REVIEW

The importance of the development of antibiotic resistance in *Staphylococcus aureus*

G. C. Schito

Institute of Microbiology, University of Genoa, Italy

ABSTRACT

Hospital- and community-acquired *Staphylococcus aureus* infections pose a substantial burden in terms of morbidity, mortality and healthcare costs. The introduction of new antibiotics to counter this pathogen has frequently been closely followed by the emergence of resistant strains. Most significantly, *S. aureus* isolates resistant to β-lactams have become common, and many of these are also resistant to β-lactamase-resistant penicillins. The rapid spread of methicillin-resistant *S. aureus* (MRSA) clones across the world often results in hospital outbreaks, but implementation of appropriate control measures usually reduces prevalence to sporadic levels. However, the recent emergence of MRSA infections in the community, affecting patients with no established risk factors for MRSA acquisition, is likely to impact significantly on future strategies for control of nosocomial MRSA. In contrast to other antibiotic classes, *S. aureus* resistance to glycopeptides did not emerge until nearly 40 years after their clinical introduction, and as a result this drug class has remained the mainstay of treatment for MRSA infections. However, a number of vancomycin-intermediate *S. aureus* isolates have emerged worldwide and four fully resistant *S. aureus* isolates have been reported in the USA. This raises the concern that the current first-line treatment for MRSA infection may become ineffective in an increasing proportion of cases in the near future. New classes of antibiotic are urgently needed to treat infections with this growing population of multidrug-resistant *S. aureus*, and the recently introduced oxazolidinone linezolid and the cyclic lipopeptide daptomycin are welcome additions to the ever-narrowing range of therapies effective against this pathogen.

Keywords Antibiotic, methicillin-resistant *Staphylococcus aureus*, review, vancomycin-resistant *Staphylococcus aureus*

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INTRODUCTION: THE CURRENT SITUATION

Staphylococcus aureus is a major cause of hospital- and community-acquired infections, and can result in serious consequences [1]. Hospital infections caused by *S. aureus* include those affecting the bloodstream, lower respiratory tract, skin and soft tissues, as well as ventilator-assisted pneumonia and central venous catheter-associated bacteraemia. These infections can lead to substantial morbidity and mortality, as well as high healthcare costs. This situation has been exacerbated by the rising incidence of strains that are less susceptible to a variety of antibiotics, making treatment of these infections more difficult.

The SENTRY Surveillance Program investigated the worldwide extent of infections caused by *S. aureus* between January 1997 and December 1999 [1]. Bloodstream isolates from 15 439 patients infected with *S. aureus* and 6350 patients infected with coagulase-negative *Staphylococcus* species were referred by SENTRY-participating hospitals in the USA, Canada, Latin America, Europe and the Western Pacific region. *S. aureus* was the most common overall cause of bacterial infections involving the bloodstream, lower respiratory tract and skin/soft tissue (Table 1) [1]. Indeed, 32–47% of all skin/soft tissue infections appeared to be caused by *S. aureus* [1].

The importance of *S. aureus* as a human pathogen, apart from its ability to cause a diverse range of life-threatening infections, is its extraordinary potential to develop antimicrobial resistance [2].

Corresponding author and reprint requests: G. C. Schito, University degli Studi Di.s.c.a.t., Sezione di Microbiologia, L.go R. Benzi, 10, Genova, 16132, Italy
E-mail: schito@unige.it

This review will focus on the development of antimicrobial resistance in *S. aureus*, including the evolution of resistant strains, their spread into the community and molecular mechanisms of resistance, with a particular emphasis on methicillin and vancomycin resistance.

EMERGENCE OF ANTIMICROBIAL RESISTANCE

Causes of antibiotic resistance

History has shown that the introduction of a new antibiotic is frequently followed by the development of bacterial antibiotic resistance, as antibiotic use provides the potential for selection of resistant strains [3]. There are several underlying reasons for this phenomenon: (1) antimicrobial use is the key driver of resistance and, paradoxically, this selective pressure comes from a combination of overuse in many parts of the world (e.g., for minor infections or in food-producing animals), misuse due to lack of access to appropriate treatment, and failure to complete treatment courses [3]; (2) inherent microbial characteristics also play a role—for example, *S. aureus* resistance to penicillin is highly prevalent, and yet *Streptococcus pyogenes* strains are uniformly susceptible to penicillin, which remains the drug of choice for treating infections caused by this organism [4]; and (3) societal and technological traits also contribute to the spread of antibiotic resistance due to substantial increases in the availability and ease of travel within and between countries [2].

MACROLIDE, LINCOSAMIDE AND STREPTOGRAMIN RESISTANCE

Macrolides, lincosamides and streptogramins, which were first introduced in 1952, constitute a group of antibiotics collectively known as MLS. They target the bacterial 50S ribosomal subunit, thereby effectively inhibiting protein synthesis [5]. As some patients were allergic to penicillin, MLS antibiotics provided welcome alternatives for treating staphylococcal infections. However, resistance to the new antibiotics emerged shortly afterwards in strains of *S. aureus*, as resistance genes were already present, and use of these antibiotics exerted a selective pressure [5,6].

Table 1. Percentage of bloodstream, lower respiratory tract and skin/soft tissue infections due to *Staphylococcus aureus* at SENTRY centres across the world, 1997–1999 [1]

	Number (%) of infections due to *S. aureus*					
Site of infection	**USA**	**Canada**	**Latin America**	**Europe**	**Western Pacific**	**All regions**
Bloodstream	4405/17 399 (25.3)[a]	739/3840 (19.2)[a]	1092/5295 (20.6)[a]	2014/10 815 (18.6)[b]	679/3148 (21.6)[a]	8929/40 497 (22.0)[a]
Lower respiratory tract	1709/6711 (25.5)[a]	379/1659 (22.8)[a]	413/1914 (21.6)[b]	526/2572 (20.4)[a]	344/1696 (20.3)[b]	3371/14 552 (23.2)[a]
Skin/soft tissue	969/2328 (41.6)[a]	278/633 (43.9)[a]	432/1353 (31.9)[a]	880/2371 (37.1)[a]	369/789 (46.8)[a]	2928/7474 (39.2)[a]

[a]*S. aureus* ranked as leading cause of infection.
[b]*S. aureus* ranked as second-highest cause of infection.

In *S. aureus*, MLS resistance has two phenotypes. The first is due to ribosomal modification by 23S rRNA methylases, mediated primarily by *ermA*, *ermB* or *ermC* (found on plasmids or chromosomes), preventing antimicrobial agents from binding to their ribosomal target site [5,6]. The second resistance type is mediated by *msrA* and involves the active efflux of the antimicrobial agent by an ATP-dependent pump, thereby maintaining intracellular concentrations below the level required for binding to ribosomes [6].

Aminoglycoside resistance

Aminoglycosides were introduced in 1944, and by the 1950s aminoglycoside-resistant strains of *S. aureus* had emerged [7]. These drugs enter bacterial cells by energy-dependent binding to the cell wall and energy-dependent transport across the cytoplasmic membrane, finally binding to one or more ribosomal sites, thus inhibiting protein synthesis [5]. Resistance in staphylococci results from any of three events: (1) a chromosomal mutation leading to altered aminoglycoside binding to ribosomes; (2) ineffective transport of aminoglycosides into the bacterial cell, producing low-level cross-resistance to most aminoglycosides; and, most commonly, (3) enzymic modification of aminoglycosides [2]. In the last case, resistant strains have the aminoglycoside-modifying genes *acc*, *aph* and *ant*, which code for aminoglycoside acetyltransferases, phosphotransferases and adenyltransferases, respectively [5,8]. The acetylated, phosphorylated or adenylated aminoglycosides do not bind to ribosomes, and thus do not inhibit protein synthesis [8].

Quinolone resistance

Although fluoroquinolones were introduced in the 1980s for the treatment of Gram-negative bacterial infections, their Gram-positive spectrum of activity meant that they were also used to treat infections caused by pneumococci and staphylococci [2]. The primary target of quinolones is bacterial DNA gyrase, without which DNA replication is inhibited [5]. Quinolone resistance emerged rapidly, particularly among methicillin-resistant strains, by the stepwise acquisition of chromosomal mutations. This involved mutations in the quinolone-resistance-determining region of the enzyme–DNA complex, reducing the affinity of quinolone for its targets (DNA gyrase and topoisomerase IV) [2].

β-LACTAM RESISTANCE

The penicillins isolated from fermentation broths of *Penicillium chrysogenum* were found to be very active against Gram-positive and Gram-negative cocci [7]. When penicillin G was initially introduced in the early 1940s, over 85% of *S. aureus* isolates were susceptible to <0.1 mg/L, but penicillin-resistant staphylococci appeared within 3 years. By 1948, up to 50% of hospital strains were resistant, with the level of resistance rising to 80% by 1957 [7]. Resistance is due to the production of a penicillinase (or β-lactamase). More than 90% of staphylococcal isolates now produce β-lactamase, which inactivates β-lactam antibiotics by hydrolysis of their β-lactam ring [2]. *BlazA* encodes β-lactamase and is part of a transposable element on a plasmid, which often also contains genes resistant to other antibiotics, (e.g., gentamicin and erythromycin) [2].

Methicillin, the first of the semi-synthetic penicillinase-resistant penicillins, was introduced in 1961 to target strains of penicillinase-producing *S. aureus* [2,8]. However, resistance to methicillin was reported very quickly after its introduction [9]. Resistance occurs following the chromosomal acquisition of novel DNA, resulting in the production of a new penicillin-binding protein, termed PBP2a, with a low binding affinity for methicillin [8]. PBP2a substitutes for all other penicillin-binding proteins, and because of its low affinity for all β-lactam antibiotics it confers resistance to all β-lactam agents, including cephalosporins [2]. PBP2a is encoded by *mecA*, part of the mobile genetic element, the staphylococcal chromosomal cassette *mec* (SCC*mec*) [8]. The source of *mecA* is unknown; however, it has been suggested that it was acquired from a coagulase-negative staphylococcal species such as *Staphylococcus sciuri* [2,10].

Waves of clonal dissemination of methicillin-resistant *S. aureus* (MRSA) strains spread rapidly across the world, accounting for varying proportions of nosocomial *S. aureus* infections in different countries [2]. Of particular interest is the prevalence of methicillin resistance among *S. aureus* isolates in various European countries (Fig. 1) [11]. Data were collected from January

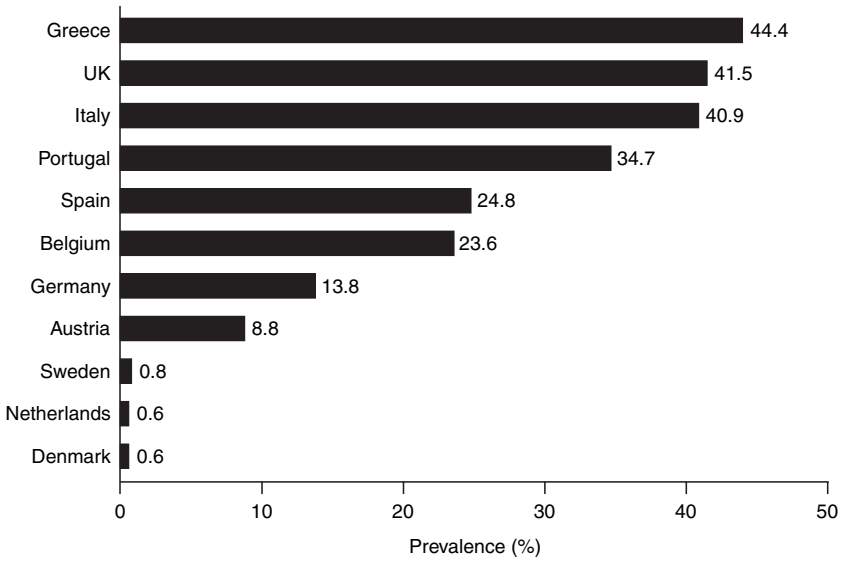

Fig. 1. The prevalence of methicillin resistance among *Staphylococcus aureus* blood isolates in Europe [11].

1999 to December 2002, and involved 50 759 isolates from 495 hospitals in 26 countries. MRSA prevalence varied almost 100-fold, from <1% in northern Europe (Sweden, Denmark and The Netherlands) to >40% in Greece, the UK and Italy. This study also showed considerable variation in MRSA proportions among hospitals within a country. This regional variation may be explained by different phenomena. The emergence of MRSA is largely due to dissemination of clonal strains, and temporary hospital outbreaks are typically due to clonal expansion [11]. However, if effective control measures are taken to prevent further MRSA transmission, MRSA prevalence may be reduced to sporadic levels [11,12]. The effectiveness of MRSA control depends on several factors, including good hygiene (e.g., hand hygiene, isolation practices and cohort nursing of patients with MRSA), level of care needed by patients (e.g., indicating host susceptibility), and antimicrobial drug prescription policies (which would influence selective pressure) [11,12]. Rapid application of these hygiene and control measures at the hospital level, as well as at the regional level, may be successful in containing the MRSA epidemic [12].

The prognosis for patients infected with MRSA is generally worse than that for patients infected with methicillin-susceptible *S. aureus* (MSSA). This was demonstrated by a meta-analysis of *S. aureus* bacteraemia that showed a significant increase in mortality associated with MRSA (OR 1.93, 95% CI 1.54–2.42; $p < 0.001$) [13]. Rather than any enhanced virulence of the MRSA strains, this difference is thought to be associated with patient age and disease severity, as well as the relative lack of effective antibiotic treatments for MRSA [2]. A study investigating cases of *S. aureus* bacteraemia during a hospital outbreak of MRSA over a 4-year period showed that patients with MRSA bacteraemia were older than those with MSSA bacteraemia (69 years vs. 54 years, respectively; $p < 0.01$) [14]. Patients with MRSA bacteraemia were also more likely than those with MSSA to have the following predisposing factors: prolonged hospitalisation (32 vs. 14 days, respectively; $p < 0.01$); prior antimicrobial therapy (61 vs. 34%, respectively; $p < 0.01$); urinary catheterisation (58 vs. 27%, respectively; $p < 0.01$); nasogastric tube placement (31 vs. 13%, respectively; $p < 0.01$); and prior surgery (45 vs. 31%, respectively; p 0.05) [14]. It is also interesting to note that mortality rates in patients with MRSA or MSSA in nosocomial pneumonia are high, even if they are correctly treated for these strains (50 vs. 34%, respectively; p 0.34) [15].

Although MRSA was until recently considered a healthcare-associated pathogen, several recent reports have documented the emergence of infections in non-healthcare settings, in patients with no established risk factors for MRSA acquisition. Methicillin resistance in these community-acquired MRSA isolates has most commonly been associated with SCC*mec* type IV [16]. In contrast to nosocomial MRSA infection, infections caused by community-acquired MRSA are often mild

and similar to those caused by MSSA, but severe infections, including necrotising fasciitis and necrotising pneumonia, have been reported [17,18]. Reported prevalence rates vary, but a recent meta-analysis found a pooled MRSA colonisation rate of 0.2% among community members without healthcare contacts [19]. It is likely that this reservoir of community-acquired MRSA will continue to expand, potentially leading to endemicity within the community—a situation that could well have a substantial negative impact on the control of nosocomial MRSA [20].

Glycopeptide resistance

Following the spread of MRSA, glycopeptides (usually vancomycin and more recently teicoplanin) have become the mainstay of treatment for MRSA infections. Vancomycin was introduced in 1958, but the first fully vancomycin-resistant *S. aureus* (VRSA) clinical isolates were found in 2002 [21]. Prior to this, vancomycin-intermediate *S. aureus* (VISA) strains were isolated, first in Japan in 1996, and then in many other countries, including the USA, prompting widespread concern. As nearly 40 years transpired between the introduction of vancomycin and the emergence of the first case of VISA, and as resistance was difficult to induce *in vitro*, many thought that vancomycin resistance was unlikely to occur in a clinical setting. However, subsequent events have disproved this hypothesis. Four clinical VRSA isolates have now been reported, all in the USA (two from Michigan, and one each from Pennsylvania and New York), each from a single patient.

In addition to worries that strains of *S. aureus* have emerged with total resistance to vancomycin, these VRSA isolates also have a different and potentially much more efficient mechanism for dissemination than VISA strains. Whereas resistance in VISA strains is thought to occur solely as a result of alterations in peptidoglycan synthesis, VRSA strains are thought to acquire additional resistance by conjugal transfer of plasmids containing the *vanA* operon from vancomycin-resistant *Enterococcus faecalis*. Resistance in VRSA strains is caused by alteration of the cell wall terminal peptide D-alanyl-D-alanine to D-alanyl-D-lactate, preventing the inhibition of cell-wall synthesis by vancomycin. VISA strains appear to be selected from a population heterogeneous for vancomycin resistance (in the presence of vancomycin, which exerts a selective pressure). These VISA strains synthesise greater quantities of peptidoglycan with more exposed D-alanyl-D-alanine residues to bind and trap vancomycin, which may then act, in addition to the thickened cell wall found in these strains, to prevent drug molecules reaching the target of the cytoplasmic membrane. The specific resistance genes for VISA and VRSA strains are, as yet, unknown.

It is difficult to assess the effect of heterogeneous VISA, VISA and VRSA on clinical outcomes, as these are relatively recent phenomena, and thus there are few published studies. However, one study has examined treatment outcomes in patients ($n = 25$) with serious infections caused by MRSA with reduced vancomycin susceptibility (MICs of 2–4 mg/L) [22]. In this study, eight patients had endocarditis, nine had bacteraemia associated with a deep-seated infection, six had osteomyelitis or septic arthritis, and two had empyema. All patients had previously received vancomycin for a median period of 15 days; this treatment failed for 19 of 25 patients (76%). Although second-line treatment was usually effective (in 24 of 25 cases; 96%), surgical interventions were required in 60% of patients ($n = 15$). Thus, second-line antibiotic therapy, especially linezolid with or without rifampicin and fusidic acid, in conjunction with surgical debulking, was effective for the majority of patients with serious infections, including endocarditis, caused by MRSA with reduced vancomycin susceptibility [22].

CONCLUSIONS

The history of the development and introduction of antibiotics, together with the often rapid emergence of resistance in *S. aureus*, shows that this organism has a remarkable capacity for adapting to new types of antimicrobial agents. As a consequence, there is a growing population of *S. aureus* that is resistant to traditional antibiotics and their derivatives. Moreover, this resistance is moving from the hospital to the community, thus posing an even greater threat than before. New classes of antibiotic are needed to overcome this threat. In 2000–2001, the oxazolidinone linezolid became the first new antimicrobial drug specifically targeting Gram-positive bacteria to enter clinical use since the glycopeptides in the 1950s. More recently, the cyclic

lipopeptide daptomycin was introduced into clinical practice in the USA in 2003 and is expected to be available in Europe early in 2006. It is hoped that these new agents will help to combat the threat posed by the spread of *S. aureus* strains resistant to the older antimicrobial agents.

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