The successful clone: the vector of dissemination of resistance in *Streptococcus pneumoniae*

Keith P. Klugman*

Department of International Health, Rollins School of Public Health and Division of Infectious Diseases, School of Medicine, Emory University, 1518 Clifton Road NE, Room 764, Atlanta, GA 30322, USA; MRC/NHLS/ Wits Respiratory and Meningeal Pathogens Research Unit, Johannesburg, South Africa

A small number of pneumococcal clones dominate the population of antibiotic-resistant pneumococci. The emergence of fluoroquinolone resistance in these clones predicts the dissemination of fluoroquinolone resistance in countries where the clones are present and community use of fluoroquinolones for respiratory infections is common. The molecular basis of resistance to a number of classes of antimicrobials can be used to predict the likelihood of dissemination of the resistance genes.

Introduction

The global emergence of antimicrobial resistance in the pneumococcus does not reflect an even distribution of resistance amongst all pneumococcal strains. A small number of highly successful clones dominate the population of antibiotic-resistant pneumococci. The emergence of resistance to a new class of antimicrobials in one of these resistant clones is therefore more likely to predict the dissemination of that resistance than the emergence of resistance in a strain not belonging to one of these clones. Little attention has been paid to the implications of resistance mechanisms for the subsequent dissemination of resistance genes.

Global antibiotic-resistant clones

A network of interested researchers, constituted under the umbrella of the International Union of Microbiological Societies, has defined the nomenclature of 16 global pneumococcal clones. Each of these strains is characterized by being resistant to at least one antimicrobial in common use; by the persistence of that clone over time; and by its wide geographical diversity within a country or internationally. The suggested nomenclature of pneumococcal clones includes the country in which the clone was first isolated, the serotype first isolated, an international clone number and the subsequent serotype of the isolated strain. An example is Spain 23F-1-19F, which describes a 19F serotype variant of the original, Spanish serotype 23F clone, named international clone 1 (Figure 1). This clone has a global distribution and is resistant to most of the major classes of antimicrobial agents used for the treatment of respiratory tract infections. When authors describe new pneumococcal clones, they are able to contact the network through the website, www.pneumo.com, and once the strains are received by the network, they are considered annually for inclusion in the list of international clones. Strains are subject to clonal analysis using box PCR, pulse-field gel electrophoresis (PFGE) and multi-locus sequence typing. These strains are then available to all researchers in the field through the American Type Culture Collection. While the global distribution of individual clones is well described, there have been few studies to date in which large collections of strains from individual countries have been subject to clonal analysis. In one such study of 328 strains from the United States, the Spain 23F-1-14,19 clone constituted 127 of these penicillin-resistant isolates (38.7%). This represents a remarkable dominance of a single clone across an entire country. A further 12.2% of fully penicillin-resistant strains (40 strains) belonged to the Spain 9V-3-14,19 clone and eight other clones comprised a further 34.1% of the isolates. Overall, 10 clones were responsible for 85% of highly penicillin-resistant pneumococcal disease in that nationwide study. A recent analysis of 672 penicillin-resistant isolates from the USA, isolated between 1994 and 2000, identified 104 different molecular types based on PFGE. In that study, 12 clones constituted 78% of the total. Five of these clones were amongst the global clones described, and it is likely that some of the others may prove to have a global distribution.
Nine of the 12 predominant types were multidrug-resistant clones and it was particularly noted that macrolide resistance increased within the four major clones during the period of the study.3

**Resistance mediated by a single base mutation in a chromosomal gene**

In the pneumococcus, resistance to at least three classes of antibiotic is mediated by a single base mutation in a chromosomal gene. It would be expected that this type of resistance could be easily selected by antibiotic therapy and that widespread use of those antibiotic classes could generate resistance problems. The widespread use of an agent may be an important selector of multiresistant strains, resistant to that agent and other antimicrobials. All of the above hypotheses are supported by published observations.

Pneumococcal resistance to trimethoprim—the more active component of co-trimoxazole against pneumococci—has been shown to be mediated by a single base change at position 100 in the dihydrofolate reductase (DHFR) chromosomal gene.4 The mutation of this nucleotide appears to be essential to resistance. Mutations at other sites in the gene may modify the resistance.5 Resistance to trimethoprim is widespread in pneumococci despite the fact that this drug is infrequently used for respiratory tract infections in developed countries.6 The eight most common clones in the study by Richter et al.3 are all resistant to trimethoprim. Trimethoprim resistance may be selected even by antibiotics with much lower activity against the pneumococcal DHFR enzyme. This includes selection by the antimalarial fansidar, in children carrying pneumococci in the nasopharynx at the time of therapy.7 Therefore, widespread trimethoprim resistance probably reflects the ease with which de novo resistant strains can be selected from a susceptible bacterial population during therapy.7

Pneumococcal resistance can be mediated by a single base change for two other classes of antibiotic that are infrequently used in the treatment of pneumococcal infections. The first is rifampicin. Pneumococcal resistance to rifampicin is mediated by a single base change in the RNA polymerase B gene.8,9 The use of rifampicin in an attempt to eradicate carriage of multiresistant pneumococci was shown to result in the rapid emergence of resistance to that agent,10 which is used only in combination therapy of pneumococcal meningitis to prevent the emergence of resistance.11

A single base change in the ribosomal protein L16 has been shown to confer reduced susceptibility to the novel antimicrobial agent evernimicin.12 This agent, which was previously under development for the treatment of multiply resistant Gram-positive infections, is no longer under development. The discovery of the single base mutation-mediated loss of susceptibility would predict that resistance could have emerged rapidly, had the agent been widely used.

**Emergence of tetracycline resistance in the pneumococcus mediated by the tet(O) gene**

This gene was originally described in 1996 in a clonal cluster from a few children in South Africa.13 Pneumococcal resist-

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**Figure 1.** Global distribution of Spain 23F-1 pneumococcal clone. Also shown is a local distinct Finnish serotype 23F clone and the Tennessee 23F-4 clone.
Clonal dissemination of resistance in S. pneumoniae

ance to tetracycline is overwhelmingly due to the tet(M) gene. Since this clone was resistant only to tetracycline, the use of which in children is extremely rare, rapid dissemination of this type of resistance in the pneumococcus would be unlikely. Indeed, there has only been one subsequent report of tetracycline resistance mediated by the tet(O) gene—from Seattle, in the United States. In an analysis of 277 tetracycline-resistant strains from Europe, none was found to harbour the tet(O) gene. The requirement for acquisition of the gene and the absence of selective pressure probably explain the lack of dissemination of this resistance determinant.

Plasmid-mediated resistance

The pneumococcus is not tolerant of plasmids, and although cryptic plasmids have been described, there has been no description of plasmid-mediated resistance in the pneumococcus to date. The pneumococcus has, however, devised a strategy to transfer plasmid-mediated resistance to the pneumococcal genome. It has recently been shown that chloramphenicol resistance in the pneumococcus, mediated by the acquisition of the gene for chloramphenicol acetyltransferase, appears to have occurred by the linearization of a staphylococcal plasmid into a transposon with the subsequent insertion of the transposon into the pneumococcal chromosome. Although chloramphenicol resistance remains rare due to lack of selective pressure with that agent, the gene may be maintained by co-resistance to other agents in multiresistant clones. It is possible that other plasmid-mediated genes may be transferable into a pneumococcus based on linearization of the plasmid and insertion via a transposon. These mechanisms of resistance could include β-lactamase production and vancomycin resistance, both of which are plasmid mediated. To date, however, neither of these mechanisms of resistance has emerged in pneumococcal strains.

Cephalosporin resistance

In an appropriate genetic background of altered pbp1A and pbp2X genes, a single base mutation at position 550 of the pbp2X gene can confer high-level cephalosporin resistance. In general, the emergence of cephalosporin resistance has been linked to penicillin resistance, and cephalosporin-resistant strains have largely been associated with penicillin resistance. A cephalosporin-resistant pneumococcus that was susceptible to penicillin (MIC 0.06 mg/L), in which the pbp2B gene was identical to that of the susceptible pneumococcal strain, R6, has recently been described. The identification of these strains implies that cephalosporins may select resistant pneumococci from the pool of all susceptible pneumococci, and not just amongst penicillin-resistant strains. This observation and the occurrence of the single base pbp2X mutation in one of the global clones (Tennessee 23F-4) mean that cephalosporin use has a high propensity to select resistance in pneumococcal strains.

Very high-level resistance to the β-lactam drugs

Most highly penicillin-resistant pneumococci are inhibited by penicillin concentrations in the range 2–4 mg/L. There is a concern that very high-level penicillin resistance (penicillin MIC ≥8 mg/L) may be emerging in the USA and elsewhere, particularly in Eastern Europe. The molecular basis of very high-level resistance has not been described in wild-type strains, although there is evidence that the murM gene is implicated in a Hungarian strain with a penicillin MIC of 16 mg/L.

Emergence of fluoroquinolone resistance in the pneumococcus

Fluoroquinolone resistance in the pneumococcus is mediated by changes in the quinolone resistance-determining regions of the parC and gyrA genes. As the population of pneumococci with single base mutations in the parC gene expands, the likelihood of selection of a second mutant in the gyrA gene increases. Of particular concern is the emergence of fluoroquinolone resistance in multiresistant pneumococcal clones. This emergence has been documented in France, Spain and Northern Ireland, as well as in Hong Kong and the USA. It appears that strains with penicillin MICs of 32 mg/L and levofloxacin MICs of 32 mg/L are present in Hungary. Although these extremely high levels of resistance do not currently co-exist in the same strains, they do occur in the same geographical region and the acquisition of both those resistances by a single multiresistant strain would lead to the emergence of a pneumococcus resistant to virtually all first-line classes of antimicrobials in current use.

Conclusions

The expansion of antimicrobial resistance in the pneumococcus is driven by the success of a few multiply resistant clones. The molecular mechanism of resistance may assist in predicting the likelihood of spread of resistance genes. An analysis of the genetic basis of the success of these clones may be critical to our understanding of the future spread of antimicrobial resistance in the pneumococcus. Pneumococcal conjugate vaccines have been shown to interrupt the transmission of antibiotic-resistant strains. It is possible that the success of a limited number of pneumococcal clones has made the pneumococcus vulnerable to attack by the widespread use of conjugate vaccine, which would target the serotypes of the multiresistant clones. Eleven of the 12 dominant clones in the
USA are vaccine serotypes or have the closely related serotype 6A.3 Rational use of antibiotics, surveillance of resistant clones and more widespread use of vaccines that interrupt carriage are, therefore, critical strategies in the control of antimicrobial resistance in the pneumococcus.

References


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