New strategies to overcome antimicrobial resistance in 
Streptococcus pneumoniae with β-lactam antibiotics

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The worldwide epidemic of antibiotic resistance in Streptococcus pneumoniae appears to advance inexorably and the measures taken to date to contain its progression have not been successful. Consideration should be given to the complex relationship between antibiotic consumption and resistance to the drug administered and also to other agents in the same and other antibiotic groups. We propose an integrated triple strategy that maximizes the use of the current antibiotic arsenal and is designed to curb the spread of resistance in S. pneumoniae. There are three main parts to this strategy: (i) reduction of prescribing, with particular emphasis on those drugs whose consumption has been shown to correlate strongly with resistance; (ii) development of new formulations or dosing schedules of those drugs whose pharmacodynamic parameters are better suited to cope with highly resistant strains; and (iii) encouragement of the use of antibiotics with the maximal capability of bacterial eradication. We believe such a strategy would reduce the spread of resistance both in the current ecological situation and in the post-pneumococcal vaccination era to come. Of all current antibiotics amoxicillin meets the above requirements, and seems to be the least ecologically disturbing oral antibiotic with regard to resistance in S. pneumoniae.

Introduction

The development of antibiotic resistance in bacteria has accompanied the therapeutic use of antimicrobial agents, with a steady erosion of antimicrobial activity against Gram-positive cocci.1 There is a pandemic of resistance among clinical isolates of Streptococcus pneumoniae,2,3 and pneumococcal disease is among the leading causes of infective mortality in children and the elderly.4,5 S. pneumoniae is the most frequent clinical isolate in respiratory tract infections (RTIs) due to its prevalence in community-acquired pneumonia (CAP) and acute otitis media (AOM) and probably in acute exacerbations of chronic bronchitis (AECB). AOM is the most frequently diagnosed disease in childhood,6 while AECB and CAP are more frequent in the elderly,7 with a 22–51% hospitalization rate,8,9 which can rise to 87% in patients admitted to emergency units.8 Owing to the frequency of these clinical conditions, any intervention directed at prevention or adequate treatment will result in a decrease in the health cost burden.

Community-acquired RTIs are very important in terms of antibiotic consumption, since up to 85–90% of antibiotics are used in the community and, of these, 80% are prescribed for this type of infection.10 The most important factor explaining emergence of resistance (both in nasopharyngeal11 and clinical isolates12,13) in S. pneumoniae is the selective pressure of antimicrobial agents,14 exerted globally in the population11,13 or individually.11 Because many pneumococcal strains are multiresistant, it is not surprising that the consumption of a given antibiotic leads to increased resistance to an unrelated antibiotic,12 with the selective pressure of oral antibiotic use in the management of outpatient RTIs13 being the most important factor.

Penicillin-resistant pneumococci (PRP) seem to have a greater potential to spread from human to human than susceptible strains,11,15 and a few clonal groups with multiple resistance determinants are mainly responsible for this spread, thus giving rise to an endemic multiresistant phenotype in some areas.14,16 The growing prevalence of penicillin resistance and increasing penicillin MICs may result in the loss of efficacy of penicillin even at maximal doses in the future4 (although the current absence of strains with very high MICs suggests that the biological cost associated with a high mutational load...

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could be a self-limiting factor in the spread of these strains\textsuperscript{3)}, making alternative treatment strategies necessary.\textsuperscript{4,17}

Against this background, the importance of the use of antibiotics with the greatest potential to eradicate multiresistant clones (thus curbing their spread within the community) is understandable, since clinical outcome is dependent on bacterial eradication in RTIs.\textsuperscript{2} The maximal reduction in bacterial load (with the ultimate aim of bacterial eradication)\textsuperscript{4} should be achieved in the infectious foci and also at colonization sites, in order to decrease within-host proliferation and between-host transmission of resistant clones.\textsuperscript{2} Inappropriate therapy has been implicated in increased carriage of PRP,\textsuperscript{11} and failure to eradicate bacteria may promote the emergence and dissemination of resistant clones.\textsuperscript{2} The bactericidal effect of an antibiotic is key because those that eradicate rapidly present a lower risk of resistance selection.\textsuperscript{18,19} The more potent the antimicrobial agent, the less likely it is to select for resistance.\textsuperscript{14}

\textbf{Current pneumococcal resistance: the need for new strategies}

Over the past decade, there has been an increase in pneumococcal resistance to the so-called ‘antibiotic group markers’, namely penicillin for \textit{β}-lactams, erythromycin for macrolides and ciprofloxacin for quinolones.\textsuperscript{20} This has compromised the choice of empirical treatments for RTI, as exemplified by the failures described in macrolide-treated RTI caused by erythromycin-resistant \textit{S. pneumoniae}\textsuperscript{21,22} or by ciprofloxacin-resistant \textit{S. pneumoniae} treated with quinolones.\textsuperscript{23} Most authors have not reported failures with PRP and \textit{β}-lactams when these were given at adequate doses,\textsuperscript{24,25} although a recent report found an increased ‘late’ mortality (after the fourth hospital day) in adults with bacteraemic pneumococcal CAP related to penicillin MICs of $\geq 4$ mg/L or cefotaxime MICs of $\geq 2$ mg/L.\textsuperscript{26}

The problem of resistance is heightened by the difficulty in choice among commonly used groups of antibiotics, since resistance to erythromycin is more prevalent among penicillin-resistant strains,\textsuperscript{12,13} and resistance to ciprofloxacin is more prevalent among penicillin-non-susceptible and erythromycin-resistant strains.\textsuperscript{12,27}

The consumption of macrolides and \textit{β}-lactams over the past three decades has been associated with erythromycin and penicillin resistance in \textit{S. pneumoniae} in Spain.\textsuperscript{28} From a different point of view, the geographical correlation between the prevalence of resistance to erythromycin in \textit{S. pneumoniae} isolates from adults with lower RTIs and in \textit{Streptococcus pyogenes} isolates from children with pharyngitis\textsuperscript{12,29} points to the consumption of macrolides as the most likely epidemiological cause of resistance to erythromycin.\textsuperscript{12}

Not all drugs have the same in vitro or epidemiological\textsuperscript{13} capability for selection of resistance in \textit{S. pneumoniae}, which appears to be higher for long half-life macrolides and oral cephalosporins.\textsuperscript{28} Owing to the multiresistance phenomenon, the role of macrolides in penicillin resistance seems greater than that of \textit{β}-lactams in erythromycin resistance, at least geographically.\textsuperscript{13} The overall ‘blame’ for erythromycin-resistant pneumococci and PRP, is, respectively, five- and three-fold higher in relative terms for macrolides than for \textit{β}-lactams at equal consumption.\textsuperscript{13} Among \textit{β}-lactams, aminopenicillins appear to be less responsible for selection of co-resistance (resistance to penicillin plus erythromycin), from both the geographical and historical points of view.\textsuperscript{13,20,28}

In the Spanish multicentre study SAUC-2 (1998–1999), the prevalence of resistance was 22\% for penicillin (with an additional 28\% of intermediate resistance), 35\% for erythromycin and 7\% (MIC $\geq 4$ mg/L) for ciprofloxacin (with an additional 28\% of intermediate resistance for MIC of 2 mg/L).\textsuperscript{12} Using the current NCCLS\textsuperscript{31} breakpoints, prevalence of resistance within \textit{β}-lactams was 5\% for aminopenicillins, 7\% for cefotaxime, 22\% for penicillin, 31\% for cefuroxime and 42\% for cefaclor.\textsuperscript{12} In other words, within oral \textit{β}-lactams, aminopenicillins appear to be the best candidates for developing new strategies to overcome antimicrobial resistance and its spread in the community.

\textbf{Pharmacodynamic considerations}

Empirical therapy is usually clinically based and bacterial eradication is only a secondary consideration. However, there is mounting evidence confirming that bacterial eradication must be the primary goal of antibiotic therapy, since eradication is the main determinant of both therapeutic outcome,\textsuperscript{2,4} and prevention of transmission of resistant clones.\textsuperscript{2} As potency is the product of both antibacterial activity and the ability to deliver the antimicrobial agent in adequate ‘on-site’ concentrations to eradicate the microorganism,\textsuperscript{13} pharmacodynamic indices such as the time for which the antibiotic concentration exceeds the MIC ($T > MIC$) or the ratio between the peak serum concentration and the MIC ($C_{\text{peak}}/\text{MIC}$) can be related to bacterial eradication, subsequent therapeutic outcome and the prevention of resistance.\textsuperscript{2} Mathematical models\textsuperscript{32} indicate that when a level of resistance is achieved in the population there is a lag before a decrease in resistance can be seen following a decrease in antibiotic use. Therefore, therapeutic strategies that influence the pharmacokinetic parameters in the pharmacodynamic equation seem more reliable at eradicating resistant bacteria. Consideration must also be given to the use of the most potent agents in a particular antimicrobial class in order to prevent the spread of resistance.\textsuperscript{14}

In the case of \textit{β}-lactams, in contrast to other antibiotics, resistance is gradual,\textsuperscript{33} because of a series of successive alterations in penicillin targets that decrease \textit{β}-lactam affinity,\textsuperscript{34} making it a concentration-dependent phenomenon.\textsuperscript{4} Owing to

\hspace{1cm} L. Aguilar et al.
New strategies to overcome resistance in *S. pneumoniae*

the molecular basis of pneumococcal resistance to β-lactams, one strategy would be to increase the $T >$ MIC (β-lactams are time-dependent drugs as regards prediction of efficacy) and/or $C_{\text{max}}/\text{MIC}$. 

MIC$_{90}$ in the SAUCE surveillance study cited earlier$^{12}$ was 2 mg/L of aminopenicillins and ≥4 mg/L of oral cephalosporins (8 mg/L of cefuroxime, ≥4 mg/L of cefixime and ≥64 mg/L of cefaclor). Only 5% of isolates were resistant to amoxicillin or co-amoxiclav (MIC ≥ 8 mg/L).$^{12}$ Penicillin non-susceptibility has consistently appeared as a better driver for non-susceptibility to macrolides and cephalosporins than to amoxicillin, because amoxicillin non-susceptibility occurs only in highly penicillin-resistant isolates, whereas cephalosporin and macrolide non-susceptibility clusters in both penicillin-intermediate and -resistant isolates.$^{12}$ With respect to *in vitro* susceptibility, pharmacodynamic indices can be best maximized with aminopenicillins.

From the clinical point of view, a strategy using aminopenicillins also seems logical since a $T >$ MIC of ~40% is needed for efficacy of penicillins; this contrasts with a $T >$ MIC of ~50% for efficacy of cephalosporins$^{2,35}$ in patients with RTIs. In pneumonia, the penetration of β-lactams in lung tissue is very good, and serum concentrations are similar to those at the site of infection, but in the case of AOM, effective therapy depends on adequate concentrations in extracellular middle ear fluid.$^{2,18,36}$ If peri-MIC concentrations of amoxicillin are achieved in middle ear fluid, therapeutic success is obtained in animal models despite values of $T >$ MIC in serum of only 25% of the dosing interval.$^{36,37}$

Once developed, antimicrobial resistance determinants in *S. pneumoniae* are constitutively expressed, and this provides a selective advantage when pneumococci are exposed to antimicrobials.$^{14}$ So, suboptimal doses of antibiotics (caused by inadequate prescribing, failure to comply with medication or insufficient dosage)$^{2,28}$ or repeated antibiotic courses$^{11}$ may promote the resistant clones. The ability to select resistance at specific concentrations (selective windows) allows for the differentiation of agents.$^{39}$ This may help explain the observations that in France and Spain the increase in the prevalence of PRP (MIC ≥ 2 mg/L) was linked to the replacement of aminopenicillins by oral cephalosporins.$^{4,28}$ many of them achieving a $T >$ MIC of <40%, resulting in inadequate killing.$^{40,41}$

Aminopenicillins are *in vitro* selectors of low-level resistance (intermediate resistance) while the cephalosporins tested were good selectors for high-level resistance,$^{25}$ probably due to their pharmacodynamic characteristics and selective antibiotic concentrations.$^{28}$ In Spain, the replacement of intermediate penicillin resistance (MIC 0.12–1 mg/L) by high-level resistance (MIC ≥ 2 mg/L) became evident after the increased consumption of oral cephalosporins,$^{28}$ although the development of a previous intermediate level of penicillin resistance is required for subsequent high-level resistance.$^{28}$ In addition, the risk of mutant selection is greater when the $C_{\text{max}}/\text{MIC}$ is <4 for penicillins compared with ≤16 for cephalosporins.$^{18,43}$ Again, any new strategy to overcome penicillin resistance with β-lactams would be helped by the use of aminopenicillins.

The maximal reduction in bacterial load should be achieved not only in the infectious foci but also at colonization sites in order to decrease the between-host transmission of resistant clones.$^{2}$ Nasopharyngeal carriage and its duration depend on serotype immunity (longer for serotypes inducing immunity poorly,$^{32}$ such as serotypes 6 and 9$^{44}$), age (inversely correlated with its increase), infection (increases during episodes of AOM), season (increases in winter), smoking and crowding.$^{45}$ The carriage of resistant phenotypes is associated with previous antibiotic treatment,$^{45}$ and ranges from 11% to 75%, with an average of 40% in children and 20–30% in adults. The most common serotypes are 6, 14, 19 and 23 in both adults and children, although with different frequencies.$^{45}$ Resistance is clustered in serotypes 6, 19, 23, 9 and 14.$^{45,46}$ The prevalence of serotypes among clinical isolates in Spain in a recent report was 14% for serotype 19, 11% for serotype 6, 10% for serotype 23 and 9% for serotype 14.$^{12}$ Amoxicillin possessed the lowest MIC$_{90}$/MIC$_{50}$ values (<1/4) of all the oral antibiotics tested for serotypes 6, 19, 23 and 9.$^{46}$ This lowers the possibility of selection of resistance in the nasopharynx when compared with oral cephalosporins or macrolides, as shown in prospective studies.$^{15,45}$

**Antibiotic pressure, development of resistance in *S. pneumoniae* and therapeutic failure: a circle?**

Aminopenicillins, in common with other β-lactams, select for PRP present in the nasopharynx, but it is possible that prescribing other antibiotics such as cephalosporins, macrolides or co-trimoxazole is more important$^{13,15}$ because relative selective pressures of different classes of antibiotic are seldom calculated.$^{15}$ In addition, because low-level penicillin resistance began after the consumption of aminopenicillins, this may have led to the overestimation of their responsibility.

In prospective studies of the effect of β-lactams on pneumococcal carriage, although the number of penicillin-susceptible strains is reduced,$^{45,47-49}$ the number of penicillin-non-susceptible *S. pneumoniae* decreases according to the potency of the β-lactam agent,$^{45}$ with the highest PRP reductions once more found with aminopenicillins. However, since the effect is greater against susceptible strains, the final relative resistance rates can only increase. Therefore, we must conclude from this observation that the final resistance increase is misleading since the overall number of subjects with non-susceptible *S. pneumoniae*, which is what really matters, is reduced with aminopenicillins.$^{45,47,48}$

Resistance to penicillin does not influence all β-lactams to the same extent in terms of the prevalence of resistance,$^{12}$ or *in vitro*$^{50-52}$ *ex vivo*$^{53}$ bactericidal activity, with a lower
influence for aminopenicillins compared with cephalosporins. In this way, decreased susceptibility to penicillin affects aminopenicillins to a lesser extent compared with oral cephalosporins in animal models of otitis and sepsis. In the latter, this effect may be due to a higher decrease in blood colony counts by amoxicillin when compared with cefotaxime, as the former requires a lower value of $T > \text{MIC}$ to achieve efficacy.

In the clinical setting, and taking the case of otitis media, the bacteriological failure rate ($<10\%$) of oral cephalosporins increased to $>50\%$ for non-susceptible pneumococci whereas amoxicillin eradicated $70\%$ of penicillin-resistant strains. The effect of cefaclor on PRP was equivalent to that seen with placebo.

Within β-lactams, consumption of oral cephalosporins may account for up to $88\%$ of the temporal evolution of PRP prevalence in Spain. Oral cephalosporins are usually prescribed for streptococcal pharyngitis, and the use of these agents may also be in part responsible for the PRP increase in Northern Ireland. Moreover, regarding the geographical prevalence in Spain. Oral cephalosporins are usually prescribed for streptococcal pharyngitis, and the use of these agents may also be in part responsible for the PRP increase in Northern Ireland. Moreover, regarding the geographical correlation in Spain, the overall blame for PRPs was 1.5 times higher for aminopenicillins compared with cephalosporins, whereas at equal consumption, the situation was reversed (1.3 times higher for cephalosporins than for aminopenicillins). In any case, small changes in consumption of macrolides will have a much greater impact on penicillin resistance than changes in consumption of β-lactams. Newer macrolides are also usually prescribed for streptococcal pharyngitis, despite a $35\%$ prevalence of resistance to erythromycin in S. pyogenes in Spain. Integrating antibiotic pressure, development of resistance and therapeutic failure, a strategy to minimize PRP prevalence is more suitable with aminopenicillins.

**Aminopenicillin strategies to overcome resistance**

In addition to the social actions to be taken concerning antibiotic consumption, with greater efficacy against PRP selection if they are directed at antibiotic groups other than β-lactams, there is a need, with respect to bacterial eradication, therapeutic outcome and resistance spread, to treat PRP with amoxicillin MICs of 4 and 8 mg/L (amoxicillin MIC$_{90}$ for penicillin-susceptible and intermediate pneumococci are 0.03 and 2 mg/L, respectively).

To achieve bacterial eradication of PRP with high MICs and to prevent the spread of these strains, the necessary $T > \text{MIC} \geq 40\%$ of the dosing interval cannot be obtained with current amoxicillin and co-amoxiclav formulations. A pharmacokinetically enhanced formulation of co-amoxiclav (16:1) covering strains with amoxicillin MICs of 4 mg/L for at least 50% of the dosing interval with twice daily administration for adults has been developed. In a previous ‘human-like’ pneumonia model in the rat, in vivo bactericidal activity ($>99.9\%$ decrease in bacterial load compared with controls) was obtained against three strains of S. pneumoniae with amoxicillin MICs of 8 mg/L (against which commercial formulations had no efficacy), with similar efficacy to the commercial formulations (8:1 and 7:1 three times daily) against strains with MICs of 4 mg/L, suggesting that probably a $T > \text{MIC} < 40\%$ is needed for bacterial eradication with aminopenicillins.

Clinical trials with this enhanced formulation are now ongoing to evaluate efficacy against S. pneumoniae strains with an amoxicillin MIC of $\geq 4$ mg/L. Available clinical data on RTI caused by PRP indicate a clinical cure rate of $94.1\%$ (16 of 17 cases in which the co-amoxiclav MIC ranged from 0.5 to 8 mg/L).

Since only 1% of strains in Spain have an MIC $> 8$ mg/L (16 mg/L), the development of this formulation would be a major breakthrough covering practically 100% of PRP, both as oral therapy or in sequential therapy following intravenous co-amoxiclav. In this way, all degrees of severity (from sinusitis to pneumonia) would be covered regardless of penicillin resistance.

In pediatrics, to be effective against strains with decreased susceptibility it is necessary to use high doses (80–100 mg/kg/day) to increase the $T > \text{MIC}$ and decrease the selective window, as recommended by the Centers for Disease Control and Prevention (Atlanta, GA, USA), with administration three times daily, at least in areas with high PRP prevalence. Only amoxicillin at high doses and cefotaxime are reliable against PRP otitis media, although in one study the use of a single dose of ceftriaxone failed to eradicate $\sim 50\%$ of PRP.

Since β-lactams act on the bacterial surface, a synergic effect with respect to bactericidal activity with non-specific immunity may be expected. This effect has been reported with clavulanate and amoxicillin in vitro, and with amoxicillin in vivo. In addition, the in vivo efficacy of subinhibitory concentrations of amoxicillin has recently been reported against a serotype 6B PRP strain in a sepsis model with mice passively immunized prior to inoculation. The $T > \text{MIC}$ associated with efficacy was reduced in the presence of specific antibodies, resulting in more rapid clearance of bacteria from blood in the presence of amoxicillin when specific antibodies were present. This finding may have clinical relevance, since PRP are clustered in infants and the elderly, the two main candidate populations for pneumococcal vaccination.

Even more, the effect of specific antibodies on the outcome of infections in animal models using PRP and treated with amoxicillin (at doses that achieve serum concentrations similar to those obtained with high doses in humans) is worth exploring as a strategy to overcome high resistance. A decrease in the amoxicillin $T > \text{MIC}$ by means of specific antibodies may contribute to a reduction in the spread of high-level resistance.
New strategies to overcome resistance in *S. pneumoniae*

The strategy of enhanced formulations of previously marketed antibiotics is suitable for β-lactams (but not with macrolides for areas where *S. pneumoniae* erythromycin resistance is due to a constitutive mechanism that gives an MIC of ≥64 mg/L).

Another pharmacological strategy to overcome resistance would be to develop new drugs with high intrinsic anti-pneumococcal activity, but this approach is at risk of two handicaps: resistance may progress more rapidly than drug development, and the selective effect of the new drug on resistance in the community would not be recognized until several years after launch of the drug. Considering the circle described above, the new drug has to have a very high intrinsic activity on a weight basis on *S. pneumoniae* to avoid selective action in the community in terms of the pharmacodynamics of resistance.

Conclusions

A pharmacological strategy to overcome resistance in the community must be based on the most potent oral β-lactam available in order to withstand multiresistance, not only at the infectious foci, but also in serotypes currently colonizing the nasopharynx, and those that could colonize the nasopharynx after vaccine introduction in the population. Within each class of antibiotics, different drugs have a different potential to select resistance, and the implications of the carriage of antibiotic-resistant pneumococci should be considered when prescribing an antibiotic for an individual patient. Amoxicillin is the most potent oral β-lactam against *S. pneumoniae* and suitable dosage regimens achieving the optimal $T > MIC$ against strains with MICs of amoxicillin of 4 and 8 mg/L will probably cover nearly 100% of PRPs. This is important since aminopenicillins seem to have the lowest ecological impact on resistance in *S. pneumoniae*.

Acknowledgements

The authors are indebted to: Fernando Baquero (Hospital Ramón y Cajal, Madrid, Spain) and Juan José Granizo (Fundación Jiménez Díaz, Madrid, Spain) for their contribution to the ‘Antibiotic Consumption-Resistance’ Program; the SAUCE (acronym for ‘Sensibilidad a los Antimicrobianos Utilizados en la Comunidad en España’ and the Spanish word for the willow tree) group for their continued efforts in updating resistance prevalence in Spain; and the PRISM-A (Pneumococcal Resistance and Immune System Modulation, and Antibiotics) group: Francisco Soriano and M. Carmen Ponte (Fundación Jiménez Díaz, Madrid, Spain) for their pharmacodynamic animal models, José Prieto and M. Luisa Gómez-Lus (Universidad Complutense, Madrid, Spain) for their studies on the effect of non-specific immunity on antibiotic activity, and Julio Casal and Asunción Fennoll (Centro Nacional de Microbiología, Majadahonda, Madrid, Spain) for their studies on the *in vivo* effect of specific immunity on antibiotic efficacy.

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New strategies to overcome resistance in S. pneumoniae


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