Douglas L. Mayers · Jack D. Sobel
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Dror Marchaim *Editors*

Antimicrobial Drug Resistance

Clinical and Epidemiological Aspects, Volume 2

Second Edition

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Preface

Antimicrobial drug resistance is a global health problem that continues to expand as micro-organisms adapt to the antibiotics we use to treat them and as new classes of antimicrobial agents have been harder to discover and advance into the clinic. The second edition of *Antimicrobial Drug Resistance* grew out of a desire by the editors and authors to provide an updated, comprehensive resource of information on antimicrobial drug resistance that would encompass the current information available for bacteria, fungi, protozoa, and viruses. The two volumes have been extensively revised with many new authors and chapters as the field of drug resistance has evolved. We believe that this information will be of value to clinicians, epidemiologists, microbiologists, virologists, parasitologists, public health authorities, medical students, and fellows in training. We have endeavored to provide this information in a style that is accessible to the broad community of persons who are concerned with the impact of drug resistance in our clinics and across broader global communities.

Antimicrobial Drug Resistance is divided into two volumes. Volume 1 has sections covering a general overview of drug resistance and mechanisms of drug resistance, first for classes of drugs and then by individual antimicrobial agents, including those targeting bacteria, fungi, protozoa, and viruses. Volume 2 addresses clinical, epidemiologic, and public health aspects of drug resistance, along with an overview of the conduct and interpretation of specific drug resistance assays. Together, these two volumes offer a comprehensive source of information on drug resistance issues by the experts in each topic.

We are very grateful to the 197 international experts who have contributed to this textbook for their patience and support as the work came together. The editors would like to especially thank Michelle Feng He for her exceptional support and encouragement to the editors in bringing this revised textbook to print. Finally, the book would never have been completed without the patience and support of our wives and families.

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Part VII

Gram Positive Bacterial Drug Resistance: Clinical

Resistance in *Streptococcus pneumoniae*

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1 Introduction

Streptococcus pneumoniae (the pneumococcus) has been an important human pathogen for over 100 years and continues to cause a wide variety of infections, ranging from mild otitis media and sinusitis to serious lower respiratory infections, as well as life-threatening invasive infections such as meningitis or pneumonia. Worldwide, morbidity and mortality due to pneumococcal infections are highest among young children below the age of 5 years, accounting for approximately one-third of the estimated 1.3 million deaths from pneumonia in 2011 [1]. The pneumococcus is a common colonizer in the respiratory tract, especially in the nasopharynx of children where it is often exposed to antimicrobials. As well as affecting the young, *S. pneumoniae* is an important cause of morbidity and mortality in the elderly; it is the most common etiological agent of community-acquired pneumonia, often resulting in hospitalization of previously healthy individuals.

Infections caused by *S. pneumoniae* were for many years traditionally treated with penicillin or ampicillin, to which this species was exquisitely sensitive when penicillin was first introduced in the 1940s. However, exposure to antimicrobials has led to the selection of resistant strains and clones, some of which have a global distribution; resistance, which was first seen in the 1960s, has continued to increase throughout the world in more recent decades. The emergence of resistance to penicillin and other beta-lactam antibiotics in pneumococci in the 1980s and 1990s led to the increased use of macrolides, fluoroquinolones (FQs), and other non-beta-lactam antibiotics for pneumococcal infections. Pharmacodynamics predicts that high doses of parenteral β-lactams with good Gram-positive activity will currently treat most penicillin-resistant pneumococci. In contrast, oral β-lactams may fail, and high doses of amoxicillin are the best oral β-lactam drugs currently available to treat penicillin-resistant infections. Neither oral nor parenteral macrolides are able to treat macrolide-resistant pneumococcal infections. Fluoroquinolone resistance remains rare, but in patients previously exposed to these drugs, there is an increased risk of treatment failure due to selection of first-step mutants. Efforts to treat pneumococcal disease in both adults and children have been complicated by this increasing resistance to antimicrobials. The increase in antimicrobial resistance rates has been in part due to the selective pressures associated with the widespread use of antibiotics [2, 3] and the clonal expansion and spread of multiresistant pneumococcal clones [4]. More recently, the introduction of conjugate vaccine immunization of infants reduces the burden of resistance by eliminating vaccine types from invasive infections, but resistance continues to be selected in non-vaccine types. New classes of antimicrobials are needed to ensure long-term treatment options for pneumococcal infections.

This chapter will describe the emergence and incidence of antibiotic resistance in pneumococci, mechanisms, clinical implications, and impact of vaccines on resistance.

2 Epidemiology of the Pneumococcus and Risk Factors for Resistance

The incidence of pneumococcal disease remains the highest in children <2 years of age and in adults >65 years of age. Other important risk factors include underlying medical conditions such as chronic heart and lung disease, cigarette smoking, and immunodeficiency states such as asplenia, HIV, and sickle cell disease.

S. pneumoniae colonizes the upper respiratory tract and is part of the normal flora of healthy individuals. Pneumococcal colonization is a dynamic process. A particular serotype can be carried for many months before being eradicated or replaced by a different serotype. Carriage increases in the

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first few months of life, and universally, carriage rates are highest in young children (40–60 %), compared with older children (12 %), adolescents (6–10 %), and adults (3–4 %) [5, 6]. Factors associated with increased carriage include winter season, situations of overcrowding such as day-care attendance, and living in crowded conditions [7]. Prior antibiotic use does not appear to alter the rate of carriage but does promote carriage with antibiotic-resistant strains, particularly to β-lactam antibiotics [8].

Investigations of serotype prevalence from various parts of the world have shown that serotype distribution varies with geographical location and age [9]. The distribution of serotypes also varies between carriage isolates and invasive disease, and antibiotic resistance (at least in the pre-conjugate vaccine era) is most frequent in pneumococcal serotypes that are carried by children (types/groups 6, 9, 14, 19, and 23) [9]. The probable reason is the frequent use of antibiotic therapy in small children and hence exposure of strains of these serotypes to antimicrobial drugs, providing a selective advantage to resistant mutants [10].

There are multiple risk factors for acquisition of infection with antibiotic-resistant pneumococci. Most of these factors have a commonality in exposure to the drugs that select the resistance. This exposure to β-lactams can be at the level of a country [11, 12], province [13], day care [14], family [15], or individual [16]. Macrolide resistance is also a function of exposure, particularly of long-acting drugs such as azithromycin [17]. The selection of resistant strains is complicated by multiple resistances where macrolides appear to be better selectors of multiresistant strains than do β-lactam drugs [16]. Antimicrobial resistance may be seasonal, with higher rates detected during increased antibiotic use in the winter months [18]. The issue of cross-resistance extends to treatment of such diverse organisms as the malaria parasite, where treatment with Fansidar selects trimethoprim-sulfamethoxazole resistance in pneumococci [19].

Most resistance is selected as mentioned above in children, but the exception is fluoroquinolone resistance which is selected in adults [20–22] as these agents are not usually given to children. In the unusual circumstance of fluoroquinolone treatment of children, for example, as treatment of drug-resistant tuberculosis, the selection of fluoroquinolone-resistant pneumococci occurs, and these strains are associated with invasive disease [23].

Children in rural settings generally have less access to antibiotics and therefore have less resistant strains [24, 25], while in some large cities, where poorer children live in the city center with less access to care and more affluent children live in the suburbs, there may be more resistance outside the city [26].

Little is known about the impact of drug dose on the selection of resistant strains, but there is a prospective study that suggests that high dose and short duration of amoxicillin therapy may select less resistance than the same total dose given over a longer period of time [27].

Nosocomial acquisition is a major risk for resistant pneumococci [28], and the first multiply resistant strains were selected in hospital [29]. Recent hospitalization is also a risk for infection with multiply resistant pneumococci [25].

HIV infection is a risk for increased resistance in pneumococcal infections due to the frequent exposure of these patients to antibiotic prophylaxis with trimethoprim-sulfamethoxazole [30], as well as the fact that these patients, especially HIV-infected women, are at risk due to the antibiotic-resistant serotypes carried by children [31].

Children exposed to conjugate vaccine, as well as adults living in countries where these vaccines are routinely administered to children, are at lower risk for pneumococcal infections due to resistant strains as described in Sect. 10.

3 The Role of Clones in Resistance

The increase in antibiotic resistance and the introduction of conjugate vaccines have focused attention on the epidemiology of *S. pneumoniae*. Molecular typing data from numerous studies over the past few decades has added to our knowledge by showing that although there is considerable diversity among resistant strains within most serotypes, a small number of highly successful clones have emerged within countries and in some cases have achieved massive geographical spread [4]. In response to this, the Pneumococcal Molecular Epidemiology Network (PMEN) was established in 1997 with the aim of standardizing nomenclature and classification of pneumococcal clones worldwide. At present, PMEN has documented 43 international clones, 26 of which are multidrug-resistant. The best characterized, and most widely spread of these international clones, is the Spain^{23F}-1 or PMEN1 originally described in Spain during the 1980s. Intercontinental spread of this clone to the USA was described in 1991 and shortly thereafter in the UK, South Africa, Hungary, and South America [32]. By the late 1990s, it was estimated that approximately 40 % of penicillin non-susceptible pneumococci circulating in the USA were members of this clone [33], and while strains belonging to this genotype continue to be isolated today in many countries all over the world, their prevalence has decreased in countries where conjugate vaccines have been introduced [34, 35]. Recent studies [32, 36, 37] looking at whole genome sequencing of pneumococci representing PMEN1 show that there is a considerable amount of genetic diversity within this lineage. This diversity, which largely results from hundreds of recombination events, indicates rapid genomic evolution and presumably allowed rapid response to selective pressures such as those imposed by vaccine and antibiotic use [36].

Clonal analyses of large surveillance collections of pneumococci have revealed the remarkable dominance of a small number of clones among the antimicrobial-resistant population. As these global clones have spread, they have been exposed to new selective pressures applied by regional variations in the use of different antibiotics. This has led to the further selection of strains belonging to these clones with varying antimicrobial resistance patterns. These resistant clones have also been exposed more recently to conjugate vaccines, and shifts in both serotype and clonal types have been documented [34, 35, 38]. For example, in the USA serotype 19A strains have been identified as the main cause of serotype replacement in both carriage and invasive disease post-PCV7 introduction; this has coincided with a significant increase in penicillin resistance and multidrug resistance among 19A clinical strains [34, 35, 39]. The majority of penicillin-resistant 19A strains belonged to emerging clonal complex 320 (CC320), which is descended from multidrug-resistant Taiwan^{19F}-14 (PMEN14). In 1999, prior to PCV7 introduction, only CC199 and three minor clones were apparent among 19A strains. In 2005 post-PCV, 11 clonal complexes were detected, including ST695 capsular variants of serotype 4 [38, 40].

4 Laboratory Detection of Resistance

Even though we can now identify pneumococci and many resistances based upon genetic features, bacterial culture and phenotypic susceptibility tests remain the gold standard approaches in clinical laboratories. Because it is a fastidious organism, however, specific methods and interpretative criteria developed by a variety of professional bodies such as the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards, NCCLS), the British Society for Antimicrobial Chemotherapy (BSAC), and the European Committee on Antimicrobial Susceptibility testing (EUCAST) must be used to ensure accurate and consistent susceptibility results [41]. Because the breakpoints are determined on the basis of microbiological, pharmacological, and clinical outcome data and since patterns of resistance to antimicrobial drugs continue to evolve, changes to breakpoints can occur during the lifetime of an antibiotic. A good example is the CLSI revised breakpoints for penicillin adopted in January 2008 to redefine the susceptibility of meningeal and non-meningeal pneumococcal isolates [42].

Culture of clinical specimens and antibiotic susceptibility testing are often slow, taking up to 48 h, and are often negative due to prior antibiotic use before sampling or autolysis of the organism. Rapid tests, based mainly on immunological or molecular techniques, have gained importance for the detection of bacteria and antibacterial resistance over the last two decades. PCR has been shown to be a useful tool for the rapid identification of *S. pneumoniae* from both clinical specimens and bacterial isolates [43, 44]. The increased use of molecular tests such as PCR for the diagnosis of bacterial infections has led in turn to an increased demand for antibiotic susceptibility testing using molecular methods. However, unlike phenotypic testing for antibiotic susceptibility, which examines all resistance mechanisms for a particular antibiotic simultaneously, molecular testing can detect only known resistance mechanisms. A variety of assays has been described to detect the presence of specific resistance genes in pneumococcal isolates and also directly from clinical specimens [44–50]. The majority of these assays are PCR based [44–47], although sequencing approaches and microarrays have also been used [49, 50].

5 Resistance to β-Lactams

With the advent of penicillin G therapy in the 1940s, the case fatality rate for pneumonia fell dramatically [51]. Pneumococcal isolates were initially extremely sensitive to the drug with MICs of ≤0.01 mg/L. Penicillin resistance was demonstrated in laboratory mutants soon after the introduction of penicillin G into clinical use but was not reported in clinical strains until 20 years later when investigators in Boston reported penicillin resistance in 2 of 200 strains [52]. Initially, the observation was not considered relevant, until a report by Hansman and Bullen [53] describing a penicillin-resistant strain (MIC 0.6 mg/L) isolated in Australia from the sputum of a patient with hypogammaglobulinemia. Subsequently, resistant strains were identified in New Guinea and Australia, and in 1974, the first clinical infection due to a penicillin non-susceptible strain was reported in the USA [54, 55]. In 1977, pneumococci resistant to penicillin began to appear in South Africa, and in 1978, the first multidrug-resistant pneumococci were documented in Johannesburg, South Africa [29, 56]. In between and after these initial reports, detection of penicillin-resistant pneumococci among clinical isolates began to be reported with increasing frequency in the clinical and microbiological setting. Today, penicillin-resistant strains are encountered in all countries in which adequate surveys are conducted. Recombination appears to be an essential mechanism in the evolution of beta-lactam resistance in nature, and resultant clonal spread of resistant strains plays an enormous role in the increase in beta-lactam resistance globally [4].

β-lactam antibiotics inhibit the growth of pneumococci by inactivation of cell wall synthesizing penicillin-binding proteins (PBPs). β-lactam resistance in pneumococci occurs by alterations in the key cell wall PBPs and the creation of *pbp* genes with decreased affinities for these antimicrobials. Six PBPs have been identified in *S. pneumoniae* (PBPs 1a, 1b,

2a, 2b, 2x, 3), of which PBP2X and PBP2B have been confirmed to be essential for cell growth [57, 58]. Resistance to β-lactams is complex and involves a multifactorial process. Depending on the selecting β-lactam, different combinations of *pbp* genes and mutations within these *pbp* genes are involved in conferring resistance. Little data exist for the role of PBPs 1b, 2a, and 3 [59, 60] as resistance determinants, and altered PBPs 2x, 2b, and 1a are the major players in the development of β-lactam resistance in most clinical isolates. The altered PBPs are encoded by genes with a mosaic structure and can undergo inter- and intraspecies recombination so that parts of the genes are replaced by allelic variants that differ by up to 20 % in DNA sequence [61]. Mosaic sequences of *pbp* genes are very difficult to classify and organize. In general, the resistance profile of particular isolates results from interactions between various combinations of altered PBPs, in conjunction with a functional *murMN* operon which encodes enzymes involved in the synthesis of branched structured muropeptides. Several other genes have been implicated in β-lactam resistance in selected clinical isolates that contribute to resistance in addition to mutations in PBP genes [61], although certain combinations of these three altered PBP genes alone appear to confer resistance.

Resistance to penicillin is associated with some degree of non-susceptibility to all β-lactam antibiotics. Mutations in PBP2x confer low-grade penicillin resistance and may be sufficient for the cell to become non-susceptible to oral cephalosporins. Alterations in PBP2b result in even higher MICs to penicillin [62], while changes in PBP1a are required for high-level penicillin resistance [60, 63] and extended-spectrum cephalosporin resistance [64, 65]. Isolates with very high levels of penicillin resistance (MICs ≥ 8 mg/L) require changes in all three PBPs (i.e., 1a, 2b, and 2x) and sometimes in additional non-PBP resistance determinants such as MurM [66].

Resistance rates reported for amoxicillin are relatively low (<5 %) as a result of the favorable pharmacodynamic properties of this agent [67, 68]. Generally, MICs to amoxicillin are equal to or two to four times less than the MIC of penicillin [69]. In the past, there have been numerous reports of strains with amoxicillin MICs (4–16 mg/L) higher than penicillin MICs (2–8 mg/L) [68, 70–72]. In particular, PBP2b appears to play a significant role in mediating the expression of this resistance phenotype [73]. In addition to typical changes in PBP1a and PBP2x, these strains have unique mutations in the 590–641 region of the PBP2b gene in close proximity to the active binding site [68, 72, 73].

Resistance to cephalosporins may develop with mutations in the pbp1a and pbp2x genes, and the close linkage of these two genes on the chromosome is conducive to the transfer of both genes in a single transformation step [64, 74]. PBP2b is not a target for cephalosporins so would remain unaltered in isolates expressing cephalosporin resistance and susceptibility to penicillin [75]. Most, but not all, extended-spectrum cephalosporin-resistant strains are also penicillin-resistant, and as with amoxicillin, the MICs of cefotaxime and ceftriaxone are usually lower than the MICs of penicillin. Newer antibiotics such as ceftaroline and ceftobiprole appear to be more active and have greater affinity for altered *pbp* genes allowing it to be active against strains with elevated MICs to other β-lactams [76, 77]. In the early 1990s in the USA, pneumococci with high-level cefotaxime and ceftriaxone (2–32 mg/L) resistance were detected [78], and this high-level resistance was due to alterations in PBPs 1A and 2X [65]. The cephalosporin MICs were in excess of the MICs of penicillin for these isolates, and specific point mutations ($Thr_{550}Ala$) in the *pbp2x* gene were associated with this phenotype [65]. These cephalosporin-resistant strains emerged within a few preexisting clones and demonstrate that point mutations as well as recombinational events are important in the development of resistance to β-lactam antibiotics in pneumococci.

6 Resistance to Macrolides

The macrolides have been used extensively to treat community-acquired respiratory tract infections worldwide, and in recent years, resistance to macrolide antibiotics (e.g., erythromycin, clarithromycin, and azithromycin) in *S. pneumoniae* has escalated dramatically. Macrolide-resistant *S. pneumoniae* are now more common than penicillin-resistant *S. pneumoniae* in many parts of the world [79]. However, both macrolide resistance rates and resistance mechanisms may vary considerably depending on location [80]. Erythromycin resistance rates range from about 15 % in Latin America to as high as 80 % recorded among isolates in Far East [81], and these differences probably reflect, in part, the variation in antibiotic prescribing behavior between different countries.

Macrolide resistance in *S. pneumoniae* is mediated primarily by two mechanisms: target modification and active efflux. The most common form of target modification is usually the result of dimethylation of the adenine residue at position 2058 on the 23S rRNA by a methylase enzyme [82]. This mechanism confers constitutive high-level resistance (MIC, >256 mg/L) to 14-, 15-, and 16-member macrolides, lincosamides, and streptogramins B, the so-called MLS_B phenotype. In *S. pneumoniae*, methylation is *erm*(B) mediated in almost all cases, although, more rarely, a methylase encoded by *erm*(A) subclass *erm*(TR) has been implicated [83]. Target modification by point mutations in domain II and V of 23S rRNA and in the genes encoding riboproteins L4 and L22 can also confer macrolide resistance and has been documented in clinical isolates from widely distributed global sites [84–86].

In certain countries, such as the USA [87], active efflux is the major mechanism for macrolide resistance. It confers low-level resistance (MIC, 1–16 mg/L) to 14- and 15-member macrolides but not to 16-member macrolides, lincosamides, and streptogramin B and is phenotypically referred to as the M phenotype. Active efflux is encoded by *mef*-class genes, which include several variants, the abundant *mef*(A) and *mef*(E), which share 90 % sequence identity, and the rare variant *mef*(I) which has only been described in two Italian clinical strains [88].

In pneumococci the three subclasses of *mef* are carried on a number of similar but distinct genetic elements. *mef*(A) is located on the defective transposon Tn*1207.1* or the closely related Tn*1207.3* [89], whereas *mef*(E) is typically carried on the mega (macrolide efflux genetic assembly) element [90]. The *mef*(I) gene exhibits 91.4 and 93.6 % homologies to the *mef*(A) gene of Tn*1207.1* and the *mef*(E) gene of the mega element, respectively [88], and is carried on a nonmobile composite structure, designated 5216IQ complex [91].

Worldwide *erm*(B) and *mef* (A or E) mechanisms account for the majority of macrolide resistance among pneumococci, and the prevalence of these genes varies considerably among countries. In recent years, the presence of both the *erm*(B) and the *mef* genes in *S. pneumoniae* clinical isolates has been increasingly recognized, particularly in Asian countries but also in Europe, S. Africa, and the USA [92, 93]. The PROTEKT study reported a 12 % global prevalence of macrolide-resistant isolates positive for both *erm*(B) and *mef*(A) in 2003–2004 [81].

The majority of dual-positive isolates exhibit multidrug resistance and are clonal lineages of Taiwan^{19F}-14, mostly multilocus sequence type 320, 271, and 236 [4, 92–94]. It appears that the global increase in macrolide-resistant strains carrying both the *erm*(B) and *mef* genes is being driven in part by the diversification and expansion of this Taiwan^{19F}-14 clone following conjugate vaccine introduction. This was especially true of the major 19A ST320 variant in the USA, which became the single most common IPD causing genetic complex in the USA prior to PCV13 implementation.

7 Resistance to Fluoroquinolones

Due to the increased rates of resistance to β-lactam and macrolide antibiotics among pneumococcal strains, fluoroquinolones (FQs) are now included among the choices for first-line therapy in clinical guidelines for the treatment of respiratory tract infections and pneumonia. A direct correlation between the use of FQs and the prevalence of resistance in *S. pneumoniae* has been described [95–97]; however, despite the increased use of FQs, the resistance of *S. pneumoniae* to the newer members of the family is uncommonly found. Reports from Europe, the USA, and Canada showed levels of resistance to levofloxacin and moxifloxacin below 2 % [95–97]. Three major events may have contributed to this low level of resistance: the replacement of the old FQ ciprofloxacin by the more active levofloxacin and moxifloxacin, the introduction of the pneumococcal conjugate vaccine, and, probably, the fact that children who are the main reservoir of pneumococci are not generally treated with FQs. This is supported by a recent study from South Africa showing a rise in FQ resistance in pneumococci isolated from children treated with FQ due to MDR tuberculosis [98]. In countries that report increasing incidence of resistance, the proportion of resistant isolates is much higher among older subjects and patients with chronic lung disease, a patient population that is frequently exposed to FQ [99].

Two mechanisms that decrease susceptibility to FQs in pneumococci have been identified: target alteration and reduced accumulation due to efflux. Resistance associated with target modification requires a combination of mutations in the quinolone resistance-determining region (QRDR) of the genes encoding the DNA gyrase and DNA topoisomerase IV subunits. First-step mutants generally result from spontaneous mutations in the preferential target for a given FQ, ParC for ciprofloxacin, and levofloxacin or GyrA for moxifloxacin, gatifloxacin, and gemifloxacin [100, 101]. Some isolates with a first-step mutation in *par*C gene have ciprofloxacin MICs that would indicate they are clinically susceptible (MIC, <4 mg/L) and these strains would not be identified using routine antibiotic susceptibility testing [102]. The population of isolates with first-step mutations is important because, compared with strains without these first-step mutations, they are more likely to develop high-level resistance during therapy with the acquisition of a second-step mutation [103, 104]. In the second-step mutants, amino acid substitutions are present in both topoisomerase IV and gyrase, most frequently affecting ParC and GyrA and less so ParE and GyrB [105].

Several mutations have been described in these enzymes, but only a few have been shown by in vitro studies to confer resistance: S81F or Y, C, or I and E85K in *gyr*A; E474K in *gyr*B; A63T, S79F, or Y or L and D83G or N in *par*C; and E474K and D435N or H in *par*E [100, 106]. Other frequently described mutations are K137N in *par*C and I460V in *par*E, which appear to not contribute to FQ resistance because they are commonly found in susceptible strains, and no evidence exists for their conferring FQ resistance in vitro [107]. A Q118K in *gyr*A together with S79F in *par*C in a FQ-resistant isolate resulted in treatment failure [108].

Another mechanism underlying non-susceptibility to FQs in some pneumococcal isolates is an increase in active efflux which affects quinolones such as ciprofloxacin [109]. In contrast to the *mef*A gene conferring macrolide resistance, the efflux mechanisms in FQ resistance are poorly characterized and have primarily been demonstrated in isolates with low-level quinolone resistance [101]. They are not encoded by

resistance genes but are thought to be overexpressed in 8–45 % of pneumococcal strains [110]. Little is known about the mechanism of the expression regulation of PmrA, but the efflux pump can be blocked by the plant alkaloid reserpine and, to a lesser degree, by verapamil [111]. Efflux may not confer complete resistance but may be able to lower intracellular FQ to sublethal concentrations, fostering the occurrence of QRDR mutations [112].

In contrast to β-lactam resistance, horizontal gene transfer and the role recombination plays in the evolution of FQ resistance are uncertain. Both intra- and interspecies transfers of FQ resistance loci have been found to occur in vivo, but the frequency of such events appears to be rare. In vitro models report a higher frequency for recombination of QRDRs between viridans group streptococci and *S. pneumoniae* compared to that of spontaneous mutations [113]; however, this level of recombination does not appear to be replicated in vivo [114]. Published studies addressing this question of recombination found evidence for horizontal gene transfer in 0–11 % of FQ-resistant isolates, and interestingly, this ratio seems to be higher in respiratory isolates than in invasive isolates [115–118].

Fluoroquinolone resistance has been reported in a number of international pneumococcal clones that have been associated with the evolution of resistance to penicillin and macrolides [119, 120]. However, the role that clonal spread plays in the increase of FQ resistance is controversial, with studies placing different significance on its importance. The increased prevalence of levofloxacin resistance that was reported from Hong Kong between 1995 and 2001 was suggested to be associated with the dissemination of strains related to the Spain^{23F}-1 clone. However, several studies have shown that clonal spread does not play a significant role in the increase of FQ resistance [120–122]. Data on levofloxacin-resistant pneumococci from 25 countries analyzed as part of the PROTEKT study (1999–2000) showed the majority were genetically unrelated, although 34 % belonged to the Spain^{23F}-1 clone [120]. These studies suggest that both clonal dissemination and the emergence of newly resistant strains contribute to the spread of FQ resistance.

8 Resistance to Newer Classes of Antibiotics

Telithromycin was the first ketolide drug approved for clinical use; however, safety issues have limited the clinical utility of this drug [123]. Both cethromycin (ABT-773) and solithromycin (CEM-101), a novel fluoroketolide, have shown improved activity against macrolide-resistant as well as telithromycin-intermediate and telithromycin-resistant organisms [124–126]. This enhanced potency shows promise for future clinical use for these compounds, subject to pharmacokinetic/pharmacodynamic, toxicity, and animal infection model studies. High-level telithromycin resistance in *S. pneumoniae* has been experimentally generated by mutations in domain II or V of 23S rRNA gene and ribosomal proteins L4 and L22 [127] and is easily created from a macrolide-resistant strain by the deletion or mutation of the region upstream of *erm*(B) [128]. In contrast, clinical telithromycin resistance in *S. pneumoniae* remains rare. Farrell reported that among a worldwide collection of 13 874 *S. pneumoniae* isolates, isolated between 1999 and 2003, only ten were resistant, with MICs ≥4 mg/L and all contained *erm*(B) gene [129]. Mutations in 23S rRNA, L4, and L22 have also been found in clinical telithromycin-resistant isolates [130, 131], and a combination of mutated genes can result in a higher telithromycin resistance than mutation of only one gene [132, 133]. Wolter and colleagues demonstrated that *erm*(B) with a deletion in the leader sequence was responsible for high-level telithromycin resistance in a strain isolated in Canada in 2007 [134].

Linezolid is the first in the class oxazolidinone that was approved for clinical use in 2000 for the treatment of nosocomial and community-acquired pneumonia. Linezolid binds to the 50S subunit of the bacterial ribosome via interactions with the central loop segment of domain V of the 23S rRNA to block the formation of protein synthesis initiation complexes. To date, linezolid non-susceptible pneumococcal strains are extremely rare [129, 135]. Recent data from the US LEADER and global ZAAPS surveillance systems show no linezolid non-susceptible isolates among 2150 *S. pneumoniae* isolates tested in 2011 [136, 137]. Reports of non-susceptibility to linezolid have been sporadic among clinical isolates of staphylococci and enterococci, and resistance has been found to be conferred by mutations in domain V of 23S rRNA [138]. In pneumococci, Wolter et al. [139] have described two clinical isolates with decreased susceptibility to linezolid (MICs 4 mg/L) which were found to contain 6-bp deletions in the gene encoding the riboprotein L4. The L4 deletions were also found to confer a novel mechanism of simultaneous resistance to macrolides, oxazolidinones, and chloramphenicol. A more recent study identified two additional linezolid non-susceptible pneumococci from the USA within the Centers for Disease Control and Prevention (CDC) Active Bacterial Core Surveillance (ABCs) program with mutations and deletions within the *rplD* gene [140]. Whole genome sequencing of linezolid-resistant laboratory-generated mutants has also revealed a role in resistance for a 23S rRNA methyltransferase (spr0333) and for the ABC proteins PatA and PatB [141]. A proteomic and transcriptomic screen suggested increased energy requirement needs associated with the burden of resistance in these laboratory-derived mutants [142]. Second-generation oxazolidinones like tedizolid, which is a protein synthesis inhibitor, are in clinical development for the treatment of

Gram-positive infections. Tedizolid has demonstrated potent in vitro activity against penicillin-resistant *S. pneumoniae*, including linezolid-resistant strains [143].

Resistance to quinupristin-dalfopristin among Gram-positive cocci has been very uncommon. Two clinical isolates among 8837 (0.02 %) *Streptococcus pneumoniae* isolates were discovered in 2001–2002 with MICs of 4 mg/L. Each had a 5-amino acid tandem duplication (RTAHI) in the L22 ribosomal protein gene (rplV) preventing synergistic ribosomal binding of the streptogramin combination [144].

9 Resistance to Other Agents

One class of antimicrobial agents previously used often in clinical practice is the tetracyclines, which are broad-spectrum bacteriostatic drugs shown to be active against pneumococci. Reflecting patterns of past usage, in some countries reported rates of non-susceptibility to tetracyclines remain the most frequently observed resistance phenotype [145]. In *S. pneumoniae* tetracycline resistance is due to the protection of the bacterial 30S ribosome subunit against antibiotic binding by the TetM or TetO [146, 147] proteins, with the *tet*(M) gene being far more common than the *tet*(O)gene in pneumococci. In streptococci, *tet*(M) is usually associated with highly mobile conjugative transposons of the T*n916*–T*n1545* type and large composite structures like T*n5253* and T*n3872*. A recent study discovered the oldest known examples of two different Tn*916*-like, *tet*(M)-containing elements identified among pneumococci dated from 1967 and 1968 [145]. These transposons often carry other resistance genes, such as *erm*(B) coding for resistance to macrolides, lincosamides, and streptogramins B which explains the persistence of tetracycline resistance (these transposons continue to be selected by macrolides). The comparison of *tet*(M) sequences in multidrug-resistant isolates reveals a high degree of allelic variation [148]. There is evidence of clonal distribution of selected alleles as well as horizontal movement of the mobile elements carrying *tet*(M) [149, 150].

The use of rifampin combined with either β-lactam antibiotics or vancomycin has been recommended for the treatment of meningitis caused by multiresistant pneumococci. Rifampin has been used in combined therapy to treat tuberculosis and resistant staphylococci, and it is extensively used in the prophylaxis of *Neisseria meningitidis* and *Haemophilus influenzae* type b exposure. The prevalence of rifampin resistance among pneumococcal isolates is low at present, and reported rates vary between 0.1 % and 1.5 % [151, 152]. Rifampin resistance has been described in several bacterial species and is caused by an alteration of the β-subunit of RNA polymerase, the target for the antibiotic. Resistance to rifampin in pneumococci has been linked to mutations in clusters N, I, II, and III of the *rpo*B gene, which encodes the β-subunit [153, 154].

Resistance to chloramphenicol in *S. pneumoniae* is due to the acetylation of the antibiotic by the production of a chloramphenicol acetyltransferase (CAT). The cat gene in pneumococcal isolates is carried on the conjugative transposon *Tn*5253, a composite transposon consisting of the tetracycline resistance transposon, *Tn*5251, and *Tn*5252 which carries the chloramphenicol resistance determinant [155]. Chloramphenicol-resistant strains have been shown to contain sequences homologous to cat_{pC194} and other flanking sequences from *S. aureus* plasmid pC194 [156].

Trimethoprim and sulfamethoxazole are used extensively in combination as the drug co-trimoxazole. Co-trimoxazole has been used in the treatment of a range of *S. pneumoniae* diseases, especially in children, because it is inexpensive and generally effective. Resistance to co-trimoxazole has increased dramatically in many regions of the world, and recent surveillance studies show rates ranging from 19 % in Europe to around 50 % associated with HIV infection in Africa and >60 % in Asia [29, 157, 158]. Resistance to co-trimoxazole is often associated with resistance to other antibiotics, especially to penicillin. Trimethoprim resistance in pneumococci has been reported to result from a single amino acid substitution (Ile-100 → Leu) in the dihydrofolate reductase (DHFR) protein [159] and often associated with mosaic alleles. Additional mutations have also been reported which seem to enhance resistance and modulate the effects of existing alterations on the affinity of DHFR for its natural substrates [160]. In many cases, resistance to sulfonamides is associated with chromosomal mutations within the gene encoding dihydropteroate synthase (DHPS). Different studies have reported the occurrence of single and/or multiple amino acid mutations in the DHPS of sulfonamide-resistant clinical isolates of *S. pneumoniae* [161–163]. The use of Fansidar therapy for malaria in Africa has been shown to increase co-trimoxazole resistance in pneumococci [19].

10 Clinical Relevance of Antibiotic Resistance

When penicillin-resistant pneumococci were first isolated from adults, there was an implicit assumption that such strains would fail intravenous penicillin therapy [164, 165]. As our appreciation of pharmacodynamics has allowed the understanding of the time-based mode of action of β-lactams, it is clear that the very high levels of penicillin achieved by intravenous therapy exceed the MICs of strains up to 8 mg/L for most of the short 4–6 h dosing interval for high-dose intravenous penicillin [166]. Such highly penicillin-resistant strains remain rare, and there is little evidence for the failure of intravenous penicillin, amoxicillin, cefotaxime, or ceftriaxone [167, 168] due to penicillin resistance. It is possible that less active intravenous agents such as cefuroxime [169]

may fail to treat penicillin-resistant infections, and β-lactams with a more Gram-negative spectrum such as ticarcillin [164] and ceftazidime [170] should not be used to treat penicillin-resistant pneumococcal infections. It is likely that oral β-lactam therapy may fail in the management of pneumococcal infections such as otitis media when the strains become intermediately (MIC ≥ 0.1 mg/L) resistant to penicillin. Poorly active cephalosporins such as cefaclor fail more often than cefuroxime [171, 172], and high-dose amoxicillin is the most active oral agent available against penicillin-resistant pneumococcal otitis media [173]. It is likely that the inferences made for otitis will be similar for sinusitis [174]. β-lactam resistance is clinically important for meningitis treatment where penicillin has been shown to fail [175, 176] even for intermediately resistant strains because of the poor penetration of penicillin through the blood-brain barrier. Extended spectrum cephalosporins fail too when there is full penicillin resistance in meningitis (MIC ≥ 2 mg/L; associated with cefotaxime or ceftriaxone MIC's ≥ 1 mg/L) [177, 178]. The empiric therapy therefore of penicillin-resistant pneumococcal meningitis is cefotaxime plus vancomycin or ceftriaxone plus vancomycin, based on the observation that these drugs in combination are able to eradicate cephalosporin-resistant pneumococci from the CSF better [178] than either drug alone [179, 180].

Macrolide resistance is associated in most instances with MICs > 2 mg/L regardless of the mechanism of macrolide resistance, and treatment of these strains with macrolides has been shown to fail [181, 182], both in the management of otitis media [171, 172] and of pneumonia [183]. These failures are in keeping with our knowledge of the pharmacodynamics of these agents [184].

Trimethoprim-sulfamethoxazole has been shown to not be able to eradicate from the middle ear, strains resistant to that agent [185].

Fluoroquinolones fail to successfully treat pneumococcal infections when preexisting resistant strains are present or even when first-step mutations in the *parC* gene are present [186]. Immunocompromised patients may be most at risk for repeated infections due to fluoroquinolones-resistant strains [187].

11 Impact of Conjugate Vaccine

The introduction of conjugate pneumococcal vaccine has not only reduced the burden of invasive disease in children [188] but has impacted on carriage and thus on the burden of disease in adults by preventing the spread of vaccine-type resistant strains to adults [189]. Direct demonstration of the impact of conjugate vaccine on antibiotic-resistant invasive disease was demonstrated in the 9-valent conjugate vaccine trial in South Africa [190], while multistate studies [191] have demonstrated a significant reduction in the proportion and absolute incidence of antibiotic-resistant pneumococci isolated from blood. Antibiotic resistance however emerged in non-vaccine-type pneumococci causing both ear infections and invasive disease following the 7-valent conjugate vaccine introduction in the USA, particularly among serotype 19A strains [192, 193]. The increase in serotype 19A post-conjugate vaccine in the USA was significantly increased among states with higher rates of community antimicrobial use in children [194]. In addition to direct protection of children from antibiotic-resistant pneumococci, and herd protection of adults to these resistant strains, through interruption of their transmission, conjugate vaccine may also contribute to reduction in selection of resistance by reducing the antimicrobial prescriptions written for vaccinated children, compared to controls [195–197].

12 Concluding Remarks

The multiply resistant pneumococcus continues to have a global distribution. Antimicrobial resistance within the pneumococcal population emerges and is maintained through a complex interplay of many factors. Attempts to reduce the burden of resistance in this pathogen are frustrated by widespread empiric therapy for respiratory infections. Both appropriate and inappropriate antibiotic uses continue to select resistance in this pathogen. Although the conjugate vaccine has reduced the burden of resistance in invasive isolates, continued antibiotic exposure is leading to the emergence of resistance in non-vaccine types.

References

1. Fischer Walker CL, Rudan I, Liu L, et al. Global burden of childhood pneumonia and diarrhoea. Lancet. 2013;381:1405–16.
2. Castanheira M, Gales AC, Mendes RE, et al. Antimicrobial susceptibility of *Streptococcus pneumoniae* in Latin America: results from five years of the SENTRY Antimicrobial Surveillance Program. Clin Microbiol Infect. 2004;10:645–51.
3. Reinert RR, Reinert S, van der Linden M, et al. Antimicrobial susceptibility of *Streptococcus pneumoniae* in eight European countries from 2001 to 2003. Antimicrob Agents Chemother. 2005;49:2903–13.
4. McGee L, McDougal L, Zhou J, et al. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. J Clin Microbiol. 2001;39:2565–71.
5. Cardozo DM, Nascimento-Carvalho CM, Andrade AL, et al. Prevalence and risk factors for nasopharyngeal carriage of *Streptococcus pneumoniae* among adolescents. J Med Microbiol. 2008;57:185–9.
6. Dagan R, Melamed R, Muallem M, et al. Nasopharyngeal colonization in southern Israel with antibiotic-resistant pneumococci during the first 2 years of life: relation to serotypes likely to be included in pneumococcal conjugate vaccines. J Infect Dis. 1996;174:1352–5.

7. Zenni MK, Cheatham SH, Thompson JM, et al. *Streptococcus pneumoniae* colonization in the young child: association with otitis media and resistance to penicillin. J Pediatr. 1995;127:533–7.
8. Mehr S, Wood N. *Streptococcus pneumoniae*—a review of carriage, infection, serotype replacement and vaccination. Paediatr Resp Rev. 2012;13:258–64.
9. Hausdorff WP, Feikin DR, Klugman KP. Epidemiological differences among pneumococcal serotypes. Lancet Infect Dis. 2005;5:83–93.
10. Klugman KP, Friedland IR. Antibiotic-resistant pneumococci in pediatric disease. Microb Drug Resist. 1995;1:5–8.
11. Bronzwaer S, Cars O, Buchholz U, et al. A European study on the relationship between antimicrobial use and antimicrobial resistance. Emerg Infect Dis. 2002;8:278–82.
12. Van Eldere J, Mera RM, Miller LA, et al. Risk factors for development of multiple-class resistance to *Streptococcus pneumoniae* strains in Belgium over a 10-year period: antimicrobial consumption, population density, and geographic location. Antimicrob Agents Chemother. 2007;51:3491–7.
13. Arason VA, Kristinsson KG, Sigurdsson JA, et al. Do antimicrobials increase the carriage rate of penicillin resistant pneumococci in children? Cross sectional prevalence study. Br Med J. 1996; 313:387–91.
14. Levine OS, Farley M, Harrison LH, et al. Risk factors for invasive pneumococcal disease in children: a population-based case-control study in North America. Pediatrics. 1999;103, E28.
15. Samore MH, Magill MK, Alder SC, et al. High rates of multiple antibiotic resistance in *Streptococcus pneumoniae* from healthy children living in isolated rural communities: association with cephalosporin use and intrafamilial transmission. Pediatrics. 2001;108:856–65.
16. Vanderkooi OG, Low DE, Green K, et al. Predicting antimicrobial resistance in invasive pneumococcal infections. Clin Infect Dis. 2005;40:1288–97.
17. Dias R, Caniça M. Emergence of invasive erythromycin-resistant *Streptococcus pneumoniae* strains in Portugal: contribution and phylogenetic relatedness of serotype 14. J Antimicrob Chemother. 2004;54:1035–9.
18. Dagan R, Barkai G, Givon-Lavi N, et al. Seasonality of antibiotic-resistant *Streptococcus pneumoniae* that causes acute otitis media: a clue for an antibiotic-restriction policy? J Infect Dis. 2008;197:1094–102.
19. Feikin DR, Dowell SF, Nwanyanwu OC, et al. Increased carriage of trimethoprim/sulfamethoxazole-resistant Streptococcus pneumoniae in Malawian children after treatment for malaria with sulfadoxine/pyrimethamine. J Infect Dis. 2000;181:1501–5.
20. Chen DK, McGeer A, de Azavedo JC, et al. Decreased susceptibility of Streptococcus pneumoniae to fluoroquinolones in Canada. N Engl J Med. 1999;341:233–9.
21. Ho PL, Tse WS, Tsang KW, et al. Risk factors for acquisition of levofloxacin-resistant *Streptococcus pneumoniae*: a case-control study. Clin Infect Dis. 2001;32:701–7.
22. Kupronis BA, Richards CL, Whitney CG, Active Bacterial Core Surveillance Team. Invasive pneumococcal disease in older adults residing in long-term care facilities and in the community. J Am Geriatr Soc. 2003;51:1520–5.
23. von Gottberg A, Klugman KP, Cohen C, et al. Emergence of levofloxacin-non-susceptible *Streptococcus pneumoniae* and treatment for multidrug-resistant tuberculosis in children in South Africa: a cohort observational surveillance study. Lancet. 2008;371:1108–13.
24. Mthwalo M, Wasas A, Huebner R, et al. Antimicrobial resistance of nasopharyngeal isolates of *Streptococcus pneumoniae* from children in Lesotho. Bull WHO. 1998;76:641–50.
25. Crowther-Gibson P, Cohen C, Klugman KP, et al. Risk factors for multidrug-resistant invasive pneumococcal disease in South Africa, a setting with high HIV prevalence, in the prevaccine era from 2003 to 2008. Antimicrob Agents Chemother. 2012;56: 5088–95.
26. Hofmann J, Cetron MS, Farley MM, et al. The prevalence of drug-resistant *Streptococcus pneumoniae* in Atlanta. N Engl J Med. 1995;333:481–6.
27. Schrag SJ, Pena C, Fernandez J, et al. Effect of short-course, high-dose amoxicillin therapy on resistant pneumococcal carriage: a randomized trial. J Am Med Assoc. 2001;286:49–56.
28. Bedos JP, Chevret S, Chastang C, et al. Epidemiological features of and risk factors for infection by Streptococcus pneumoniae strains with diminished susceptibility to penicillin: findings of a French survey. Clin Infect Dis. 1996;22:63–72.
29. Jacobs MR, Koornhof HJ, Robins-Browne RM, et al. Emergence of multiply resistant pneumococci. N Engl J Med. 1978;299: 735–40.
30. Soeters HM, von Gottberg A, Cohen C, et al. Trimethoprim-sulfamethoxazole prophylaxis and antibiotic nonsusceptibility in invasive pneumococcal disease. Antimicrob Agents Chemother. 2012;56:1602–5.
31. Buie KA, Klugman KP, von Gottberg A, et al. Gender as a risk factor for both antibiotic resistance and infection with pediatric serogroups/serotypes, in HIV-infected and -uninfected adults with pneumococcal bacteremia. J Infect Dis. 2004;189:1996–2000.
32. Wyres KL, Lambertsen LM, Croucher NJ, et al. The multidrug-resistant PMEN1 pneumococcus is a paradigm for genetic success. Genome Biol. 2012;13:R103.
33. Corso A, Severina EP, Petruk VF, et al. Molecular characterisation of penicillin-resistant *Streptococcus pneumoniae* isolates causing respiratory disease in the United States. Microb Drug Resist. 1998;4:325–37.
34. Richter SS, Heilmann KP, Dohrn CL, et al. Changing epidemiology of antimicrobial-resistant Streptococcus pneumoniae in the United States, 2004–2005. Clin Infect Dis. 2009;48:23–33.
35. Simões AS, Pereira L, Nunes S, et al. Clonal evolution leading to maintenance of antibiotic resistance rates among colonizing Pneumococci in the PCV7 era in Portugal. J Clin Microbiol. 2011;49:2810–7.
36. Croucher NJ, Harris SR, Fraser C, et al. Rapid pneumococcal evolution in response to clinical interventions. Science. 2011;331:430–4.
37. Hiller NL, Eutsey RA, Powell E, et al. Differences in genotype and virulence among four multidrug-resistant Streptococcus pneumoniae isolates belonging to the PMEN1 clone. PLoS ONE. 2011;6, e28850.
38. Beall BW, Gertz RE, Hulkower RL, et al. Shifting genetic structure of invasive serotype 19A pneumococci in the United States. J Infect Dis. 2011;203:1360–8.
39. Beall B, McEllistrem MC, Gertz Jr RE, et al. Pre- and post-vaccination clonal compositions of invasive pneumococcal serotypes for isolates collected in the United States in 1999, 2001, and 2002. J Clin Microbiol. 2006;44:999–1017.
40. Pai R, Moore MR, Pilishvili T, et al. Postvaccine genetic structure of Streptococcus pneumoniae serotype 19A from children in the United States. J Infect Dis. 2005;192:1988–95.
41. Edson DC, Glick T, Massey LD. Susceptibility testing practices for *Streptococcus pneumoniae*: results of a proficiency testing survey of clinical laboratories. Diagn Microbiol Infect Dis. 2006;55:225–30.
42. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement. CLSI document M100–S18. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
43. Carvalho Mda G, Tondella ML, McCaustland K, et al. Evaluation and improvement of real-time PCR assays targeting lytA, ply, and psaA genes for detection of pneumococcal DNA. J Clin Microbiol. 2007;45:2460–6.

44. Harris KA, Turner P, Green EA, et al. Duplex real-time PCR assay for detection of Streptococcus pneumoniae in clinical samples and determination of penicillin susceptibility. J Clin Microbiol. 2008;46:2751–8.
45. Srinivasan V, du Plessis M, Beall BW, et al. Quadriplex real-time polymerase chain reaction (lytA, mef, erm, pbp2b(wt)) for pneumococcal detection and assessment of antibiotic susceptibility. Diagn Microbiol Infect Dis. 2011;71:453–6.
46. Fukushima KY, Yanagihara K, Hirakata Y, et al. Rapid identification of penicillin and macrolide resistance genes and simultaneous quantification of Streptococcus pneumoniae in purulent sputum samples by use of a novel real-time multiplex PCR assay. J Clin Microbiol. 2008;46:2384–8.
47. Kearns AM, Graham C, Burdess D, et al. Rapid real-time PCR for determination of penicillin susceptibility in pneumococcal meningitis, including culture-negative cases. J Clin Microbiol. 2002;40:682–4.
48. Zettler EW, Scheibe RM, Dias CA, et al. Determination of penicillin resistance in *Streptococcus pneumoniae* isolates from southern Brazil by PCR. Int J Infect Dis. 2006;10:110–5.
49. Cassone M, D'Andrea MM, Iannelli F, et al. DNA microarray for detection of macrolide resistance genes. Antimicrob Agents Chemother. 2006;50:2038–41.
50. Haanperä M, Huovinen P, Jalava J. Detection and quantification of macrolide resistance mutations at positions 2058 and 2059 of the 23S rRNA gene by pyrosequencing. Antimicrob Agents Chemother. 2005;49:457–60.
51. Austrian R, Gold J. Pneumococcal bacteremia with special reference to bacteremic pneumococcal pneumonia. Ann Intern Med. 1964;60:759–76.
52. Kislak JW, Razavi LM, Daly AK, et al. Susceptibility of pneumococci to nine antibiotics. Am J Med Sci. 1965;250:261–8.
53. Hansman D, Bullen MM. A resistant pneumococcus. Lancet. 1967;1:264–5.
54. Hansman D, Glasgow H, Sturt J, et al. Increased resistance to penicillin of pneumococci isolated from man. N Engl J Med. 1971;284:175–7.
55. Naraqi S, Kirkpatrick GP, Kabins S. Relapsing pneumococcal meningitis: isolation of an organism with decreased susceptibility to penicillin G. J Pediatr. 1974;85:671–3.
56. Appelbaum PC, Bhamjee A, Scragg JN, et al. *Streptococcus pneumoniae* resistant to penicillin and chloramphenicol. Lancet. 1977;2:995–7.
57. Hakenbeck R, Ellerbrok H, Briese T, et al. Penicillin-binding proteins of penicillin-susceptible and -resistant pneumococci: immunological relatedness of altered proteins and changes in peptides carrying the (-lactam binding site. Antimicrob Agents Chemother. 1986;30:553–8.
58. Kell CM, Sharma UK, Dowson CG, et al. Deletion analysis of the essentiality of penicillin-binding proteins 1A, 2B and 2X of *Streptococcus pneumoniae*. FEMS Microbiol Lett. 1993;106:171–5.
59. Smith AM, Feldman C, Massidda O, et al. Altered PBP2A and its role in the development of penicillin, cefotaxime and ceftriaxone resistance in a clinical isolate of *Streptococcus pneumoniae*. Antimicrob Agents Chemother. 2005;49:2002–7.
60. Reichmann P, Kőning A, Marton A, et al. Penicillin-binding proteins as resistance determinants in clinical isolates of *Streptococcus pneumoniae*. Microb Drug Resist. 1996;2:177–81.
61. Hakenbeck R, Brückner R, Denapaite D, et al. Molecular mechanisms of β-lactam resistance in *Streptococcus pneumoniae*. Future Microbiol. 2012;7:395–410.
62. Grebe T, Hakenbeck R. Penicillin-binding proteins 2b and 2x of *Streptococcus pneumoniae* are primary resistance determinants for different classes of β-lactam antibiotics. Antimicrob Agents Chemother. 1996;40:829–34.
63. Dowson CG, Johnson AP, Cercenado E, et al. Genetics of oxacillin resistance in clinical isolates of *Streptococcus pneumoniae* that are oxacillin resistant and penicillin susceptible. Antimicrob Agents Chemother. 1994;38:49–53.
64. Munoz R, Dowson CG, Daniels M, et al. Genetics of resistance to third-generation cephalosporins in clinical isolates of *Streptococcus pneumoniae*. Mol Microbiol. 1992;6:2461–5.
65. Coffey TJ, Daniels M, McDougal LK, et al. Genetic analysis of clinical isolates of *Streptococcus pneumoniae* with high-level resistance to expanded-spectrum cephalosporins. Antimicrob Agents Chemother. 1995;39:1306–13.
66. Smith AM, Klugman KP. Alterations in MurM, a cell wall muropeptide branching enzyme, increase high-level penicillin and cephalosporin resistance in *Streptococcus pneumoniae*. Antimicrob Agents Chemother. 2001;45:2393–6.
67. Vanhoof R, Brouillard J, Damee S, et al. High prevalence of penicillin resistance and comparative in vitro activity of various antibiotics in clinical isolates of *Streptococcus pneumoniae* isolated in the Province of Hainaut during winter 2004. Acta Clin Belg. 2005;60:345–9.
68. Cafini F, del Campo R, Alou L, et al. Alterations of the penicillin-binding proteins and *mur*M alleles of clinical *Streptococcus pneumoniae* isolates with high-level resistance to amoxicillin in Spain. J Antimicrob Chemother. 2006;57:224–9.
69. Butler DL, Gagnon RC, Miller LA, et al. Differences between the activity of penicillin, amoxycillin, and co-amoxyclav against 5,252 *Streptococcus pneumoniae* isolates tested in the Alexander Project 1992–1996. J Antimicrob Chemother. 1999;43:777–82.
70. Schrag SJ, McGee L, Whitney CG, et al. Emergence of *Streptococcus pneumoniae* with very-high-level resistance to penicillin. Antimicrob Agents Chemother. 2004;48:3016–23.
71. Doit C, Loukil C, Fitoussi F, et al. Emergence in France of multiple clones of clinical *Streptococcus pneumoniae* isolates with high-level resistance to amoxicillin. Antimicrob Agents Chemother. 1999;43:1480–3.
72. Kosowska K, Jacobs MR, Bajaksouzian S, et al. Alterations of penicillin-binding proteins 1A, 2X and 2B in *Streptococcus pneumoniae* isolates with amoxicillin MICs are higher than penicillin MICs. Antimicrob Agents Chemother. 2004;48:4020–2.
73. Du Plessis M, Bingen E, Klugman KP. Analysis of penicillin-binding protein genes of clinical isolates of *Streptococcus pneumoniae* with reduced susceptibility to amoxicillin. Antimicrob Agents Chemother. 2002;46:2349–57.
74. Gasc AM, Kauc L, Barraillé P, et al. Gene localization, size, and physical map of the chromosome of *Streptococcus pneumoniae*. J Bacteriol. 1991;173:7361–7.
75. Smith AM, Botha RF, Koornhof HJ, et al. Emergence of a pneumococcal clone with cephalosporin resistance and penicillin susceptibility. Antimicrob Agents Chemother. 2001;45:2648–50.
76. Davies TA, Flamm RK, Lynch AS. Activity of ceftobiprole against Streptococcus pneumoniae isolates exhibiting high-level resistance to ceftriaxone. Int J Antimicrob Agents. 2012;39:534–8.
77. Flamm RK, Sader HS, Farrell DJ, et al. Antimicrobial activity of ceftaroline tested against drug-resistant subsets of *Streptococcus pneumoniae* from U.S. medical centers. Antimicrob Agents Chemother. 2014;58:2468–71.
78. McDougal LK, Rasheed JK, Biddle JW, et al. Identification of multiple clones of extended-spectrum cephalosporin-resistant *Streptococcus pneumoniae* isolates in the United States. Antimicrob Agents Chemother. 1995;39:2282–8.
79. Felmingham D, Reinert RR, Hirakata Y, et al. Increasing prevalence of antimicrobial resistance among isolates of Streptococcus pneumoniae from the PROTEKT surveillance study, and comparative in vitro activity of the ketolide, telithromycin. J Antimicrob Chemother. 2002;50(Suppl S1):25–37.
80. Klugman KP, Lonks JR. Hidden epidemic of macrolide-resistant pneumococci. Emerg Infect Dis. 2005;11:802–7.