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Olga Genilloud *Editors*

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New and Old Molecules in the Fight Against Multi-Resistant Bacteria

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Preface

The positive impact of antibiotics in human health has been challenged in the past decade by the emergence and prevalence of antibiotic resistant pathogens either in the hospitals or in the community, requiring renovated efforts to identify and develop therapeutic alternatives. The current medical need to identify antibiotics with novel structures and unexploited mode of action is triggering the development of new strategies for the discovery of natural and synthetic molecules, providing new options in the never-ending battle against ever-evolving resistant bacteria.

The objective of this book is to present an updated review of the status of all major classes of antibiotics, especially focusing on most recent advances in already known chemical classes, including new analogs and semi-synthetic derivatives, as well as the recent new classes that have reached the clinic in the past years or are in clinical and preclinical development phases. This work is divided into two major sections covering both the clinical impact of bacterial pathogens and the current trends in antibiotic discovery and development.

The first section opens with a review by Davies (Chap. 1) on the origin and evolution of antibiotics emphasizing the need to understand their role in the environment and their chemical and biological evolution to successfully exploit their pharmaceutical potential. Rossolini et al. (Chap. 2) review the evolution of the clinical impact of Gram-positive pathogens, and especially the multiresistant ones, in health care-associated and community-acquired bacterial infections, whereas Paitan and Ron (Chap. 3) analyze the rising prevalence of resistant Gram-negative pathogens, including their various resistance mechanisms, prevalence, risk factors, as one of the major clinical problem given the lack of treatment options.

The second section contains a series of 13 chapters covering the status of different classes of antibiotics, including both novel candidates in development as well as mature compounds. The emergence of pan-resistant pathogens challenging the development of new β-lactams and the most recent advances in the understanding of the action of this family of antibiotics are accurately reviewed by Leemans et al. (Chap. 4). The chemical diversity of peptide antibiotics has been classified into five different classes of compounds. Glycopeptides are extensively described by Marcone and Marinelli (Chap. 5), whereas Baltz (Chap. 6) presents the specific characteristics of daptomycin and other related lipopeptides. Lantibiotics is another emerging family of peptides with no evident cross-resistance

with any of the major classes of antibiotics (Cortes, Chap. 7). Vaara reviews the status of old and new analogs of polymyxin against Gram-negative pathogens (Chap. 8), whereas Carter and McDonalds present the recent developments in the biosynthesis and medicinal chemistry of uridyl peptide antibiotics (Chap. 9). The recent development of new aminoglycosides within the review of traditional aminoglycosides by Kirst and Marinelli provides an extensive coverage of the evolution of this old class (Chap. 10). Similarly, the chapters on traditional macrolides (Kirst, Chap. 11) and tetracyclines (Genilloud and Vicente, Chap. 12) include recent progress in the development of semi-synthetic and synthetic analogs. The last four chapters include reviews on the class of oxazolidinones (Zappia et al., Chap. 13) with description of the antibacterial activity and chemistry of this synthetic new antibiotics, the development of actinonin and its analogs as peptide deformylase inhibitor (East, Chap. 15), the status of other smaller classes of protein synthesis inhibitors (Kirst, Chap. 14), and novel bacterial topoisomerase inhibitors (Pucci and Willes, Chap. 16).

The book concludes with an extended review by Genilloud and Vicente of recent strategies developed in the pharma and academic sectors to respond to emerging medical needs (Chap. 17), ranging from the use of selected old and new targets to novel screening approaches involving the implementation of alternative technologies and mode of action studies.

The editors thank the contribution of all authors, with a special mention of Herbert Kirst, who greatly supported in the preparation and revision of the last chapters ensuring the final completion of the work.

Flavia Marinelli
Olga Genilloud

Contents

Part I
Current Trends in Antibiotics, Pathogens and Medical Needs

Chapter 1
The Origin and Evolution of Antibiotics

Julian Davies

Abstract Microbes are the most prevalent living organisms in the biosphere; they constitute about 50 % of the Earth's weight. They are also the most prolific in terms of the production of antibiotics and other bioactive small molecules. This rich store of chemical diversity (termed the Parvome) provides an inexhaustible source of therapeutic agents that has barely been investigated. Devising new ways of harvesting these compounds is a major challenge that requires developing new insights into their origin and evolution and also predictions of their roles in chemical and biological evolution. Only with this information will it be possible to exploit their pharmaceutical potential to the full.

1.1 Introduction

The biosphere is populated with an enormous collection of low-molecular weight organic compounds with an extraordinary diversity of molecular structures produced by living organisms (the Parvome) (Davies and Ryan 2012). Although a significant proportion of these compounds may be products of the normal processes of biodegradation, the majority are made by defined, regulated biosynthetic pathways and are involved in many of the functions and interactions of cells, tissues, organs, and organisms (both positive and negative). These molecules have highly specific interactions with cellular targets (although very few have been identified), and may act both extra- and intracellularly. It is likely that all living beings make bioactive small molecules for these purposes. It has been suggested that the "central dogma of biology" is more than just the triumvirate of DNA, RNA, and protein and should include the wealth of bioactive small molecules

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(Schreiber 2005). They play essential, but as yet, largely unidentified roles in the maintenance of the biosphere.

One of the best recognized groups of bioactive small molecules are those that have antibiotic activity; they are produced principally by bacteria, fungi, plants, and sponges, but in all probability most living organisms, including humans and elephants, etc., make such molecules. They are critically important, not because of their number or distribution, but because of their demonstrated therapeutic properties. However, it is likely that, they represent only a small fraction of the Parvome.

The word "antibiotic" was coined by Selman Waksman (Strohl 1997) soon after the discovery of streptomycin in the early 1940s. This compound, together with penicillin (historically and therapeutically), presaged the most successful medical epoch ever, the age of antibiotics. Infectious diseases became curable and billions of humans have been saved from the historical and modern plagues.

Although Waksman's definition was convenient, it ignored the vast number of bioactive compounds without detectable therapeutic potential that are produced by living organisms. In reality, the word antibiotic defines a property or an activity and not a compound.

1.2 What's in a Name?

The word antibiotic is often incorrectly used. At the present time, almost any small molecule made by a microbe is termed an antibiotic: this is loose-thinking! What can these products be called when their functions are so broad and when they have such a wide range of structures, activities, and biochemical origins?

The name "secondary metabolite" has frequently been used for bioactive compounds; this term was employed originally with reference to plant products. The simplest definition comes from "Wikipedia:" "Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism."

It is true that antibiotic activity is most frequently detected when the logarithmic phase of growth begins to slow down, when a microorganism is no longer dividing exponentially (Campbell 1984). However, although antibiotic activity cannot be detected earlier, bioactive compounds could well be produced, at concentrations not detected by inhibition. This definition also seems somewhat derogatory, since the compounds produced play many critical roles and can in no sense be referred to as "secondary"! The fact is, secondary metabolites are mostly synthesized using primary metabolites as precursors. The timing of their production with respect to growth is of no real consequence.

The word "idiolyte" has also been used as a substitute for secondary metabolite; this refers to an association with a production phase late in microbial growth. This word has not really caught on, perhaps because of its use in immunology.

1.3 Many Sources, Uses, and Functions

Bioactive small molecules have many properties and applications in medicine, industry, agriculture, and other uses (Demain and Sanchez 2009); these go far beyond Waksman's definition which states that "an antibiotic is a chemical substance produced by a microorganism which has the capacity, in dilute solutions, to inhibit the growth of, or to kill other microorganisms." It was not realized at the time that, at even lower dilutions, antibiotics might actually stimulate growth or influence other biochemical functions in microorganisms. For example, the formation of biofilms.

In order to discuss the evolution of microbially produced bioactive compounds in the context of the evolution of the cell, it is essential to realize that bioactive small molecules can, and do have many sources and probably played numerous roles in the origins of living organisms.

Thus, the Parvome components have evolved to serve numerous ecological functions in different organisms and under many circumstances. The biosynthetic pathways for small molecules may share many commonalities and probably evolved in similar ways, but their roles in the organism that produces them are often intimately related to the lifestyle of the producer. Bioactive compounds may play different roles depending on their hosts. For example, the adrenergic hormones: these are relatively simple organic molecules that have specific functions depending on the circumstances. They have been characterized as hormones produced by and playing important roles in humans and animals, but recently they have been shown to affect the properties of bacterial populations.

Norepinephrine acts as a stress hormone and a neurotransmitter in animals, but also stimulates bacterial growth and enhances bacterial virulence functions. This catecholamine was one of the first compounds identified in studies of the growing discipline of microbial endocrinology (Freestone et al. 2008). Similar activities have been reported for other congeners of this family of compounds. Interestingly, the mammalian protein hormone insulin has been shown to enhance the growth of certain bacterial pathogens (Plotkin and Viselli 2000). These are good examples of the extensive biological plurality of functions exhibited by bioactive small molecules. This is especially true for bacterial products that were first characterized for their antibacterial activity and used therapeutically. When tested employing a range of assays and concentrations for their biological activity, most so-called antibiotics prove to have a surprising range of concentration-dependent biochemical activities (Davies et al. 2006). In addition, most antibiotics have multiple toxic side effects due to their ability to bind to specific human receptors of one kind or another. Yet, for our convenience, they remain labeled as antibiotics.

Biologically active compounds with a diversity of biological activities (including antibiosis) have been present in the biosphere for eons. For example, the lichens, which are ancient mutualistic associations of fungi/algae/bacteria produce a large number of bioactive low molecular weight compounds that may originate from any of the symbionts. Evidence suggests that they play roles in the

maintenance of the lichen structure, but many have antibacterial activity (and are called antibiotics) (Muller 2001). Plants are also prolific sources of bioactive small molecules with a huge range of functions and applications (Firn and Jones 2003).

The Parvome refers to the enormous diversity of organic compounds in the biosphere: these chemical entities must be essential but what roles do they play in their natural habitats? It is becoming increasingly obvious that microbes exist in all living organisms as communities or microbiomes (Banfield and Young 2009): Bioactive small molecules are likely involved in the establishment and maintenance of microbial communities through inter-species signaling activities. These broad ecological functions are not well understood and studies of their activities in situ are still in their infancy. It is unfortunate that the pervasive notion of small molecules as weapons of attack and defence has suppressed their recognition as ubiquitous agents of communication in biology.

1.4 How Old Are "Antibiotics"?

It does not make sense to discuss the evolution of antibiotics without some consideration of their origins. How old are these compounds and in what way and when, did their biosynthetic pathways evolve? This is distinct from the commercial evolution of antibiotics taking place at this time, driven by the competition between the pharmaceutical industry and resistant pathogens.

Calculations show that a biosynthetic pathway responsible for making a complex non-ribosomal peptide antibiotic (NRP) is at least one billion years old (Baltz 2010). The biosynthetic gene cluster for daptomycin is 128 kB in size (see Baltz, this volume). However, the precursor amino acids for their synthesis must have been present in the biosphere from earlier times. The NRPs include both protein-associated amino acids and other amino acids that have only been found in NRP structures. Thus, both protein and non-protein amino acids are very old and are thought to have been delivered to the Earth as organic components of meteorites. Meteorites have been shown to transport a number of different amino acids, both protein and NRP-associated (Pizzarello and Shock 2010) into the biosphere, and they likely played roles in prebiotic chemistry (van der Gulik et al. 2009). As an example, the components of the pharmaceutically important NRP antibiotic daptomycin with 10 different amino acids, including the rare 3-methylglutamic acid, have been detected in meteorites.

1.5 Antibiotic Myths

The notion that the antibiotic activities of small molecules are used as competitive weapons is mentioned frequently, but is largely unproven. After all, there are many natural products, chemically related to the compounds used in the clinic that have no antibiotic activity and could not have been identified in conventional screens.

Similarly, there is a long-held belief that Streptomycetes and related spore-forming Actinomycetes produce the majority of useful antibiotics. This also, is not correct: the large family of *Actinobacteria* are possibly the most fruitful microbes in terms of small molecule production (Miao and Davies 2010). Even the Pseudomonads and Firmicutes produce many bioactive small molecules; not all have demonstrated antibacterial or antiviral (phage) properties but many of the compounds play roles in pathogenesis and in various signaling processes. The eukaryotes also have their champions: the Fungi are rich in small molecule production and chemical diversity and have been exploited extensively by the pharmaceutical industry.

If the truth be known it is probable that all microbes, prokaryotic and eukaryotic, produce bioactive small molecules that may exhibit antibiotic activity under certain conditions. One defining feature is that all of these products are made by large and often complex, tightly regulated biosynthetic pathways. The gene clusters vary considerably: That for tetracycline (see Genilloud and Vincente, this volume) is around 30 kB and for pristinamycin (see Kirst, this volume), more than 200 kB.

Is chromosomal DNA of high G+C composition a prerequisite for small molecule production? A number of microbes with low G+C content (Firmicutes such as Staphylococci) are known to make non-ribosomal peptides but, in general, genomes with higher G+C content appear to have the greatest potential for small molecule production. There could be a reason for this: GC-rich genomes might be considered more "ancient."

Considering the evolution of bioactive molecules without having a clear idea of their true biological roles is difficult: in most cases their small molecule productivity appears to endow no specific selective advantage to the producing host. What roles might they have played in biochemical evolution? Until exhaustive small molecule screening and genome mining have been employed to investigate the microbial world, such questions will remain unanswered.

1.6 Mode of Action and Evolution of Targets

Assuming that the majority of bioactive small molecules are ancient (possibly as old as amino acids), what types of selection pressure determined their evolution? And what can be said about the development of their complex biosynthetic pathways? The evolution of the biosynthetic pathways for molecules such as daptomycin, tetracycline, and other well-known antibiotics is of great interest (Fischbach et al. 2008; Ridley et al. 2008). It is easy to say that they are old, but how did these complex genetic systems evolve and over what period of time? There has been much speculation over the evolution of "simple" biosynthetic pathways such as those for the protein amino acids (Teichman et al. 2001).

What are the benefits of small molecules to the producing organism? There are countless microbial natural products that cannot be detected using conventional

screening approaches. What are the evolved functions for this large number of fascinating molecules?

With respect to the process of chemical evolution, low molecular weight compounds (monomers) are likely to be ancient and were used as precursors to generate more complexity: peptides followed amino acids (van der Gulik et al. 2009). The same is true for the evolution of complex organelles found in cells: ribosomes and cell walls, for example. It is now generally accepted that "early" RNA was a ribozyme and this was the precursor of the protein-rich ribosome and other catalytic RNA structures (Noller 2012).

One can imagine that the primordial synthesis of simple polymers, such as peptides/proteins required that small molecule effectors bound to the catalytic RNA and so facilitated polymerization reactions. Under certain conditions, protein synthesis inhibitors can actually enhance peptide bond formation. Similar in vitro studies may mimic the primordial catalytic reactions of RNA. In an RNA world, activities and binding sites for effectors on the RNA could eventually become the binding sites for antibiotic inhibition in ribosomes (Davies et al. 1992). This suggests that structural relationships exist between small molecule binding sites on pro- and eukaryotic organelles such as ribosomes or nucleic acid synthesis complexes. Non-ribosomal peptides may have played roles as catalysts of primitive reactions by binding to nucleic acid fragments and enhancing the activity of ribozymes. They might have evolved into site-specific binding functions that led to their subsequent activity as inhibitors. Similar evolutionary transitions might have resulted in the formation (or conservation) of small molecule binding sites on human and animal hormone receptors (Catnach and Fairclough 1992).

Modern-day antibiotics have been shown to have a wide range of biological activities depending on the concentrations used. This phenomenon, referred to as hormesis (low concentration: positive effect, high concentration: negative effect) probably applied to all bioactive molecules throughout evolution (Kendig et al. 2010). Hormesis is the key to identifying true biological activity. It can be assumed that primordial bioactive molecules appeared in the environment at low concentrations and interacted with different target molecules/structures at concentrations well below inhibitory levels (before defined biosynthetic pathways had evolved). Many relics of these reactions remain: binding of low concentrations of antibiotics to the translation system can, under some circumstances, stimulate peptide bond formation. The peptidyl transferase reaction can be enhanced by some antibiotics. The same is true for nucleic acid processes, that required ribozymes: small molecules could have modulated their activity.

1.7 Parallel Chemical and Protein Evolution

Antibiotic "evolution" during the past 60 years has essentially been a synthetic chemical process. Almost all drugs have undergone successive rounds of chemical remodeling in efforts to overcome the appearance of pathogens with acquired

resistant to the current generation of antibiotics. This has been a typical "catch-22" situation. The mechanisms of resistance have been well characterized: inactivation/destruction of inhibitor, protection of target, secretion from cell, and others. The best-studied and most dramatic example, that of the β-lactam antibiotics, has seen the evolution by mutation and selection of over 1000 β-lactamase enzymes, each with subtle variations in active site (see Leemans et al. this volume). This has occurred in response to rounds of chemical improvements of the penicillin and cephalosporin antibiotics (Bush and Jacoby 2010).

1.8 Conclusions

Microbial small molecules are ancient, huge in number, and diverse in structure and function. This brief overview of the origins of bioactive small molecules leaves many unanswered questions, particularly with respect to evolutionary mechanisms. What are the evolved natural functions for these fascinating molecules? A discussion of the evolution of these compounds without having a clear sense of their true biological roles is difficult. How many different roles might they have? They did not evolve to challenge chemists, amuse biochemists or microbiologists, or to cure diseases that were absent on the Earth before the advent of man.

The following are recommendations for future studies:

(a) Low molecular weight organic molecules played important roles in the evolution of the biology of the cell. A better understanding of these processes will lead to the identification of new receptors and compounds that bind to them.
(b) Harvesting the Parvome, using genome mining and heterologous gene cluster expression, will revolutionize the pharmaceutical industry. There is no shortage of novel compounds!
(c) Studies of small molecule activity should focus on cell–cell signaling rather than on antagonistic activities.
(d) Creative studies on the activities of bioactive small molecules in microbiomes will aid in the understanding of all aspects of health and disease.

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Chapter 2
Novel Infectious Diseases and Emerging Gram-Positive Multi-Resistant Pathogens in Hospital and Community Acquired Infections

Gian Maria Rossolini, Fabio Arena and Simona Pollini

Abstract Gram-positive pathogens are a major cause of healthcare-associated and community-acquired bacterial infections. Staphylococci (mostly *Staphylococcus aureus* but also coagulase-negative staphylococci), enterococci, streptococci, and *Clostridium difficile* are the most important species of clinical interest. Antibiotic resistance issues are common among Gram-positive pathogens, especially among staphylococci and enterococci. Methicillin-resistant *Staphylococcus aureus* (MRSA) and glycopeptide-resistant enterococci (GRE) are paradigms for difficult-to-treat multi-resistant pathogen capable of global-scale diffusion, with remarkable impact on morbidity, mortality, and healthcare-associated costs. MRSA, in particular, is the most relevant Gram-positive multi-resistant pathogen in terms of diffusion and overall clinical impact, being a leading cause for healthcare-associated infections worldwide, as well as an emerging cause of community-acquired infections that are often associated with novel MRSA strains. Resistance to anti-MRSA and anti-VRE drugs remains uncommon or exceptional among the respective species. However, invasive infections caused by MRSA strains resistant to glycopeptides, linezolid, or daptomycin, and by VRE strains resistant to linezolid or daptomycin have increasingly been reported, especially after prolonged

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drug exposure, and a transferable resistance mechanism to linezolid and other antiribosomal agents has recently emerged among staphylococci and enterococci. This evolving scenario underscores the need for continuing efforts aimed at surveillance and control of infections caused by multi-resistant Gram-positives, and at the discovery and development of new drugs active against these pathogens.

2.1 Introduction

Gram-positive bacterial pathogens remain a very common cause for healthcare-associated infections (HCAIs) and for community-acquired infections, and represent a major target for antimicrobial chemotherapy. The most important Gram-positives of clinical interest are staphylococci, enterococci, streptococci, and *Clostridium difficile*, although other species (e.g., *Listeria* and corynebacteria) may also play a role in some settings. The spectrum of infections caused by Gram-positives is very broad, including skin and skin structure infections (SSSIs), upper and lower respiratory tract infections, bloodstream infections (BSIs) and endocarditis, surgical site infections (SSIs), bone and joint infections, diabetic foot infections, central nervous system infections, urinary tract infections (UTIs), and intestinal infections. Central venous catheters and other artificial devices are also a common site for Gram-positive infections, mostly caused by coagulase-negative staphylococci.

Antibiotic resistance issues are common among Gram-positive pathogens, especially among staphylococci and enterococci. Methicillin-resistant *Staphylococcus aureus* (MRSA) and glycopeptide-resistant enterococci (GRE) are well-known paradigms of difficult-to-treat Gram-positive multi-resistant pathogens capable of global-scale diffusion, which have attained high proportions in several epidemiological settings (see below). Resistance problems remain overall lower with streptococci and other Gram-positives, although relatively high proportions of penicillin- and/or macrolide-resistant pneumococci are reported in many countries (EARS-Net 2010; Linares et al. 2010; Darabi et al. 2010).

The scope of this chapter is to provide an overview of the most important multi-resistant Gram-positive pathogens emerging as causes of HCAIs and community-acquired infections, i.e., MRSA and GRE, and to briefly discuss some aspects related with *Clostridium difficile* infection.

2.2 Methicillin-Resistant *S. aureus* as a Cause of Hospital- and Community-Acquired Infections

Among Gram-positives, methicillin-resistant *S. aureus* (MRSA) is by far the most relevant resistant pathogen, being a leading cause for SSSIs, BSIs, and hospital-acquired pneumonia (HAP) worldwide (Boucher and Corey 2008). MRSA strains have acquired a *mecA* gene encoding a peculiar penicillin-binding protein (PBP),

named PBP2a, which is not inhibited by methicillin, oxacillin, and other conventional β-lactams available for clinical use (see Leemans et al., this volume), and can take over the functions of the resident staphylococcal PBPs (Fuda et al. 2004). Thus, expression of PBP2a results in clinical resistance to those compounds (which are normally the first choice for treatment of *S. aureus* infections), and anti-MRSA antibiotics (which are often more toxic and expensive, see below) become the mandatory treatment option (Welte and Pletz 2010).

Overall, MRSA poses a global healthcare challenge affecting both industrialized and low-income countries. Proportions of MRSA infections can reach values higher than 50–60 % of *S. aureus* infections in some settings (Stefani et al. 2012), although with significant geographical and institutional differences which largely depend on the efficacy of infection control practices adopted at the nationwide or local level. In Europe, for instance, the proportion of MRSA among invasive isolates of *S. aureus* was reported to vary between 0.5 and 52 % in different countries, according to the most recent data from the EARS-Net surveillance system (EARS-Net 2010). In some countries (e.g., the United Kingdom and France) the enforcement of strict infection control strategies has apparently been successful in curbing the dissemination of MRSA in recent years (EARS-Net 2010; Johnson et al. 2012). However, MRSA proportions continue to be very high in several countries, and MRSA remains one of the leading multi-resistant pathogens in terms of clinical burden (EARS-Net 2010; Kock et al. 2010).

MRSA infections were originally detected in the 1960s (i.e., soon after introduction of methicillin in clinical practice) and their epidemiology has undergone significant evolution during the past decades. Initially, these infections emerged as typical hospital-acquired infections (HA-MRSA infections), and exhibited a remarkable ability at spreading both in acute-care hospitals and in long-term care facilities where strict infection control practices were not enforced (DeLeo and Chambers 2009; Kock et al. 2010). The mortality rate associated with invasive HA-MRSA infections varies considerably between different settings, but in some cases may exceed 50 % (Klevens et al. 2007; Kock et al. 2010). In the mid-2000s, in the United States, it was calculated that the yearly in-hospital mortality attributable to MRSA infections was overall comparable with the mortality associated with HIV/AIDS, viral hepatitis and tuberculosis taken together (Boucher and Corey 2008). A recent European study has confirmed the substantial clinical burden associated with MRSA BSIs in terms of mortality rates and length of hospital stay (De Kraker et al. 2011), underscoring the impact and the public health relevance of this resistant pathogen. HAP caused by MRSA also represents a major clinical challenge, with high mortality rates particularly in ventilated patients (Kollef et al. 2005; Welte and Pletz 2010). Since recent global-scale surveillance data indicate that *S. aureus* is the leading cause of HAP in the United States and Europe, being associated with approximately one-third to one-fourth of cases, respectively (Jones 2010), this further underscores the impact of MRSA in hospital-acquired infections of the lower respiratory tract. Spreading of HA-MRSA typically follows a clonal pattern. A limited number of very successful HA-MRSA clonal complexes (CCs) have disseminated internationally, with CC5 and

CC8 being the most prevalent worldwide and CC22, CC30 and CC45 being less frequently detected and limited to specific areas (Stefani et al. 2012).

More recently, MRSA infections have also emerged as community-associated (CA) infections (CA-MRSA infections) (DeLeo et al. 2010). Unlike HA-MRSA infections, CA-MRSA infections are often encountered among young and otherwise healthy subjects lacking the risk factors that are typically associated with HA-MRSA infections (i.e., long hospitalization periods, prolonged antimicrobial therapy, chronic cardiovascular, and pulmonary diseases, diabetes) (Liu et al. 2011). SSSIs are the most common presentation of CA-MRSA infections (approximately 90 % of all clinical manifestations), with many of them being mild to moderate (DeLeo et al. 2010; Skov et al. 2012). However, CA-MRSA may also cause severe infections, such as necrotising cellulitis or fasciitis and necrotising pneumonia, associated with high mortality rates (up to 75 % in case of necrotizing pneumonia) (Li et al. 2011). Noteworthy, most of the CA-MRSA strains involved in severe infections necrotising infections produce potent cytotoxins, such as the Panton–Valentine leukocidin, the a-hemolysin or the α-type phenol-soluble modulins, which are believed to play an important role in the pathogenesis of these infections (David and Daum 2010). CA-MRSA has experienced a remarkable diffusion in North America, while these infections have remained overall less common in Europe, although with an increasing trend (Otter and French 2010). CA-MRSA also disseminates with a clonal pattern, but a higher diversity has been observed in the population structure, with clonal complexes differing in different geographic areas and some being quite characteristic of specific areas or continents. For instance, while CC1 and CC8 are mostly detected among CA-MRSA from the United States and Canada, ST80 appears to circulate in Europe (DeLeo et al. 2010; Rolo et al. 2012). Unlike HA-MRSA strains, which usually exhibit complex multi-resistant phenotypes including non β-lactam agents (e.g., fluoroquinolones, macrolides, and lincosamides, see Leemans et al.; Pucci and Wiles; Kirst, this volume), CA-MRSA strains often remain susceptible to these drugs, and this peculiar resistance profile, together with the presence of certain classes of SCC*mec* elements carrying the *mecA* gene (e.g., SCC*mec*IV types and SCC*mec*V), have been regarded as biological markers for CA-MRSA strains (David and Daum 2010). However, in recent years, the spread of CA-MRSA clones in the hospital setting and the movement of typical HA-MRSA clones (such as CC5) in the opposite direction has increasingly been reported (Campanile et al. 2012; David and Daum 2010; Maree et al. 2007; Otter and French 2011; Song et al. 2011; Valsesia et al. 2010), blurring the original distinction between CA-MRSA and HA-MRSA infections and making typical CA-MRSA clones a potential cause for HA infections.

Since the early 2000s, livestock-associated (LA) MRSA infections in humans were also reported, caused by MRSA strains of CC398 which are commonly found among pigs and cattle (Crombe et al. 2012; Porrero et al. 2012; Schaumburg et al. 2012; van Cleef et al. 2011). LA-MRSA infections caused by CC398 strains have mostly been reported from Europe and only sporadically from Asia and the United States (Monecke et al. 2011). These infections appear to be common only in

individuals having frequent contact with livestock and living in geographical areas with high density of farms (van Cleef et al. 2011), and may range from mild SSSIs to severe infections such as BSIs, endocarditis, pneumonia, and necrotising fasciitis (Mammina et al. 2010; Soavi et al. 2010; van der Mee-Marquet et al. 2011). Recent studies indicate that LA-MRSA is not significantly spreading into hospital settings in Europe, and that invasive infections are quite uncommon (Grundmann et al. 2010; Wassenberg et al. 2011).

The most popular options for MRSA infections include vancomycin, teicoplanin (see Marcone and Marinelli, this volume), linezolid (see Zappia et al., this volume), and daptomycin (see Baltz, this volume). Tigecycline (see Genilloud and Vicente, this volume) is also very active against MRSA, while telavancin (a new lipoglycopeptide, see Marcone and Marinelli, this volume) and ceftaroline (a new cephalosporin endowed with high binding affinity to PBP2a, see Leemans et al., this volume) have been the most recent additions in the repertoire of anti-MRSA drugs. Moreover, a number of novel anti-MRSA agents of various classes are found at various developmental stages of the pipeline (e.g., dalbavancin, oritavancin, razupenem, omadacycline, and nemonoxacin) (Hait et al. 2011; Kihara et al. 2008; Li et al. 2010; Zhanel et al. 2010; see Marcone and Marinelli; Leemans et al.; Genilloud and Vicente; Pucci and Wiles, this volume).

Vancomycin and teicoplanin (see Marcone and Marinelli, this volume) are normally considered the first choice for infections caused by MRSA, although with some limitations related with slow bactericidal activity, potential toxicity (especially for vancomycin), and individual pharmacokinetic variability which mandates for therapeutic drug monitoring at least in severe infections (Liu et al. 2011). Despite an increased use in clinical practice since almost three decades (due to the global emergence of MRSA), resistance to glycopeptides has remained very uncommon among MRSA strains. In fact, *S. aureus* has evolved two mechanisms of glycopeptide resistance, of which one is mediated by chromosomal mutations that alter the cell wall structure and physiology limiting the access of glycopeptides to the D-ala-D-ala target in peptidoglycan precursors, while the other is mediated by acquisition of a *van* gene cluster which is responsible for the synthesis of modified peptidoglycan precursors with reduced affinity for glycopeptides. The former mechanism has been described since the late 1990s (Hiramatsu et al. 1997) and is associated with a moderate increase in MIC values (usually up to 4–8 mg/L for vancomycin, the so-called VISA phenotype) (Howden et al. 2010). In some cases the VISA phenotype is only expressed by a subpopulation in a background of susceptible bacterial cells (the so-called hVISA phenotype) (Howden et al. 2010). The emergence of VISA and hVISA strains appear to be typically associated with prolonged exposure to glycopeptides, and such strains are often recovered from patients with vancomycin treatment failure (Bae et al. 2009; Howden et al. 2010; Khatib et al. 2011). Indeed, isolates exhibiting the VISA phenotype have been identified belonging to many epidemic MRSA clonal lineages, including the hospital acquired ST5 and ST8 (Gardete et al. 2008; Hageman et al. 2008; Howe et al. 2004), but their overall proportions has remained low and significant epidemic diffusion has not been observed. Several mutations associated with the

VISA phenotype have been characterised (Gardete et al. 2012; Howden et al. 2010), and it has been demonstrated how the stepwise accumulation of mutations can lead first to the hVISA phenotype and that to a homogeneous VISA phenotype (Neoh et al. 2008). Noteworthy, mutations involved in the resistance phenotype can also be responsible for the repression of some virulence-related properties (such as the quorum sensing regulator Agr, the α-type phenol-soluble modulins, α-hemolysin and protein A), which may help the resistant bacteria to evade the host immune system (Gardete et al. 2012) but could also be associated with reduced fitness and poor in vivo survival (McCallum et al. 2006) accounting for the low propensity to epidemic diffusion exhibited by VISA strains.

Glycopeptide resistance mediated by acquisition of a *van* gene cluster is typically associated with higher MICs (vancomycin MICs are usually >16 mg/L; the so-called VRSA phenotype). This resistance mechanism was first detected in an MRSA strain isolated in 2002 in the United States (Bartley 2002) and raised considerable concern. However, only a few additional VRSA isolates have been reported thus far, including 11 isolates from the United States (Sievert et al. 2008, http://www.cdc.gov/HAI/settings/lab/vrsa_lab_search_containment.html), one from India (Saha et al. 2008) and 1 from Iran (Aligholi et al. 2008), showing no propensity to cross-transmission and epidemic diffusion, and in no case VRSAs were involved in severe bacteremic infections (most isolates were from infected ulcers or wounds, or simply colonizers). This was likely due to a fitness defect associated with the modified cell wall structure. In fact, competition experiments between an MRSA recipient of CC5 (a lineage prone to the acquisition of resistance traits) and its isogenic VRSA transconjugant revealed that, in the absence of vancomycin, the transconjugant had a significant fitness disadvantage (Kos et al. 2012). GRE were the most likely source of the *van* operon found in VRSA strains, as suggested by the similarity of their genetic contexts and by results of in vitro and in vivo transfer experiments (Perichon and Courvalin 2009). Indeed, in many cases of VRSA isolation, a GRE had also been co-isolated from the patient (Perichon and Courvalin 2009).

The most recent anti-MRSA drugs may offer advantages in terms of pharmacokinetic properties, clinical efficacy, and/or reduced toxicity and usually retain activity against glycopeptide non-susceptible MRSA strains (with the exception of daptomycin, which exhibit reduced activity against some VISA strains (Yang et al. 2010). Linezolid (see Zappia et al., this volume) is the most popular anti-MRSA option (in alternative to glycopeptides) due to oral bioavailability and improved clinical outcomes reported in some infections such as nosocomial pneumonia (Wunderink et al. 2012) and complicated SSSIs (Itani et al. 2010).

Linezolid resistance is still very uncommon among staphylococci, with susceptibility rates close to 100 % among MRSA, and slightly lower (98 %) among methicillin-resistant CNS (Flamm et al. 2012; Jones et al. 2009; Ross et al. 2011). Resistance to linezolid can be due to mutational modification of the ribosomal target (23S rRNA or L3 and L4 ribosomal proteins) (Long and Vester 2012), and in case of rRNA mutations can increase in a stepwise manner with the accumulation of mutated copies of the 23S rRNA genes in the bacterial chromosome

(Besier et al. 2008). This type of resistance has mostly been reported following prolonged exposure to the drug (e.g., in osteomyelitis or in cystic fibrosis patients (Benefield et al. 2012; Endimiani et al. 2011), while resistant strains do not exhibit significant propensity for cross-transmission and spreading (Long and Vester 2012). A transferable resistance mechanism to linezolid, mediated by ribosomal methylation via the plasmid-encoded Cfr protein, has also been detected in MRSA and in methicillin-resistant coagulase-negative staphylococci (Bongiorno et al. 2010; Bonilla et al. 2010; Long et al. 2006; Morales et al. 2010; Sanchez-Garcia et al. 2010). The ribosomal modification carried out by the Cfr protein is associated with resistance to several anti-ribosomal agents including phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A (the $PhLOPS_A$ phenotype), suggesting that Cfr production could be co-selected by different antimicrobial agents used both in clinical and in veterinary practice (Long et al. 2006; see Kirst; Zappia et al., this volume). The emergence of the *cfr* gene in MRSA is a matter of major concern, since Cfr-positive MRSA strains may exhibit high linezolid MICs (up to 64 mg/L) and their potential for cross-transmission and causing nosocomial outbreaks with invasive infections (e.g., ventilator-associated pneumonia and BSIs) has been documented (Morales et al. 2010; Sanchez-Garcia et al. 2010).

Also daptomycin resistance (see Baltz, this volume) is very uncommon among MRSA, although some VISA strains may exhibit reduced susceptibility to this drug. Resistance is achieved via accumulation of multiple chromosomal mutations contributing to the increase in MIC values (Mishra et al. 2009; Yang et al. 2009). Some of these mutations, affecting cell-wall thickness, are apparently involved in cross-resistance with glycopeptides and account for the reduction of daptomycin activity against VISA strains (Cafiso et al. 2012; Yang et al. 2009). However, mutations that alter the cell surface charge (e.g., mutations in *yycFG* and *mprF,* and mutations that upregulate the *dltABCD* operon) were also found to be associated with decreased susceptibility to daptomycin (Yang et al. 2009, 2010), underscoring the notion that resistance to daptomycin can be achieved by multiple mechanisms. Daptomycin-resistant MRSA strains are usually selected following prolonged exposure to the drug (e.g., in osteomyelitis and orthopedic prosthesis infections) (Enoch et al. 2007) and thus far have not shown propensity to cross-transmission and epidemic diffusion.

Resistance to telavancin (see Marcone and Marinelli, this volume) and ceftaroline (see Leemans et al., this volume) has not been reported from clinical infections. However, prolonged in vitro exposure of MRSA to subinhibitory concentrations of telavancin resulted in the selection of mutants with televancin MICs of 2 mg/L (Kosowska-Shick et al. 2009), while the presence of multiple mutations in PBP2a from some MRSA isolates can result in decreased binding affinity of ceftaroline, with increased MIC values (1–4 mg/L) (Mendes et al. 2012). Altogether, these findings suggest that resistance to these new molecules could arise by mutation in a stepwise manner.

2.3 Infections Caused by Glycopeptide-Resistant Enterococci

Enterococci are gut commensals that can act as opportunistic pathogens and are a leading cause for HCAIs including UTIs, BSIs and endocarditis, SSIs, complicated intra-abdominal infections, and infections of catheters and other medical devices (Malani et al. 2002). *Enterococcus faecalis* and *Enterococcus faecium* are the two most relevant species, although infections by unusual species, such as *Enterococcus gallinarum*, have occasionally been described (Contreras et al. 2008).

Enterococci are intrinsically resistant to many antibiotics and exhibit a remarkable ability to acquire resistance to anti-enterococcal agents. From the clinical perspective, the most important resistance issue is represented by acquired resistance to glycopeptides, which are the drugs of choice for enterococcal infections caused by ampicillin-resistant strains, which are now quite prevalent (Arias et al. 2012; EARS-Net 2010; Hidron et al. 2008).

Acquired glycopeptide resistance is due to the synthesis of a modified peptidoglycan target with reduced affinity to glycopeptides following the acquisition of a set of genes (*van* genes) that encode the several functions required for modified peptidoglycan biosynthesis (Reynolds and Courvalin 2005). Several variants of such gene clusters have been discovered (e.g., *vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, *vanN*) that can be associated with variable glycopeptide resistance phenotypes and are often carried on transposable elements such as *Tn1546* (Lebreton et al. 2011; Raynolds and Courvalin 2005; Sujatha et al. 2012; Xu et al. 2010; see Marcone and Marinelli, this volume).

Glycopeptide resistance in enterococci was originally reported in the late 1980s (Uttley et al. 1989) and has undergone a global diffusion during the past two decades, especially in *E. faecium*. In the United States, a remarkable dissemination of GRE has been observed, with proportions of up to 60 % reported among *E. faecium* isolates from BSIs (Deshpande et al. 2007). In Europe, the proportion of GRE is quite variable depending on the country (from 2 to 35 % for invasive isolates of *E. faecium*), and mixed trends (increasing or decreasing) have been reported in different countries (EARS-Net 2010 report).

Molecular epidemiology has identified a lineage of *E. faecium* belonging in CC17 as the leading cause of infections, and outbreaks caused by this pathogen have been reported worldwide (Willems et al. 2005). Strains of this lineage have adapted to the hospital niches and acquired virulence genes (e.g., esp_{Efm} and hyl_{Efm}) (Billström et al. 2008; Leavis et al. 2004), and are usually resistant to penicillins and often to glycopeptides.

Very few options (and not all of them approved) are available for treating infections caused by GRE, including linezolid (see Zappia et al., this volume), tigecycline (see Genilloud and Vicente, this volume), daptomycin (see Baltz, this volume), and quinupristin-dalfopristin (only for *E. faecium* strains, see Kirst, this volume).