Review

The clinical significance of *Streptococcus pneumoniae* resistance in community-acquired pneumonia

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SUMMARY. As resistance to *Streptococcus pneumoniae* has escalated dramatically over the past decades, the efficacy of the three major classes of antibiotics most commonly used for the empirical treatment of community-acquired pneumonia (CAP), (i.e., β-lactams, macrolides and respiratory quinolones) is under investigation. According to recently published international data 21.8% of strains of *S. pneumoniae* are penicillin non-susceptible and 36.3% are resistant to azithromycin. Rates of quinolone resistance remain low, but clonal spread of resistant strains has been reported in closed communities. The precise clinical impact of antimicrobial resistance is difficult to assess, but treatment failures due to antibiotic-resistant *S. pneumoniae* have been documented. Comparison of the relatively small number of failures with the magnitude of confirmed resistance reveals a paradox that has not been clarified and possibly involves both pharmacokinetic and pharmacodynamic parameters. It is evident that the final outcome of CAP depends not only on the therapeutic regime but also on a variety of factors including the genetic characteristics of the bacterial strain and the background of the patient. Knowledge of the mechanisms of the emergence and spread of resistance is necessary for the rational selection of appropriate antibiotics. Current data suggest that the possibility of penicillin resistance should not be a leading factor for the choice of the therapeutic regime in CAP. In Greece, monotherapy of CAP with a macrolide poses clinical risks, while quinolones should be used with caution. In the setting of increasing resistance the administration of the appropriate antimicrobial therapy is essential for the prevention of emerging infections due to resistant *S. pneumoniae* strains, which apart from the increased cost of treatment may lead to an unfavourable outcome. ***Pneumon 2011, 24(4):376-388.***

INTRODUCTION

Streptococcus pneumoniae is a Gram-positive aerobic or facultative anaerobic coccus, member of the *Streptococcaceae* family, which was discovered in 1881. The major virulence factor of *S. pneumoniae* is its capsular polysaccharide, which provides the antigenic target for antibody production[1]. Ninety-one distinct serotypes are currently recognized[1,2]. This exclusively human pathogen is a common inhabitant of the upper respiratory tract where serotypes may succeed one another. The carrier state usually lasts for weeks in adults or months in children. Most people have been carriers of *S. pneumoniae* at some point in their lives. Airway colonization by pneumonococci is detectable in about 10% of adults, 20-40% of children and 60% of infants in day-care settings. Day-care centres are significant pools of resistant strains in the community[2]. Occasionally *S. pneumoniae* may spread from the nasopharynx of a colonized person to other parts of the body and cause disease[2].

S. pneumoniae infections are divided into invasive and non-invasive, depending on whether or not *S. pneumoniae* is detected in normally sterile areas. Meningitis and bacteraemia are invasive infections, in contrast to pneumonia, sinusitis and acute otitis media[3].

Pneumonia is a major infectious disease with millions of cases diagnosed every year worldwide. It is one of the 10 leading causes of death in all age groups and the leading cause of death due to an infectious disease[4]. Approximately 50% of hospitalized patients with pneumonia have community-acquired pneumonia (CAP)[5]. *S. pneumoniae* is the predominant pathogen of CAP, regardless of severity, and the most common cause of death due to CAP[6-8].

Based on the above information, the antimicrobial agents included in the empirical treatment of CAP should be active against *S. pneumoniae*. Current guidelines focus on three classes of antibiotics, β-lactams, macrolides and respiratory quinolones[7]. The use of these agents is currently under continuous investigation since antimicrobial resistance has escalated dramatically during the past 40 years[9].

THE EPIDEMIOLOGY OF RESISTANT *S. PNEUMONIAE*

Although resistant strains of *S. pneumoniae* are detected universally, the incidence of resistance varies markedly between countries and regions[10]. Penicillin G, the drug of choice since 1942, is still used for the treatment of pneumococcal pneumonia[11]. Low levels of penicillin resistance were first described in Australia in 1960[12], and higher levels [minimum inhibitory concentration (MIC) ≥2μg/mL] were reported in South Africa in 1970[13,14]. From these areas penicillin-resistant clones of *S. pneumoniae* spread rapidly across the world. As a result by the late 1990s the incidence of penicillin non-susceptible *S. pneumoniae*, PNSSP) had increased significantly in Europe[15,16], Asia[17-19], and North[20-23] and South America[24]. Subsequently, strains with very high resistance levels to penicillin (MIC≥8μg/mL) emerged in the late 1990s[9].

Several surveillance studies have tracked the evolution of resistance among strains of *S. pneumoniae*. The PROTECT study obtained more than 20,000 isolates from 39 countries; overall 21.8% of isolates were PNSSP during the period 1999-2003[25]. Resistance rates to penicillin were highest in France, Spain, South Africa, the USA and the Far East[25]. Resistance rates to penicillin were over 50% in some of these regions, whereas in other parts of the world such as Finland, Sweden and Germany rates were below 5%[9]. PNSSP strains accounted for 21.6% of *S. pneumoniae*, in Canada in 2006[9] and for 32.5% in the USA in 2004-05[26]. Despite global increase in resistance, in some countries such as Spain decline in resistance has been reported[9,27]. This phenomenon may be the result of campaigns targeting the reduction in antibiotic usage. Alternatively there may be a resistance ceiling in *S. pneumoniae*, which the organism cannot overcome without a significant metabolic cost. In Greece PNSSP has increased in adults from 27.4% in 2001-04 to 47.8% in 2005-08[28], and in children 44.6% of *S. pneumoniae* strains are PNSSP[29].

During recent decades a paradox has gradually emerged between the penicillin susceptibility breakpoints that define resistance and its clinical consequences. More specifically, it has become evident that infections due to strains of *S. pneumoniae* formerly considered non-susceptible can be treated successfully with the usual (but not inadequate) doses of β-lactam antibiotics. This paradox has led to the revision of penicillin susceptibility breakpoints[8].

Strains of *S. pneumoniae* were initially susceptible to penicillin with MICs for penicillin ranging from 0.015-0.03μg/mL. With the emergence of strains with higher MICs, the susceptibility breakpoints were set as follows: susceptible MIC ≤0.06μg/mL, intermediate 0.12-1μg/mL and resistant ≥2μg/mL. This was changed in 2008 and for non-meningeal infections the breakpoints are currently: susceptible MIC ≤2μg/mL, intermediate MIC 4μg/mL and resistant MIC ≥8μg/mL[30,31]. Penicillin G is administered

intravenously at 12 million units/day in 6 divided doses for sensitive strains, but 18-24 million units are required for intermediate resistant strains. *S. pneumoniae* does not produce β-lactamase, therefore MIC breakpoints for amoxicillin and amoxicillin-clavulanate are the same. PNSSP strains usually remain susceptible to amoxicillin[32-34].

Macrolide resistance among *S. pneumoniae* strains escalated dramatically worldwide in the 1990s. In many regions macrolide resistance in *S. pneumoniae* is more common than penicillin resistance[9], which may be attributed to the extensive use of macrolides for CAP as monotherapy[35]. According to the PROTECT study, macrolide resistance increased from 31% in 1999 to 36.3% in 2004[25]. Azithromycin resistance reached 31% in Canada in 2006[9], while erythromycin resistance was 29.1% in United States in 2004-05[26]. In Europe the rates of macrolide resistance are highly variable among countries, ranging from 6.9% in Norway to 57% in Greece[36]. Based on Greek studies macrolide-resistant *S. pneumoniae* accounts for 38.9% in adults and 48.6% in children[28,29].

Rates of quinolone resistance remain low (<2%). However, in the USA quinolone resistance is increasing in contrast to other antibiotic classes[37]. In addition clusters of infections due to resistant strains have been reported[9,38], mainly in closed communities such as Hong Kong where clonal spread of resistant strains led to a significant increase of resistance to 13.3%[39]. In South Africa, where quinolones are widely used for the treatment of multidrug-resistant (MDR) tuberculosis, several cases of invasive *S. pneumoniae* disease due to nosocomial spread of strains resistant to levofloxacin have been reported[40]. Based on this evidence, the emergence of clinically significant quinolone resistance is a possibilty that should not be ignored. Resistance rates are generally higher for ciprofloxacin than for levofloxacin and moxifloxacin[9,26]. In Greece resistance to respiratory quinolones has not been reported but according to published data in 23.3% of *S. pneumoniae* strains the MIC for ciprofloxacin is >2μg/mL[41].

MECHANISMS OF RESISTANCE

In Gram (+) bacteria resistance is acquired through transfer of genetic material. Transfer may be accomplished by bacteriophages (i.e., bacterial viruses with the ability to pick up host genes from one strain and move them to a recipient)[42], by the movement of plasmids (i.e., circular DNA segments that are physically separated from the bacterial chromosome)[42] or by transposons (i.e DNA segments without replication functions that have the ability to move between plasmids or chromosomes and can insert into bacterial genetic material in areas flagged by certain gene sequences, known as insertion sequences)[42]. Macrolide resistance is disseminated among *S. pneumoniae* via transposons[43], and in addition *S. pneumoniae* has the ability to absorb free DNA from the environment. This ability, called transformation, was first described in 1944 and leads to the acquisition of penicillin resistance[44]. In contrast to the above mechanisms of clonal spread of resistance to β-lactam and macrolide antibiotics, resistance to quinolones is usually the result of de novo mutations, although clonal spread of quinolone-resistant strains has been also reported[45].

Beta-lactams

β-lactam antibiotics interact with target sites i.e. penicillin-binding proteins (PBP), which are integral to bacterial cell wall synthesis. Six different PBPs have been identified in *S. pneumoniae* (1a, 1b, 2a, 2b, 2x, 3). Resistance to β-lactams results from alterations in PBPs. When 2b, 2x, or 1a are altered, binding ability to β-lactams is reduced and higher MICs are required. Extremely high MICs are the result of mutations to all three PBPs. Resistance to penicillin is associated with some degree of resistance to all β-lactam antibiotics. Resistance to cephalosporins develops through alterations in 2x and 1a, since 2b is not a target for cephalosporins. Most cephalosporin-resistant strains are also penicillin-resistant[1,9].

Macrolides

Macrolides inhibit protein synthesis by binding to ribosomal sites, and therefore mutations leading to alterations in the binding site confer resistance to macrolides. Two major mechanisms of macrolide-resistance have been described:

- Methylation of the ribosomal site [*erm (B)*] leads to high-level resistance (MIC >64μg/ml) and concomitant resistance to lincosamides and streptogramins (MSL phenotype)[9].
- Active efflux [*mef (E), mef (A)*], leading to lower-level resistance (MIC 1-32μg/ml), without affecting other antibiotic classes.

Erythromycin resistance is generally associated with azithromycin and clarithromycin cross-resistance[9,43]. The genes *erm (B)* and *mef (E or A)* are responsible for 97% of macrolide resistance in *S. pneumoniae*, and both genes may be present in some strains. The prevalence of these

genes varies widely among countries, *erm (B)* being the predominant mechanism in Europe and *mef (E or A)* in North America[9].

Quinolones

Fluoroquinolones inhibit DNA synthesis by interacting with specific sites in topoisomerases II and IV, called QRDR[46]. Quinolone-resistance is the result of de novo mutations in QRDR leading to reduced affinity to quinolones. The *ParC* and *gyrA* genes are responsible for A subunits of topoisomerases, while *parc* and *gyrB* are responsible for B subunits[47]. Efflux-mediated resistance may also occur[48].

Concomitant resistance

Although resistance mechanisms for each antibiotic class are different, resistance genes may be transferred together, leading to multiple-resistant *S. pneumoniae*. PNSSP is more commonly resistant to non β-lactam drugs, such as macrolides, clindamycin, tetracyclines, chloramphenicol and trimethoprime/sulfamethoxazole than sensitive strains of *S. pneumoniae*[9,49,50]. Conversely, quinolone resistance is usually independent of penicillin resistance. MDR in *S. pneumoniae* is defined as resistance to at least 3 antibiotic classes[51]. The rates of MDR over the globe are highly variable. According to a study in 15 European countries during 2004-05 overall MDR is 15.8% (0% in Denmark and 42.9% in Greece[36]. MDR is more common when macrolide resistance results from the *erm(B)* gene than from the *mef(A)* gene[9].

THE CLINICAL SIGNIFICANCE OF RESISTANCE

Despite its escalation worldwide, the clinical consequences of *S. pneumoniae* resistance are difficult to assess. This contradiction rises from the fact that antimicrobial resistance in bacteria can be defined in two ways. First, microbiological resistance is the presence of a mechanism of resistance to a certain antibiotic that is either innate, or acquired either from the environment or through automatic mutation. The latter mechanism differentiates the resistant strain (non-wild-type) from the wild type bacteria that have never been exposed to the antibiotic drug. The level of microbiological resistance is defined by a laboratory cut-off, such as the MIC, which is independent of clinical circumstances[53]. However it should be underlined that use if the MIC has inherent weaknesses in the assessment of resistance. MIC is defined as the minimum concentration of an antibiotic in the presence of which no *visible* bacterial growth is observed for *16-20 hours* in culture medium. Bacterial sensitivity, however, is not homogeneous and MIC cannot estimate possible inhibitory results after exposure to the drug. Additionally MIC variability is 100% (i.e. an MIC of 2μg/mL may actually be 1μg/mL or 4μg/mL) and MIC does not reflect the site of infection and does not take into account phenomena such as post-antibiotic effect, post-antibiotic leukocyte enhancement or serum bactericidal effect.

The second definition of resistance is the clinical resistance. Clinical resistance is defined by the level of antimicrobial activity associated with a high likelihood of therapeutic failure. Clinical resistance relates to the adverse outcome of a patient receiving an antibiotic while suffering from an infection due to a resistant strain. This aspect of resistance estimates whether exposure of a sensitive strain to a drug in question will lead to a better outcome compared that of to a resistant strain[53]. Whether this is the case with *S. pneumoniae* in CAP remains controversial, but it is clear that microbiological failure (i.e., failure of microbial eradication) is not necessarily associated with clinical (i.e., therapeutic) failure[54].

The divergence between *in vitro* resistance and its clinical consequences becomes evident with assessment of resistance over time and in different parts of the world. Five-day mortality due to invasive *S. pneumoniae* pneumonia has remained unchanged since 1964, despite the significant escalation of *S. pneumoniae* resistance[55-57]. Although resistance rates are increasing, the percentage of invasive disease due to *S. pneumoniae* has remained stable[58], and no significant differences in mortality are observed among countries with variable resistance rates[59].

This paradoxse may be explained by the multiplicity and the interaction of the factors that determine the final outcome of pneumonia. These factors include age, gender, serotype, previous intake of steroids, hospitalization during the last 12 weeks, immunodeficiency, comorbidities [such as chronic obstructive pulmonary disease (COPD)], the clinical condition, the presence of pleural effusion, infiltrations in multiple lobes, and genetic predisposition, along with bacterial resistance[27,60-69].

The mortality is thus dependent on the bacterial strain, the antibiotic regime and the characteristics of the patient. Some of factors affecting the final outcome, such as the antibiotics, may be modifiable while others are not. Obviously, the association between *in vitro* activity and clinical efficiency is not linear. As bacterial resistance increases, however, it is expected that more and more cases of adverse outcome in CAP due to resistant *S. pneumoniae* will be reported.

Another factor that should be taken into account is the virulence of the resistant *S. pneumoniae*. As described above, resistance is the result of either genetic alteration or genetic material acquisition. In the first case an evolutionarily optimized mechanism deviates from its functional optimum, while in the second, at least in many instances, the cost of the carriage of foreign elements must be paid. It is reasonable to conclude that resistance to antibiotic drugs requires the expense of a fitness cost[70,71]. In that setting, in animal models resistant *S. pneumoniae* strains are less virulent than sensitive strains, and strains isolated from cases of bacteraemia are less resistant than those isolated from mucosal infections or from carriers[29,70,72].

B-lactams

A considerable number of studies have explored the impact of penicillin resistance on the outcome of pneumonia, but their findings are conflicting. Relatively few studies indicate that penicillin resistance is associated with a poor outcome[57,61,73]. In 6,570 cases of bacteraemic pneumonia due to *S. pneumoniae,* a correlation between the number of deaths after the 4th day of hospitalization and infection by strains with an MIC ≥4μg/mL for penicillin and ≥2μg/mL for cefotaxime was observed[57].

A meta-analysis of hospitalized patients with *S. pneumoniae* pneumonia produced the conclusion that the relative risk for mortality was higher for resistant than for sensitive *S. pneumoniae* (OR=1.37), after adjustment for age, comorbidities and severity of illness[73]. This study also examined the impact of discordant therapy (i.e., administration of a single empirical antimicrobial agent that was inactive *in vitro*) compared to concordant therapy (i.e., administration of at least one empirical antimicrobial agent with *in vitro* activity). Discordant therapy did not result in increased mortality compared to concordant therapy[73]; thus pneumonia due to PNSSP is associated with higher mortality independently of the *in vitro* activity of the antimicrobial agents. The authors conclude that there is no need to change the current empirical treatment for pneumonia, but that penicillin resistance is possibly a negative prognostic factor for pneumonia outcome.

On the other hand, a considerable number of reports suggest that the outcome of infections due to *S. pneumoniae* is not determined by penicillin resistance. Several studies examined CAP exclusively[27,67,74,75], some of which detected a non-significant trend towards increased mortality in pneumonia due to PNSSP, but penicillin resistance does not seem to be associated with the severity or outcome of bacteraemic CAP[76]. In this setting Falcó et al noted a non-significant trend towards increased mortality in the cases of PNSSP in 247 patients with bacteraemic pneumonia[77]. Similar findings have been reported for bacteraemia[63,78,79] and invasive *S. pneumoniae* disease in adults and children[60,62,80-83]. Maugein et al studying 919 patients with bacteraemia due to *S. pneumoniae* showed that mortality was independent of the level of resistance (21.3% at MIC <0.1μg/mL, 16.7% at MIC 0.1-1 μg/mL, 25.6% at MIC 1μg/mL and 20.9% at MIC ≥2μg/mL)[65]. In accordance with the previous studies quoted, cases of microbiological failure in respiratory infections treated with β-lactam antibiotics were scarce, despite the high prevalence of penicillin resistance[84].

Most of the above studies take into account the previous susceptibility breakpoints and include only small numbers of infections due to strains that are currently considered resistant (MIC ≥8μg/mL). Even in the case of invasive disease due to strains of *S. pneumoniae* with a very high MIC (currently defined as resistant) the outcome did not differ from that of other *S. pneumoniae* infections, although duration of hospitalization was longer[85].

It appears, therefore, that the administration of β-lactam antibiotics in pneumonia due to PNSSP does not affect the course of the disease compared to the results with agents that are active *in vitro*[73,77]. The reasons for which penicillin continues to be active against non-susceptible strains require further investigation. One possible explanation is based on pharmacokinetic and pharmacodynamic parameters. For penicillins, the dynamic parameter that better relates to their efficiency is the time period during which drug concentration exceeds the MIC. Mortality is low when this period is greater than 40-50% of the dose interval[86]. As a result, a high penicillin serum concentration could retain its activity even against strains with a high MIC (Figure 1)[11]. The MIC of the vast majority of *S. pneumoniae* strains (>95%) is such that high-dose penicillin is effective[31]. Furthermore, 99% of the currently active strains have an MIC≤4μg/mL[31].

It may therefore be concluded that intermediate strains can be effectively treated with high-dose penicillin, while truly resistant strains are rare and their possible presence should not alter everyday therapeutic decisions. Resistant *S. pneumoniae* is not a cause of therapeutic failure when the antibiotic regimen is appropriate and the MIC for penicillin is <4μg/ml[87]. This observation is enhanced by the fact that failures of bacterial eradication with penicillin or amoxicillin have never been documented. One case of failure with tircacillin administration has been reported and this involved a strain with an extremely high MIC[84].

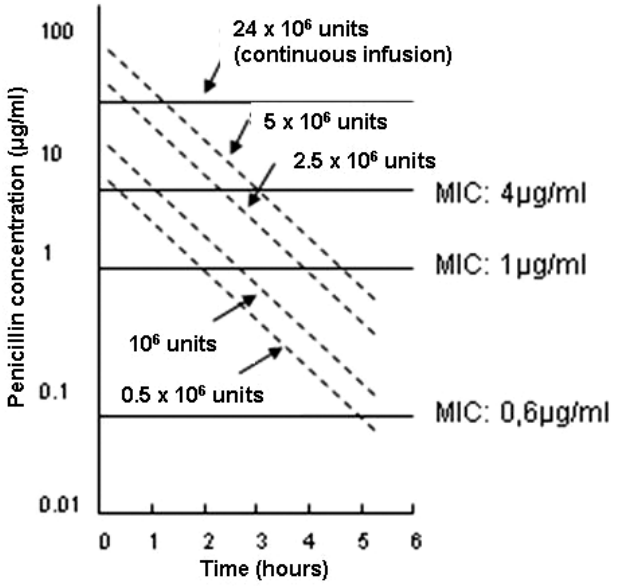

FIGURE 1. Serum levels of penicillin in relation to dosage for different levels of resistance of *S. pneumoniae*. From reference 11.

Conversely, failures have been observed with β-lactam antibiotics with poor activity against *S. pneumoniae*, such as cefazolin, cefuroxime and ceftazidime[84].

As mentioned above, the discordance between microbiological and clinical resistance to β-lactam antibiotics may be explained by the fact that previous susceptibility breakpoints were set too low, thus defining as resistant strains that were clinically susceptible[8]. This lack of clinical relevance has led to the raising of susceptibility breakpoints for nonmeningeal infections.

In this context the recommendations by IDSA/ATS published in 2007 underline that for CAP, the current levels of resistance to β-lactam antibiotics do not result in therapeutic failure when the appropriate antimicrobial agents (amoxicillin, ceftriaxone, cefotaxime) are used. According to the same guidelines the clinically relevant level of penicillin resistance is set at MIC of at least 4μg/mL. It should be noted, however, that the proposed doses are much higher than those usually used, since according to IDSA/ATS outpatients with comorbidities or risk factors for infection with resistant *S. pneumoniae* should be treated with amoxicillin 1g 3 times daily or amoxicillin/clavoulanic 2g twice daily[7]. The Greek guidelines for treatment of CAP in ambulatory patients recommend the administration of 1g amoxicillin 4 times daily[88].

Despite the factors mentioned above that contribute to the discordance between microbiological and clinical resistance, caution is needed for two main reasons: Firstly, if instead of stabilizing, resistance levels continue to raise, the clinical outcome will eventually be affected[89]. Secondly, in the case of other *S. pneumoniae* infections, such as acute otitis media and meningitis, the outcome is adversely affected by resistance. In acute otitis media, high levels of clinical failure have been observed with resistant *S. pneumoniae*[90,91]. In addition the treatment of meningitis has been significantly changed during the past 20 years, and penicillin is no longer the drug of choice; the empirical regime currently recommended comprises vancomycin and the third generation cephalosporins[92].

Apart from resistance, two other characteristics that may affect the ability of *S. pneumoniae* to survive in the presence of penicillin in high concentrations are heteroresistance and tolerance. Heteroresistance is usually defined as the presence of one or several bacterial subpopulations which can grow at higher antibiotic concentrations than predicted by MIC. This ability may give the microorganisms the opportunity to explore growth at higher penicillin concentrations without paying the fitness cost, thus serving as a tool during evolution to resistance[93]. Tolerance is the decreased susceptibility towards the killing effect of β-lactams[95]. In an animal model with bacteraemic pneumonia due to *S. pneumoniae,* the administration of amoxicillin at a dose simulating 1g/8 hours in humans led to rapid killing of sensitive strains. In the case of strains with a MIC at 2 or 4 μg/ml, however, bactericidal activity depended not only on resistance but also on *S. pneumoniae* tolerance[95].

Risk factors for PNSSP infection are previous antibiotic use (during the last 3 months), recent hospitalization, residence in a health care facility, nosocomial pneumonia, pneumonia during the past 12 months, alcoholism, age extremes, attendance at a day-care centre, contact with small children, comorbidities, HIV infection, immunodeficiency, haematological malignancies, splenectomy and infection with serotypes 14 and 19[77,96]. Previous antibiotic use need not exclusively involve β-lactams[9]. More specifically, penicillin use increases the possibility of infection due to non-sensitive strains by a degree of 2.47, trimethoprime/sulfamethoxazole by 5.97 and azithromycin by 2.78[97].

Concerning cephalosporins, the clinical impact of *S. pneumoniae* resistance in non-meningeal infections varies. Cefotaxime and ceftriaxone are highly active against *S. pneumoniae* and the prevalence of resistance (MIC ≥4μg/mL) very low. In contrast, second generation cephalosporins are much less active and the use of cefuroxime in

bacteraemia caused by *S. pneumoniae* has been reported to be associated with increased mortality[98,99].

Macrolides

In contrast to the β-lactams, numerous cases of microbiological and clinical failure and even deaths, due to macrolide-resistant *S. pneumoniae* have been reported[84,100]. In some patients breakthrough bacteraemia has been observed[84,101-105]. For this reason, despite the clear role of macrolides as part of a combination empirical treatment in CAP, the widespread prevalence of macrolide resistance worldwide renders monotherapy with a macrolide difficult to justify[84].

It is evident that discordance between microbiological and clinical failure also applies for the macrolides, as the reported therapeutic failures are disproportionately few compared to the tremendous increase in resistance rates[84]. In a recent study, 59 patients with mild or moderate CAP received azithromycin 500mg daily for 3 days. In 17 patients a macrolide-resistant *S. pneumoniae* was isolated and MIC was at >2μg/mL in 12 of the 14 strains detected. Although persistence of *S. pneumoniae* was observed in case of high-level resistance (≥256μg/mL), only one patient with high-level resistance and 3 patients with moderate resistance had poor clinical response. The authors concluded that microbiological and clinical outcome are not always concordant and that in several cases successful monotherapy with azithromycin had been achieved even in the presence of high-level resistance[106].

This paradox between *in vitro* resistance and clinical outcome may be explained in two ways. The first involves macrolide pharmacodynamics and pharmacokinetics. The pharmacodynamic parameters best associated with macrolide activity have not been clarified. For erythromycin and clarithromycin the critical parameter appears to be the time during which the drug concentration is above the MIC, while for azithromycin the ratio AUC/MIC is a better predictor of activity. Current breakpoints tend to underestimate macrolide activity, since a ratio of AUC/MIC >25 is achieved for azithromycin only if MIC is at <0.25 μg/mL, when the sensitivity breakpoint is set at 2 μg/mL[107].

Concentration at the site of infection appears to be more important than that in the plasma. Macrolides are characterized by an excellent pulmonary tissue penetration, in contrast to that in other areas of the body. It is known that macrolides concentrate in the cytoplasm of the macrophages and neutrophils,from where they are subsequently released. These cells thus act as macrolide storage sites[108,109,110]. The concentration of clarithromycin is much higher in epithelial lining fluid (ELF) than in plasma and in addition, clarithromycin has an active metabolite that possible confers to its antimicrobial effect. The concentration of azithromycin in ELF is not as high, but it increases in the case of infection and it is impressively increased in the alveolar macrophages[107]. This advantage of macrolides does not apply in the case of bacteraemia, which may be the reason for the emergence of break-through bacteraemia during the course of treatment with macrolides for infections due to resistant *S. pneumoniae*[104,107,111].

Several authors believe that due to its very long half-life (69 hours), azithromycin is responsible for the emergence of resistance to a greater degree than other macrolides[112]. Azithromycin traces can be detected even 28 days after administration and as a result of this persistance its concentration remains below the MIC for a prolonged period, representing a mutant selection window.

It is of note that the mechanism of resistance may be associated with its clinical relevance. In high-level resistance (caused by *erm B* - MIC≥64μg/mL) the concentrations required in both plasma and ELF are higher than those achieved. In contrast, macrolide concentration at the site of infection may overcome the *mef* mediated resistance[9]. It is thus scientifically correct to evaluate the impact of macrolide resistance on outcome according to the mechanisms of resistance that predominate in that particular region. It is now known that the high-level resistance due to methylation of the binding site is more common in Europe, while the low-level, efflux-mediated resistance is more prevalent in North America[113].

Based on the above evidence the US (IDSA/ATS) guidelines recommend that in regions such as Greece, where the prevalence of highly resistant *S. pneumoniae* is >25% (≥16μg/mL) macrolide monotherapy is not appropriate even for patients without comorbidities, and instead, the administration of combination therapy or monotherapy with a respiratory quinolone is necessary[7]. Accordingly, the Greek guidelines do not recommend empirical monotherapy with macrolides for any group of patients with CAP[88].

A second explanation for the discordance between *in vitro* activity and clinical outcome is the observation that macrolide concentrations below the MIC may affect the virulence of *S. pneumoniae*. Clarithromycin and azithromycin in low concentrations inhibit pneumolysin production and activity *in vitro*, and inhibit pneumolysin production in animal models, thus increasing survival[114]. Roxithromycin modifies the inflammatory response in

animal models, favouring the initial reaction against macrolide-resistant *S. pneumoniae* strains[115].

It is well known that macrolides share a number of properties other than their antimicrobial activity. Their immunomodulatory effects have not only been observed *in vitro* but have been established in the treatment of diffuse panbronchiolitis, bronchiolitis obliterans (BO) syndrome[116] and cystic fibrosis (CF)[116,117]. Rapamycin (sirolimus), which is widely used in transplantation medicine, is a macrolide[118]. The exact mechanisms of these anti-inflammatory properties have not been fully elucidated but it appears that the macrolides can decrease pro-inflammatory cytokine production, inhibit neutrophil chemotaxis and migration[119], reduce mucus production and improve mucus clearance[120]. In addition, rapamycin appears to exhibit antitumour properties[121], while azithromycin initially increases and subsequently decreases the immune response in sepsis[122].

Quinolones

Data on quinolone resistance are sparse, but cases of therapeutic failure in pneumonia due to quinolone-resistant have been reported[123-125]. In a recent observational study on CAP during the period 2002-2006, 38% of outpatients received quinolones (70% moxifloxacin, 19% levofloxacin and 9% ciprofloxacin). Of the 163 isolates none exhibited quinolone resistance, 1.2% contained a first-step mutation and 6.7% exhibited an efflux phenotype. The absence of fluoroquinolone resistance in the context of high-dose usage was attributed by the authors to the frequent use of third-generation fluoroquinolones with enhanced activity against *S. pneumoniae*[126]. It should be noted, however, that in contrast to that of the β-lactams, quinolone resistance, although rare, has a clear and significant clinical impact[125].

It is well known that quinolone resistance may emerge rapidly (within days) even without previous exposure. As described above, quinolone resistance is the result of de novo mutations occurring in two steps. The first-step mutation is fairly frequent. In an infected lung 10^5-10^7 cocci could be expected with the first-step mutation[125]. These mutations are usually silent and do not result in an increase in MIC, and therefore they are often undetected by conventional laboratory methods[127]. However they are of great clinical importance, as the development of a first-step mutation appears to facilitate the emergence of a clinically important high-level, second-step mutation[125]. If the quinolone concentration is not adequate to kill strains with a first-step mutation, then the second-step mutation is likely to occur[125]. Additionally, since first-step mutations are often non-detectable, spreading of these strains may be not be noticed, leading to therapeutic failures.

The choice of the appropriate quinolone should be made with caution. The AUC/MIC ratio is the parameter best associated with quinolone efficacy and for it should exceed 30. The administration of ciprofloxacin (750mg) leads to a AUC/MIC ratio of 21, levofloxacin to a ratio of 36 and moxifloxacin (400mg) to a ratio of 128. Quinolone activity from lowest to highest is: ciprofloxacin < levofloxacin < gatifloxacin < moxifloxacin < gemifloxacin[128].

Choosing the appropriate quinolone is important not only in terms of activity but also in the context of the emergence of resistance. One of the most significant pathways for creating resistant strains is the use of quinolones with limited anti- *S. pneumoniae* activity. The less potent quinolones, such as ciprofloxacin, may enhance the emergence of resistant strains[9], while moxifloxacin has been demonstrated better than levofloxacin *in vitro* whereresistance is concerned[129].

Prevention of the emergence of quinolone resistance is a significant issue as it may lead to adverse clinical outcomes. It is advisable that quinolone use for CAP be limited to certain indications, such as recent use or allergy to β-lactams, probable or established resistance to β-lactams or the possibility of decreased patient compliance with combination treatment.

CONCLUSIONS

The main conclusions on the resistance of *S. pneumoniae* to the three major classes of antibiotics in CAP are:

B-lactams: based on the discordance between microbiologically detected resistance and its clinical consequences, and on the rarity of truly resistant strains, the possibility of penicillin-resistance should not be a leading factor in the choice of the therapeutic regime for CAP.

Macrolides: Despite the lower degree of discordance between microbiological and clinical resistance, the high prevalence and the high level of macrolide resistance in Greece render monotherapy with a macrolide inadvisable for all patients with CAP.

Quinolones: As microbiological resistance to quinolones often leads to adverse clinical outcomes, and based on the fact that prevention of the first-step mutation is of high significance for the emergence of resistance, quinolones should be used with caution and only according to certain specific indications.

Despite the discordance between *in vitro* activity and

clinical outcome, especially in the case of penicillin resistance, decisions about the appropriate antibiotic regimen in CAP should be made with caution. The prevalence of *S. pneumoniae* resistance correlates with the overall use of antibiotics[130]. Justified or not, antibiotic use inevitably leads to microbial resistance. Inappropriate antibiotic use decreases more rapidly the period of time during which specific antibiotics remain useful. Inappropriate use includes administration of antibiotics in non-bacterial infections, such as acute bronchitis, administration of an antibiotic that either does not cover the responsible pathogen or which has a spectrum that is far too wide, and administration of the wrong dosage for the wrong period of time[35]. The use of recommended antimicrobial therapy, not only in CAP, but in general, is very important for the prevention of the emergence of resistant *S. pneumoniae* strains. In this context it is imperative that:

- Antibiotics should be administered only for bacterial infections
- Diagnostic methods, such as procalcitonin[131] measurement, should be used for the recognition of bacterial infections, thus reducing overall antibiotic use
- Antibiotics should be administered in the appropriate dosage and for the right period of time
- The local data on resistance should be taken into account.

Now, more than ever, it is evident that the effective treatment of an infection such as CAP should be accomplished at the lowest possible cost. As P.S. McKinnon pointed out, however, "the most expensive antibiotic is the one that does not work" (ECCMID 2003).

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