRSA rates in hospitals. Measures to control MRSA generally concentrate upon interrupting transmission through hand hygiene initiatives and other methods of infection control but these interventions prevent specific analysis of any antibiotic restriction policies within an overall control package. There is relatively little data to support antibiotic restriction as a single control policy against MRSA. However, some studies report reductions in MRSA rates following restrictions in both cephalosporin and quinolone consumption. Despite the lack of data, programmes to control prescribing of selected classes of antibiotics should be encouraged as an additional measure to infection control interventions in order to control outbreaks of MRSA.

Bacterial virulence: underlying molecular mechanisms

When an organism proliferates, the signs and symptoms of infection become more obvious. This is assumed to be due to the sheer quantity of bacterial cells provoking the usual inflammatory reaction, but pathogens are able to summon something more by which to advance infection. Having colonized a site and established themselves, they can initiate various virulence determinants in order to facilitate invasion and thus survival. Some strains of MRSA can be virulent whether encouraged by inappropriate antibiotics or not, but there is evidence to suggest that an inappropriate antibiotic will enhance and even accelerate its virulence in vivo, including strains that might only be regarded as commensals. Some examples of these mechanisms are described below.

(i) Quorum sensing

Expanding bacterial populations are subject to a chemical signalling mechanism called quorum sensing. Quorum sensing allows bacteria to detect the density of their own species and alter their genetic expression in order to take advantage of this information. This switch confers a survival advantage for the bacteria, in that the original requirement for colonization is superseded by a requirement for deeper penetration into the tissues once the number of cells in the colony reaches a set level.

Uncontrolled proliferation would soon result in a compromise, since there is a limit on space and nutrients at a single bodily site. The survival of the colony requires access to other

![Figure 1. Mortality rates of staphylococcal bacteraemia over time. Data taken from Rubin et al., Cosgrove et al. and Fridkin et al.](image-url)
sites in search of essential stores. The need to express genes coding for adhesion becomes less important; these genes are then repressed and others are activated that will encourage spread into the tissues and particularly into the systemic circulation. Access to the bloodstream, causing bacteraemia for the patient, allows the bacteria to find additional sites where they can perpetuate themselves.

Along with many other pathogens, *S. aureus* relies upon quorum sensing to establish itself *in vivo*. Cell density control of staphylococcal virulence is mediated by an octapeptide pheromone. This chemical sensor is released by staphylococcal cells, all of which have surface receptors to detect it. The more bacteria at the site releasing the pheromone, the higher its local concentration and the more the surface receptors are stimulated. At a predetermined concentration, the cell initiates an intracellular mechanism to repress the expression of adhesion genes and activate those able to facilitate invasion. These include the *agr* group of genes, which allow the transcription of genes responsible for encoding a variety of toxins. Activation of the *agr* locus leads to secretion of known virulence determinants such as α-toxin, β-toxin and δ-toxin.

Inappropriate or insufficient antibiotic therapy, by removing susceptible commensals, would encourage the growth of MRSA and thereby accelerate the quorum-sensing process, turning a few newly acquired colonies into virulent invaders. By its very nature, however, the system offers some additional targets for potential future therapy. Natural and artificial peptide inhibitors of the quorum-sensing response have already been evaluated *in vitro*. One group has produced a modified version of the octapeptide that binds to receptors on the surface of the organism without activating them. If this is added to *S. aureus* in culture, it stops producing at least two exotoxins known to contribute towards pathogenicity. Biostable peptide blockers might not eradicate the targeted pathogen but would allow more time for conventional antibiotics to exert their effect before the virulence switch is activated.

**(ii) Staphylococcal toxins**

It has been known for some time that antibiotics are capable of modifying the metabolic processes of bacteria when they are incorporated into culture media at subinhibitory concentrations. This includes the expression of virulence-associated genes in pathogens. Not all antibiotics exert the same effect, however, since there appears to be a differential effect dependent upon the pathogen and antibiotic pair under investigation. Since some of the products of virulence-associated genes can be measured, it is possible to rank individual antibiotics in order of their effect upon the production of toxins and other virulence determinants.

*S. aureus* produces many toxins, one of which, the staphylococcal α-toxin, is a major virulence determinant encoded by the *hla* gene. It has been shown that growing *S. aureus* in the presence of the β-lactam antibiotic, nafcillin, induces α-toxin expression and increases the lethal activity of broth filtrates in rats. These findings led to the speculation that β-lactam therapy might enhance the virulence of some *S. aureus* strains, in turn worsening the symptoms of serious staphylococcal infection. Other antibiotics were subsequently tested by measuring the induction of *hla* expression after exposure to different strains of *S. aureus*. There was a strong induction of *hla* expression by subinhibitory concentrations of several β-lactam antibiotics, including the cephalosporins and imipenem. Fluoroquinolones slightly stimulated expression, glycopeptide antibiotics had no effect, and erythromycin and aminoglycosides reduced expression. Clindamycin almost completely inhibited the expression of α-toxin. Furthermore, methicillin-induced *hla* expression appears to be a common phenomenon of α-toxin-producing strains of both MSSA and MRSA. Some MRSA strains produced up to 30-fold more α-toxin in the presence of 10 μg of methicillin per mL than in its absence.

*S. aureus* also produces a toxin called the Panton-Valentine leukocidin (PVL), now an established virulence factor linked to community-acquired MRSA strains. PVL has been associated with specific human infections in skin, soft tissue and necrotizing pneumonia, where the mortality rate is ~75%. Exposing strains that produce this toxin to subinhibitory concentrations of antibiotics yields similar findings to those described for α-toxin-producing strains. Clindamycin, linezolid and fusidic acid inhibit PVL production, vancomycin has roughly no effect, but subinhibitory concentrations of oxacillin enhance the release of PVL.

These *in vitro* findings suggest that α-toxin production might also be increased along with PVL toxin following exposure to selected antibiotics. The endothelial damage seen in necrotizing pneumonia, for example, would be encouraged by a combination of these two toxins and perhaps others produced by *S. aureus*. At the very least, antibiotics that inhibit PVL production would be better for the treatment of severe infections due to PVL-producing strains of *S. aureus*.

Another toxin associated with *S. aureus* is the toxic shock syndrome toxin (TSST), originally described in conjunction with tampon use in women. There is little evidence linking inappropriate or inadequate antibiotic therapy with increased production of TSST, but it is of interest that a recent report of two paediatric cases of toxic shock syndrome both received cephalosporin antibiotics before their rapid deterioration forced a change to more effective therapy. It has been reported that prior antibiotics may encourage non-menstrual toxic shock syndrome, as well as recurrent episodes of the syndrome. Yet more staphylococcal toxins, the enterotoxins, have been associated with post-operative enteritis caused by MRSA; staphylococcal enteritis following antibiotic therapy was originally described during the 1960s. It is possible that some cases of antibiotic-associated diarrhoea due to enterotoxin-producing *S. aureus* and MRSA go unrecognized at the present time, since many laboratories would not test a stool specimen routinely for *S. aureus* or MRSA.

Certain antibiotics obviously have the capacity for inducing the release of exotoxins, which enhance *S. aureus*-related toxic syndromes. Inadvertent use of β-lactam antibiotics to treat MRSA infections may therefore contribute to worse outcomes. Other agents appear to actively inhibit toxin production and thus attenuate virulence. In addition, these agents down-regulate the pro-inflammatory host response as well. The streptogramin antibiotic, quinupristin/dalfopristin, and the oxazolidinone, linezolid, dose-dependently reduce the induction of tumour necrosis factor-releasing activity by *S. aureus* towards host cells.

**(iii) SOS response**

Bacterial DNA damage occurs when bacteria are subjected to unfavourable environmental conditions. The global reaction to such damage is called the SOS response and its function is to
up-regulate genes involved in DNA repair and cell survival. It is known that exposure to antibiotics will initiate the SOS response, but it has only recently been shown that the response itself is capable of generating the horizontal transfer of mobile genetic elements, such as plasmids, bacteriophages, pathogenicity islands, transposons and various insertion sequences. These elements play a crucial role in spreading antibiotic resistance and virulence genes among bacterial populations.

β-Lactam antibiotics such as penicillin, ampicillin, cloxacillin and ceftriaxone induce the SOS response in S. aureus. This results in promotion of replication and high-frequency horizontal transfer of pathogenicity island-encoded virulence factors. These pathogenicity islands carry genes for virulence determinants such as TSST, other superantigenic toxins and biofilm promoters. Fluoroquinolones and trimethoprim have also been implicated in similar SOS induction in staphylococci.

It appears that non-lethal use of many antibiotics can induce the SOS response and potentially enhance the transmission not only of resistance but of virulence factors as well. Since MRSA continues to increase in hospitals, there is concern that heterogeneous populations of S. aureus will serve as a reservoir of virulence genes awaiting transfer to their meticillin-resistant counterparts.

(iv) Adhesion potential

Bacterial adhesion plays an important role in staphylococcal colonization and infection. S. aureus adheres to plasma proteins such as fibrinogen and fibronectin, which coat implanted biomaterials such as indwelling catheters and orthopaedic devices during the early stages of infection. It has been shown that sub-inhibitory concentrations of antibiotics can enhance staphylococcal binding to fibrinogen and collagen.

Exposure of highly fluoroquinolone-resistant S. aureus to sub-inhibitory levels of ciprofloxacin significantly increases the expression of fibronectin adhesins. This leads to increased attachment of the bacterial cells to immobilized fibronectin in an in vitro model. Increased adhesion also occurs with other strains of staphylococci, including MRSA and MSSA. Indeed, staphylococcal expression of surface adhesins is altered following the acquisition of the meticillin resistance element mecA. This antibiotic-promoted increase in adhesion might Even though proven to be effective against S. aureus in vitro, certain antibiotics may not necessarily protect infected host cells from S. aureus-mediated cell death. These include oxacillin, gentamicin, ciprofloxacin, trimethoprim/sulfamethoxazole and vancomycin. Linezolid, rifampicin, clindamycin and erythromycin suppress the cytotoxic action of S. aureus but most of these will only do so for as long as the antibiotic pressure is maintained. Except for rifampicin, intracellular S. aureus will regain its cytotoxic activity and kill the host cells following withdrawal of antibiotics. Linezolid and clindamycin can even induce a state of intracellular persistence of viable S. aureus. Thus, antibiotics commonly used in the management of S. aureus infections may encourage invasive intracellular strains, which may play an important role in the persistence and recurrence of infection.

Long-term intracellular persistence of small colony variants of S. aureus has been described in association with chronic osteomyelitis, cystic fibrosis, prosthetic joint and skin infections. These staphylococcal variants are able to persist under antibiotic pressure in vivo. It has been proposed that repeated treatment failures with standard antibiotic protocols might be linked with the emergence of S. aureus small-colony variants. Exposure to different classes of antibiotics frequently contributes to the selection of these variants both in vitro and in vivo, and they are undoubtedly difficult to diagnose and treat. A recent report describes the occurrence of small colony variants of MRSA during exposure to silicone impregnated with triclosan.

Small colony variants may be found in association with biofilms, an interacting conglomeration of organisms attached to both naturally occurring and synthetic surfaces. Biofilms serve as protective niches for pathogens within a host or as a means of survival in the environment. Small colony variants of staphylococci within biofilms may be highly resistant to the bactericidal action of oxacillin or vancomycin.

In most cases, treatment with antibiotics slows down biofilm progression by eliminating planktonic cells and interfering with biofilm metabolism. However, neither the biofilm nor the infection is eliminated effectively, and there is growing concern about the cross-resistance exhibited by antibiotic-resistant strains to other antimicrobial agents, including disinfectants. Strains of S. aureus that harbour plasmids coding for resistance to penicillin demonstrate resistance to quaternary-ammonium-chloride-containing disinfectants.

Virulence and antibiotic resistance

There has been much debate over whether MRSA is more virulent than MSSA. Numerous reports have linked meticillin resistance with a worse clinical outcome, although it is possible that increased morbidity and mortality due to MRSA infections could be due solely to the fact that they are more difficult to manage, and not necessarily because they are more virulent. It has been said that the most important reason for the conflicting
results is probably the heterogeneic nature of the resistant population, and that congenic strains of MRSA and MSSA should be used to correlate the genetic background with the phenotypic expression of virulence.74 Given the subject of this review, these investigations should include exposure to antibiotics, since it is possible that a resistant strain is more likely to be exposed to an inappropriate agent and thus more likely to exhibit enhanced virulence.75 Perhaps it is the case that the main reason for the perceived increased virulence, and thus mortality, in MRSA is due to initial antibiotic exposure and not because MRSA is more pathogenic than MSSA.75

One study investigated MRSA isolates obtained after clinical failure of vancomycin.76 Sequential isolates demonstrated physiological changes when compared with the original parent strain. Analysis of the virulence regulatory group of agr genes from the initial bloodstream isolate showed little β-haemolysin activity. However, after 9 months of vancomycin and a switch to linezolid, β-haemolysin expression increased noticeably. There was also a decrease in autolysis, reduced killing by vancomycin in vivo and increased biofilm formation in isolates obtained after prolonged exposure to vancomycin.76 A possible link between pathogenicity and vancomycin tolerance in MRSA has already been suggested, since the discovery that the agr group of genes is implicated in the expression of penicillin-binding proteins that help establish the VISA phenotype.77

Staphylococcal resistance contributes towards the pathogenesis of wound infections. Resistant subpopulations of staphylococci, particularly those producing β-lactamase, may account for a significant proportion of apparent prophylaxis failures. This may be due to the fact that a popular choice for antibiotic prophylaxis includes the cephalosporins, most of which are ineffective against MRSA as well as encouraging β-lactamase-producing borderline oxacillin-susceptible S. aureus.78,79

It may be relevant to note that of four paediatric deaths attributed to community-acquired MRSA, all had received prior therapy with cephalosporins on admission to hospital.80 Two other children with MRSA infections required surgical management following failed treatment with oral cephalosporins.81 The authors warn of the need to consider MRSA as a potential cause of infection in community-based patients with no obvious risk factors, including previous hospitalization.81 More recently, there have been cases of severe MRSA community-acquired pneumonia associated with influenza.82 Three children out of 10 cases (age range: 4 months to 48 years) described in the report were all initially treated with ceftriaxone, including one with a previous history of an MRSA abscess who received additional vancomycin. These children died, along with 3 others from the 10 confirmed cases. MRSA isolates from five patients carried the SCCmec-type IVa resistance gene cassette and the PVL toxin genes.82 Given the in vitro findings from work already described, it is possible that initial cephalosporin therapy encouraged the production of the PVL toxin, thereby accentuating its necrotic effects in the lungs of influenza victims.38,41

Does antibiotic therapy encourage staphylococcal transmission?

There is little data to support the premise that antibiotic therapy enhances staphylococcal transmission, although it would seem reasonable given the increase in MRSA in hospitals across the world. It is unlikely that poor infection control is the only reason for this global increase. Countries reporting higher antibiotic consumption tend to have higher rates of MRSA.83 However, we know that staphylococcal carriers have their resistant strain replaced soon after hospital admission, often with a more resistant version, and there is a possibility that this is encouraged by exposure to antibiotics.16,84 A study from 40 years ago examined the impact of tetracycline on patient carriers.85 Not only did tetracycline select for resistance in habitual carriers, its consumption also encouraged staphylococcal transmissibility. There was an impressive increase in the overall carrier rate in the antibiotic treated group, including patients previously shown to be non-carriers.84

It would be difficult to repeat this study nowadays, since MRSA is endemic in most hospitals and many more antibiotics are routinely used on the wards, but it would be interesting to document whether certain classes of antibiotics are more likely to encourage carriage of resistant strains among habitual carriers and non-carriers alike. We already know, as described earlier, that consumption of cephalosporins, quinolones and macrolides is associated with increased rates of MRSA acquisition, although we do not necessarily know whether this is specifically linked to initial colonization of major carrier sites in hospital patients.16–20,22,60,61 These antibiotics would encourage staphylococcal shedding, since exposing a patient to a drug ineffective against colonizing MRSA would facilitate proliferation of the organism at all sites, including superficial carrier sites. It has been shown that patients with established infections, particularly in wounds and urine, appear to shed more MRSA into the environment, with or without the help of antibiotics.85

Conclusions

There appear to be several explanations for the fact that patients with MRSA infections do not do as well as patients with MSSA infections. Limited antimicrobial choice for MRSA, lack of awareness by prescribers and ignorance of carrier status results in individuals progressing rapidly from colonization to infection in our hospitals. In addition, it is possible that antibiotic pressures in the healthcare environment, and not necessarily poor infection control, might be the main driver for increasing MRSA. Compounding this are the points raised in this review that drugs ineffective against MRSA could actually predispose to a more unfavourable outcome for a patient with an undiagnosed MRSA infection.

Much of the evidence presented is based on in vitro studies and is therefore far from proven in vivo. There is justification for these molecular mechanisms, however, from global reports on antibiotic consumption and rising MRSA, and detailed reports of individual cases that fail to receive appropriate agents for their MRSA infections initially and suffer the consequences. Demonstrating increasing virulence in vivo will not be easy, since we still do not understand the full range of virulence determinants employed by S. aureus and the nature of all its interactions with the human host. It is also important to emphasize the fact that many of the examples described in this review relate to in vitro experiments using MSSA and not MRSA. There may well be even more differences in the expression of virulence and other factors that are not necessarily attributable
to strains demonstrating increased resistance. More work needs to be done on both susceptible and resistant strains derived from a common predecessor to establish exactly what happens at both molecular and clinical levels when a patient receives an antibiotic.7,8,31

Trying to dissect out the effects of antibiotics versus infection control deficits when examining the spread of MRSA is almost impossible. The two are inextricably intertwined. It is possible that environmental contamination with MRSA is primarily responsible for first acquisition in healthcare institutions,27 but progression from colonization to infection, and from microbial proliferation to shedding, is more likely to be due to antibiotic pressures in an immunocompetent host. However, separating out definitive roles for everything we do in the name of infection control, including antimicrobial prescribing, may never be fully elucidated. This leaves us with no option but to apply the whole package of infection control measures at our disposal, in conjunction with supporting local and generic policies on the use of antibiotics.

Despite the speculative nature of the views presented in this article, clinicians should consider their antibiotic choices carefully. Further education on the environmental effects of prescribing and the adverse events seen in patients would help, but it is possible that the only intervention that would have an effect in hospitals would be prescribing penalties for those who do not adhere to policies or seek expert advice. Challenging a prescribers’ right to prescribe requires courage, determination and authority, but until the environmental and ecological consequences of antimicrobial consumption are fully realized, restricting established prescribing habits will remain controversial.8,31

Transparency declarations

None to declare.

References


