Flavia Marinelli
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.

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Flavia Marinelli
Olga Genilloud

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Part I
Current Trends in Antibiotics, Pathogens and Medical Needs

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).

Resistance to linezolid, which is the most popular option for GRE infections, is still uncommon among enterococci but has been increasingly reported since 2002 (Auckland et al. 2002; Gonzales et al. 2001; Ntokou et al. 2012). Resistance is usually due to ribosomal target modification following mutations of the 23S rRNA or, less frequently, of the L3 and L4 ribosomal proteins (Prystowsky et al. 2001; Saager et al. 2008). Mutants are often selected from susceptible strains following prolonged drug exposure (Rahim 2003; Swoboda 2005), but linezolid-resistant strains have also been isolated from patients with no previous exposure to the drug, indicating that these strains have some potential for cross-transmission and dissemination even in the absence of antibiotic pressure (Schutle et al. 2008). Most recently, the plasmid-encoded Cfr ribosomal methylase, which mediates transferable resistance to linezolid and other anti-ribosomal agents in staphylococci (see above), has also been detected in a linezolid-resistant strain of *E. faecalis* isolated from a patient that had received long-term linezolid treatment (Diaz et al. 2012). This observation highlights that transferable linezolid resistance mediated by Cfr could also spread among enterococci.

Enterococcal strains non susceptible to daptomycin remain relatively rare but have been reported since 2003, mostly among GREs (Kelesidis et al. 2011). Resistance can occur either in isolates exposed to prolonged drug treatment or in isolates from patients with no previous exposure to daptomycin (Lesho et al. 2006; Kelesidis et al. 2012), suggesting the possibility of cross-transmission and dissemination even in the absence of antibiotic pressure. Various mutations, either in a regulatory system involved in the cell envelope response to antibiotics (liaFSR) or in genes encoding proteins involved in phospholipid metabolism have been associated with daptomycin resistance (Arias et al. 2011; Munita et al. 2012; see Baltz, this volume), but the mechanism of resistance remains unclear. Currently, there are no known transferable determinants that confer resistance to daptomycin.

Concerning tigecycline (see Genilloud and Vicente, this volume), resistance is very uncommon among enterococcal isolates (Bérenger et al. 2011; Hope et al. 2010; Zhao et al. 2012). However, emergence of strains with increased tigecycline MICs during therapy has been occasionally documented (Werner et al. 2008), and the recent detection of enterococcal isolates with reduced susceptibility to tigecycline in different reservoirs, including animals for food consumption, suggests that selection of tigecycline-resistant isolates by antibiotics other than tigecycline might occur in non-clinical settings (Freitas et al. 2011).

2.4 Issues with *Clostridium difficile* Infections

Clostridium difficile infection (CDI) is a leading cause of nosocomial diarrhea and one of the most relevant HCAIs worldwide, with a significant burden on inpatients morbidity and mortality (Miller et al. 2011). During recent years, CDI has shown increasing trends in incidence and severity in many countries. For instance, data from US vital records indicate that the number of death certificates with

enterocolitis due to *C. difficile* listed as the primary cause of death increased from 793 in 1999 to 7483 in 2008 (Lessa et al. 2012). This dramatic increase in the incidence and severity of *C. difficile* infections has largely been attributed to emergence and global spread of hypervirulent epidemic strains, such as BI/NAP1/027 (McDonald et al. 2005). Dissemination of these strains has apparently been promoted, at least in part, by the overuse of some very popular drugs, such as the fluoroquinolones (see Pucci and Wiles), to which these hypervirulent strains are resistant.

Resistance to first-line drugs for CDI, including metronidazole for treatment of mild to moderate cases and vancomycin for treatment of severe episodes, has been reported but remains rare and is not regarded as a major clinical problem (Huang et al. 2009; Shah et al. 2010). However, a reduced response to these standard treatments and a relatively high incidence of recurrences (up to 20–25 %) have been reported (Louie et al. 2011; Tenover et al. 2012).

Rifaximin is a non-absorbable rifamycin derivative characterized by potent activity against *C. difficile* (Hecht et al. 2007; Shah et al. 2010), considered as an alternative regimen in refractory CDI and in recurrences after successful treatment with vancomycin. Recently, it has been suggested that rifaximin could also be considered as a first-line agent for mild CDI cased (Rubin et al. 2011). Resistance to rifamycins, which occurs by mutational amino acid substitutions in the β-subunit of the bacterial RNA polymerase, is overall uncommon but has occasionally been reported at high rates (Curry et al. 2009; Huang et al. 2009), and the possibility of resistance should be considered especially in patients previously exposed to rifampin or rifaximin (O'Connor et al. 2008).

Fidaxomicin is a new macrocyclic antibiotic that targets RNA polymerase, specifically developed for treatment of CDI. Fidaxomicin has potent activity against *C. difficile*, including "hyperepidemic" strains, while exhibiting a narrow spectrum of activity with low interference on the commensal microbiota and reaching high concentrations in the gut in absence of systemic absorption (Baines et al. 2008). Resistance to fidaxomicin has been described after in vitro exposure (Baines et al. 2008), but was found to be very uncommon in clinical trials (Goldstein et al. 2011). Resistance associated with mutations in *rpoB* and *rpoC* genes, encoding the β and β' subunits of bacterial RNA polymerase respectively, but these mutants are not cross-resistant to rifamycins nor cross-resistance with fidaxomycin has been reported for rifampin-resistant mutants (Babakhani et al. 2011; Baines et al. 2008).

2.5 Conclusions

Microbial drug resistance has become a public health problem of global dimension. Resistance issues affect both Gram-positive and Gram-negative pathogens. Although multi-resistant Gram-negatives are now emerging as a major clinical challenge due to the dramatic shortage of new treatment options available against

these pathogens, the overall burden caused by multi-resistant Gram-positives, and of MRSA in particular, remains of primary importance. In fact, MRSA rates continue to be high in most settings, and infections caused by MRSA and VRE strains resistant to the principal backup drugs (linezolid, daptomycin and—for MRSA—glycopeptides) have increasingly been reported. This dynamic resistance scenario, together with the ability of MRSA to evolve different epidemiological patterns (e.g., CA-MRSA and, most recently, LA-MRSA) 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.

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