Limiting the Spread of Resistant Pneumococci: Biological and Epidemiologic Evidence for the Effectiveness of Alternative Interventions

STEPHANIE J. SCHRAG,* BERNARD BEALL, AND SCOTT F. DOWELL
Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

INTRODUCTION

Streptococcus pneumoniae infections are a leading cause of respiratory illness in young children, the elderly, and persons with chronic medical conditions. Pneumococcal infections range from otitis media and bacteremia to pneumonia and meningitis. Although penicillin has traditionally been an effective treatment for pneumococcal infections, in recent years the effectiveness of antibiotic therapy has been compromised by the increasing prevalence of penicillin-resistant pneumococci (56). A smaller but growing number of pneumococcal isolates are resistant to multiple antibiotics, leaving vancomycin as a drug of last resort (53).

The clinical impact of pneumococcal resistance varies with the site of infection, the degree of antibiotic penetration to that site, and the ability of the immune response to clear the infection. Pneumococcal resistance can lead to treatment failures in patients with meningitis and acute otitis media (26, 34, 55). The impact of pneumococcal resistance on the treatment of pneumonia has been more difficult to determine (40, 55), although there is recent evidence that increased morbidity and mortality are associated with high-level β-lactam resistance (40, 88).

As antibiotic resistance increases, there is an increasing need for interventions that minimize opportunities for the development and spread of resistant pneumococci. In this review we evaluate proposed interventions to reduce the spread of resistant pneumococci. First we provide a framework for understanding the biological and epidemiologic factors that contribute to the spread of pneumococcal resistance. We then evaluate evidence for the following interventions: judicious antibiotic use programs; modifications of antibiotic treatment regimens; and the use of a conjugate pneumococcal vaccine.

* Corresponding author. Mailing address: Respiratory Diseases Branch, MS-C23, Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333. Phone: (404) 639-4646. Fax: (404) 639-3970. E-mail: zha6@cdc.gov.
We conclude by highlighting key areas for future research into methods of reducing the impact of pneumococcal resistance.

**BIOLOGICAL FOUNDATIONS OF PNEUMOCOCCAL RESISTANCE**

**Ecologic Niche of S. pneumoniae**

The human nasopharynx is the primary reservoir for *S. pneumoniae* and the main source of person-to-person transmission. Pneumococcal infections are preceded by bacterial colonization of the nasopharyngeal mucosa (44, 96), where the bacteria can persist as part of the commensal flora without causing disease. The nasopharyngeal flora is complex, consisting of normally nonpathogenic bacteria such as aerobic α-hemolytic streptococci, anaerobic streptococci, and *Prevotella melaninogenica* in addition to more commonly pathogenic organisms such as *S. pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* (11).

Nasopharyngeal carriage of *S. pneumoniae* is more common in young children than adults and varies by geographic region. Variation in the prevalence of nasopharyngeal carriage may be due to genetic differences in the host that affect the likelihood of nasopharyngeal colonization and also to socioeconomic conditions such as crowding, sanitation, family size, and day care contact (92). The duration of carriage also varies, depending on the host’s age and on the serotype of the colonizing strain, and typically ranges between 1 and 17 months (44).

The factors responsible for the transition from carriage to disease remain poorly understood. In a longitudinal study of infants in Alabama, 15% of colonization events resulted in infection (44). Disruption of natural barriers, for example by damaged bronchial epithelium following influenza virus infection, can lead to invasive pneumococcal infections (75). Some viruses and cytokines also enhance bacterial adherence in vitro and may have a similar effect in vivo (75). Additionally, some strains of *S. pneumoniae* have virulence determinants, for example, different capsular polysaccharide types, that enhance their likelihood of causing disease.

**Genetic Basis of Pneumococcal Resistance**

The genetic basis of resistance plays a key role in determining how resistance develops and spreads. A number of biological features distinguish pneumococci from many other pathogens with acquired drug resistance, such as mycobacteria and *Escherichia coli*. First, both sensitive and resistant pneumococci can be carried asymptptomatically in the nasopharynx, which is also the primary source of person-to-person transmission (46, 86). Second, for the majority of commonly used antibiotics, de novo pneumococcal resistance is rarely the result of single point mutations or plasmid carriage (86). Trimethoprim-sulfamethoxazole resistance and low-level fluoroquinolone resistance are notable exceptions in which resistance conferred by single point mutations is an important mechanism in clinical pneumococcal isolates (Table 1). In contrast, resistance to most major classes of antibiotics (Table 1) is acquired either by transformation (the uptake of free DNA from closely related strains or species, which is then incorporated by homologous recombination) or transfer via conjugative transposons (large segments of DNA encoding antibiotic resistance and self-transfer functions).

Additionally, resistant strains can vary widely in their degree of resistance to certain drugs, a graded phenomenon measured as the MIC of antibiotic required to inhibit growth. Different MICs are associated with different genetic alterations. For the β-lactam antibiotics, high-level resistance requires sequential transformation events which may then be followed by point mutations, whereas intermediate resistance can be conferred by a single transformation event (Table 1). Recently, some clinical pneumococcal isolates were found to survive in the presence of vancomycin, although they could not grow in the presence of the drug and thus were considered tolerant rather than resistant (73). This vancomycin-tolerant phenotype was associated with tolerance to a range of antibiotics in addition to vancomycin (73).

These biological characteristics directly influence the population dynamics of pneumococcal resistance. In contrast to diseases that do not have a carrier state, asymptomatic carriers of pneumococci do not contribute more to pneumococcal transmission than infected individuals. Thus, reducing the number of infected individuals in a population does not necessarily reduce the potential for transmission of resistant strains within the community. Moreover, because the preconditions for acquiring resistance by transformation and conjugative transposons are stringent and de novo resistance does not result from single point mutations for the majority of antibiotic classes, de novo evolution of resistant pneumococci from an initially sensitive strain occurs only rarely. However, evolution of increased MICs in individuals initially infected with intermediate-resistant strains may be possible, particularly for drug classes such as fluoroquinolones, for which increases in MIC can be achieved by single point mutations. For most of the major antibiotic classes, resistant pneumococci spread primarily by clonal amplification (48), and resistant pneumococcal infections result primarily from the acquisition of resistant strains from other nasopharyngeal carriers in the community.

**Association between Resistance Profile and Serogroup**

There are 90 serogroups of *S. pneumoniae*, classified on the basis of antigenic differences in the capsular polysaccharide. Currently, most clinical isolates with high-level resistance to β-lactam antibiotics belong to serogroups 6, 9, 14, 19, and 23 (56). In particular, resistance is associated with serotypes 6B, 9V, 9A, 14, 19F, and 23F. Although each serotype encompasses genetic diversity, resistant isolates often belong to prevalent, well-documented clonal groups (41, 48). For reasons not well understood, a majority of these clonal groups have also acquired resistance to other drugs, including erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline.

It is not yet clear why these particular serotypes have a higher probability of containing antibiotic resistance determinants. One possibility is that these serotypes, which are commonly isolated from children, may be carried for longer durations and thus exposed to increased antibiotic pressure. It is also possible that barriers to transformation play a role in preventing some serotypes from acquiring horizontally spread resistance determinants. The proportion of clinical isolates that are transformable in the laboratory has not been determined. Competence for genetic transformation is a transient property, optimal competence conditions differ between strains, and various capsular types may reduce or totally inhibit transformation. Differences in the competence regulon may limit the acquisition of resistance to particular clonal groups (76, 93).

Despite evidence of a strong association between resistance patterns and serotype, the genes encoding capsular serogroup can also be exchanged between strains by transformation (18). Thus, it is possible that highly resistant clones may become members of serogroups that are currently not associated with
multidrug resistance. The potential for capsular switching between strains with different resistance profiles was recently demonstrated in laboratory mouse experiments in which a multidrug-resistant serotype 23F strain acquired a serotype 3 capsular switching has particular implications for the conjugate pneumococcal vaccine, which confers protection against a limited number of common serotypes.

**LABORATORY METHODS OF DETECTING RESISTANCE**

Laboratory analysis of pneumococcal susceptibility profiles plays a key role in evaluating the impact of interventions to prevent the spread of resistant pneumococci. New molecular methods also shed light on transmission patterns of resistant strains and help identify potentially important genetic events, such as capsular switching, in which strains of a particular lineage acquire the capsular polysaccharide genes associated with an unrelated serogroup. Characterization of susceptibility profiles and serogroup can also help identify how antibiotic pressure leads to the increased incidence of resistant strains. The advantages and disadvantages of various laboratory tests for detecting pneumococcal infection and resistance are summarized in Table 2.

**Transport Medium for Nasopharyngeal Specimens**

Because the nasopharynx is the site of person-to-person transmission of pneumococci, characterizing the susceptibility profile of nasopharyngeal pneumococci is essential to assessing interventions to limit the spread of pneumococcal resistance. A recently developed transport medium for nasopharyngeal specimens enables nasopharyngeal specimens to be preserved for future pneumococcal isolation rather than requiring immediate processing (M. A. Bronsdon, K. L. O’Brien, P. Yagupsky, et al., 38th Intersci. Conf. Antimicrob. Agents Chemother. 1998, abstr. D-5 and D-25). The medium consists of skim milk, treptone, glucose, and glyceral and has been shown to permit high rates of pneumococcal recovery when maintained at cold temperatures. Use of this transport medium facilitates field investigations and large-scale studies of pneumococcal carriage.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genetic basis of resistance</th>
<th>Putative origin</th>
<th>Frequency among isolates resistant to that drug class</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate β-lactam resistance</td>
<td>PBP gene alterations</td>
<td>Transformation with PBP genes from resistant, closely related species</td>
<td>All known penicillin-resistant clinical isolates</td>
<td>46, 86</td>
</tr>
<tr>
<td>High-level penicillin resistance</td>
<td>PBP gene alterations involving at least ( pbp1a, pbp2x ) and ( pbp2b )</td>
<td>Sequential transformation events, can also be followed by spontaneous mutation events conferring incremental resistance</td>
<td>All known clinical isolates have mosaic structures for 3 PBP genes; altered ( pbp2b ) gene required for high-level penicillin resistance</td>
<td>46, 86</td>
</tr>
<tr>
<td>High-level extended-spectrum cephalosporin resistance</td>
<td>PBP gene alterations involving ( pbp1a ) and ( pbp2x )</td>
<td>Transformation may occur by a single transformation event through cotransformation of closely linked ( pbp1a ) and ( pbp2x ) resistance-conferring alleles</td>
<td>All known clinical isolates have mosaic forms of ( pbp1a ) and ( pbp2x ); ( pbp2b ) is not a target for extended-spectrum cephalosporins</td>
<td>47</td>
</tr>
<tr>
<td>Intermediate and high-level trimethoprim resistance</td>
<td>Dihydrofolate reductase (( dhf )) gene mosaics and/or point mutant alleles</td>
<td>Transformation or single point mutation</td>
<td>Both mechanisms appear common; potential ( dhf ) mosaics possibly more frequently observed than simple alterations involving 3 or fewer bases</td>
<td>1, 41, 80</td>
</tr>
<tr>
<td>Intermediate erythromycin resistance</td>
<td>( mefE ) efflux mechanism</td>
<td>Unknown, probably originated through transformation or conjugative transfer from another species, since ( mefE ) is not found in sensitive strains</td>
<td>Common</td>
<td>85</td>
</tr>
<tr>
<td>High-level erythromycin resistance</td>
<td>( ermAM ) gene</td>
<td>Conjugative transfer of transposons, including Tn1545 and Tn1545 deletion derivatives, and Tn3872</td>
<td>Common</td>
<td>21</td>
</tr>
<tr>
<td>High-level tetracycline resistance</td>
<td>( tetM )</td>
<td>Conjugative transfer of Tn1545 and its derivatives and Tn253</td>
<td>Majority of ( tetR ) isolates</td>
<td>6</td>
</tr>
<tr>
<td>High-level tetracycline resistance</td>
<td>( tetO )</td>
<td>Unknown, probably transformation or transposition</td>
<td>Less common</td>
<td>94</td>
</tr>
<tr>
<td>High-level chloramphenicol resistance</td>
<td>( cat ) gene</td>
<td>Conjugative transfer of Tn253, common</td>
<td>Common</td>
<td>6</td>
</tr>
<tr>
<td>Low-level fluoroquinolone resistance</td>
<td>Point mutations in DNA topoisomerase IV subunit genes (( parC ) and/or ( parE ))</td>
<td>Single point mutation or transformation</td>
<td>Identified only in some serotype 9V isolates</td>
<td>41, 52</td>
</tr>
<tr>
<td>High-level fluoroquinolone resistance</td>
<td>( parC ) and ( gyrA ) double mutants</td>
<td>Multiple point mutations or transformation</td>
<td>Identified only in some serotype 9V isolates</td>
<td>41</td>
</tr>
<tr>
<td>Vancomycin tolerance</td>
<td>( vncS )</td>
<td>Newly recognized, details not currently known</td>
<td>Identified only in some serotype 9V isolates</td>
<td>73</td>
</tr>
</tbody>
</table>

**TABLE 1. Genetic mechanisms of pneumococcal resistance to various classes of antimicrobial agents**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genetic basis of resistance</th>
<th>Putative origin</th>
<th>Frequency among isolates resistant to that drug class</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate β-lactam resistance</td>
<td>PBP gene alterations</td>
<td>Transformation with PBP genes from resistant, closely related species</td>
<td>All known penicillin-resistant clinical isolates</td>
<td>46, 86</td>
</tr>
<tr>
<td>High-level penicillin resistance</td>
<td>PBP gene alterations involving at least ( pbp1a, pbp2x ) and ( pbp2b )</td>
<td>Sequential transformation events, can also be followed by spontaneous mutation events conferring incremental resistance</td>
<td>All known clinical isolates have mosaic structures for 3 PBP genes; altered ( pbp2b ) gene required for high-level penicillin resistance</td>
<td>46, 86</td>
</tr>
<tr>
<td>High-level extended-spectrum cephalosporin resistance</td>
<td>PBP gene alterations involving ( pbp1a ) and ( pbp2x )</td>
<td>Transformation may occur by a single transformation event through cotransformation of closely linked ( pbp1a ) and ( pbp2x ) resistance-conferring alleles</td>
<td>All known clinical isolates have mosaic forms of ( pbp1a ) and ( pbp2x ); ( pbp2b ) is not a target for extended-spectrum cephalosporins</td>
<td>47</td>
</tr>
<tr>
<td>Intermediate and high-level trimethoprim resistance</td>
<td>Dihydrofolate reductase (( dhf )) gene mosaics and/or point mutant alleles</td>
<td>Transformation or single point mutation</td>
<td>Both mechanisms appear common; potential ( dhf ) mosaics possibly more frequently observed than simple alterations involving 3 or fewer bases</td>
<td>1, 41, 80</td>
</tr>
<tr>
<td>Intermediate erythromycin resistance</td>
<td>( mefE ) efflux mechanism</td>
<td>Unknown, probably originated through transformation or conjugative transfer from another species, since ( mefE ) is not found in sensitive strains</td>
<td>Common</td>
<td>85</td>
</tr>
<tr>
<td>High-level erythromycin resistance</td>
<td>( ermAM ) gene</td>
<td>Conjugative transfer of transposons, including Tn1545 and Tn1545 deletion derivatives, and Tn3872</td>
<td>Common</td>
<td>21</td>
</tr>
<tr>
<td>High-level tetracycline resistance</td>
<td>( tetM )</td>
<td>Conjugative transfer of Tn1545 and its derivatives and Tn253</td>
<td>Majority of ( tetR ) isolates</td>
<td>6</td>
</tr>
<tr>
<td>High-level tetracycline resistance</td>
<td>( tetO )</td>
<td>Unknown, probably transformation or transposition</td>
<td>Less common</td>
<td>94</td>
</tr>
<tr>
<td>High-level chloramphenicol resistance</td>
<td>( cat ) gene</td>
<td>Conjugative transfer of Tn253, common</td>
<td>Common</td>
<td>6</td>
</tr>
<tr>
<td>Low-level fluoroquinolone resistance</td>
<td>Point mutations in DNA topoisomerase IV subunit genes (( parC ) and/or ( parE ))</td>
<td>Single point mutation or transformation</td>
<td>Identified only in some serotype 9V isolates</td>
<td>41, 52</td>
</tr>
<tr>
<td>High-level fluoroquinolone resistance</td>
<td>( parC ) and ( gyrA ) double mutants</td>
<td>Multiple point mutations or transformation</td>
<td>Identified only in some serotype 9V isolates</td>
<td>41</td>
</tr>
<tr>
<td>Vancomycin tolerance</td>
<td>( vncS )</td>
<td>Newly recognized, details not currently known</td>
<td>Identified only in some serotype 9V isolates</td>
<td>73</td>
</tr>
</tbody>
</table>
Hybridization using PCR detection of \( \text{VOL. 13, 2000 LIMITING THE SPREAD OF RESISTANT PNEUMOCOCCI 591} \)

result that is not always adequate for characterization of labor-intensive to perform. However, it yields a qualitative simple, does not require specialized equipment and is not possible based on known breakpoints. This test is technically zation of organisms as sensitive or resistant to particular agents not be used to estimate MICs directly (95). Instead, categori-
on the rate of diffusion of the agent through the agar and on inversely related to the MIC, but because this method depends on the zone of inhibition intersects with the strip. This test combines the simplicity of disk diffusion with the quantitative results of dilution methods. However, the preformed plastic strips are significantly more expensive than the reagents necessary for the above methods.

An additional limitation of culture-based methods for esti-
mating MICs is that they cannot detect vancomycin-tolerant strains, which would be misclassified as sensitive because of their inability to grow in the presence of vancomycin.

**Rapid Methods for Detecting Resistance**

Currently, isolating pneumococci from clinical specimens and determining penicillin susceptibility takes 2 to 3 days. More rapid approaches are being developed that will allow identification of pneumococcal isolates and resistance profiles in shorter times. Some approaches still require a primary bacterial isolation step, whereas others do not. Unique features of these more rapid methods under development are summarized in Table 2.

<table>
<thead>
<tr>
<th>Method</th>
<th>Potential uses</th>
<th>Benefits</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture-based antibiotic dilution</td>
<td>Determination of MICs; appropriate for all drugs</td>
<td>Gives precise MIC results; proven reliability</td>
<td>Slow compared to molecular methods; Cannot detect vancomycin tolerance</td>
</tr>
<tr>
<td>PCR using “sensitive” and “resistant” ( \text{pbp2b} ) and ( \text{pbp1a} ) oligonucleotides</td>
<td>Diagnosis of pneumococcal infection and detection of resistance or susceptibility to penicillin</td>
<td>Potentially very rapid for simultaneous pneumococcal diagnosis and deduction of ( \beta )-lactam resistance/sensitivity or for latter use alone</td>
<td>Ability to detect entire array of penicillin resistance alleles unknown</td>
</tr>
<tr>
<td>PBP gene fingerprinting</td>
<td>Diagnosis of pneumococcal infection and detection of resistance or susceptibility to ( \beta )-lactam antibiotics</td>
<td>Potentially rapid for simultaneous pneumococcal diagnosis and deduction of resistance/sensitivity to ( \beta )-lactam antibiotics or for latter use alone; useful as stable, discriminating marker for molecular epidemiology; certain PBP gene fingerprints very common and reliably indicate high level of resistance</td>
<td>Some mosaic alleles can give “false-sensitive” results</td>
</tr>
<tr>
<td>( \text{dbhf} ) gene fingerprinting</td>
<td>Screening of trimethoprim-sulfamethoxazole resistance</td>
<td>Potentially rapid method for deduction of resistance; useful as stable, discriminating marker for molecular epidemiology; certain ( \text{dbhf} ) fingerprints are very common and reliably indicate high level of resistance to trimethoprim-sulfamethoxazole</td>
<td>Method not sensitive enough to detect point mutations in ( \text{dbhf} ) conferring resistance</td>
</tr>
<tr>
<td>PCR detection of resistance genes using specific primers</td>
<td>Determination of resistance to erythromycin, tetracycline, kanamycin, and chloramphenicol</td>
<td>Rapid and reliable for determining resistance (positive PCR result) or probable sensitivity (negative PCR result) of identified organism in pure culture</td>
<td>Negative PCR result requires internal positive PCR control</td>
</tr>
<tr>
<td>Hybridization using specific gene probes</td>
<td>Determination of resistance to erythromycin, tetracycline, kanamycin, and chloramphenicol</td>
<td>Rapid and reliable for determining resistance (positive PCR result) or probable sensitivity (negative PCR result) of identified organism in pure culture</td>
<td>Negative hybridization result requires internal positive hybridization control</td>
</tr>
</tbody>
</table>

**Culture-Based Methods**

The National Committee for Clinical Laboratory Standards recommends the agar and broth antibiotic dilution techniques for measuring pneumococcal MICs. These methods require isolating the pneumococcus as a pure culture and an additional culture step for the actual susceptibility determination. Antimicrobial agents are typically tested at twofold serial dilutions, and the lowest concentration that inhibits bacterial growth is the MIC. MICs estimated by these tests are highly accurate and the lowest concentration that inhibits bacterial growth is

\[
\text{MIC} = \frac{1}{2^n}
\]

where \( n \) is the number of dilutions. MICs estimated by these tests are highly accurate and important in determining appropriate antibiotic therapy.

**Determination of MICs**

- **Disk Diffusion Method** - A simple, inexpensive method used to determine MICs. However, its accuracy is limited by variations in growth rates and the presence of nonsusceptible strains.
- **Broth Microdilution Method** - A more precise method that uses a broth-based medium to determine MICs. However, it requires specialized equipment and is labor-intensive.
- **Agar Dilution Method** - Another method used to determine MICs. It involves inoculating the test organism onto an agar plate and adding different concentrations of antibiotics. The MIC is determined as the lowest concentration of antibiotic that inhibits bacterial growth.

**Rapid Methods for Detecting Resistance**

Currently, isolating pneumococci from clinical specimens and determining penicillin susceptibility takes 2 to 3 days. More rapid approaches are being developed that will allow identification of pneumococcal isolates and resistance profiles in shorter times. Some approaches still require a primary bacterial isolation step, whereas others do not. Unique features of these more rapid methods under development are summarized in Table 2.

**PCR for \( \beta \)-lactam and trimethoprim-sulfamethoxazole resistance** - PCR-based methods can provide rapid assessment of
pneumococcal resistance profiles and information on the relatedness of various resistant isolates. To identify pneumococcal isolates and distinguish among penicillin-resistant and -sensitive strains, PCR protocols using oligonucleotides specific to sensitive and nonsusceptible penicillin-binding protein (PBP) gene allele sequences have been developed (36, 37, 89). Although potentially a powerful approach, these methods have not yet been tested using a defined strain collection that includes major internationally dispersed clones.

Another rapid method under development is based on observations that DNA sequences of genes associated with penicillin and trimethoprim-sulfamethoxazole resistance are highly uniform in sensitive pneumococcal isolates, whereas they vary among resistant isolates. Consequently, resistance genes specific amplicons generated by PCR can in most cases be distinguished from sensitive amplicons by restriction enzyme cleavage patterns. Fingerprinting of PBP genes, associated with penicillin resistance, and of the dhf gene, associated with trimethoprim-sulfamethoxazole resistance, in combination with a method such as pulsed-field gel electrophoresis that reflects overall genomic relatedness between isolates provides a tool for rapidly distinguishing sensitive and resistant pneumococci (41). Isolates that are identical by a method that assesses overall genetic relatedness (e.g., multilocus enzyme electrophoresis, pulsed-field gel electrophoresis, or multilocus sequence typing) and that have the same altered PBP and dhf genes are considered clonal. In contrast, strains with identical mosaic resistance genes (resistance genes with multiple alterations) in genetically unrelated backgrounds are likely to have arisen by horizontal transfer of PBP genes or dhf genes. Similarly, strains with different serotypes that are otherwise identical in terms of resistance genes and pulsed-field gel electrophoresis results most likely arose by horizontal transfer of capsular type-specific genes. While this method works well for mosaic resistance genes that result from transformation, it is unable in most instances to detect point mutations.

Thus, PCR analysis of PBP genes using pneumococcus-specific primers has the potential to permit rapid detection of pneumococci in clinical specimens and assessment of β-lactam and trimethoprim-sulfamethoxazole susceptibility profiles. However, because PCR-based methods of susceptibility determination cannot generally quantify MICs, culture-based microdilution methods remain the gold standard.

Tetracycline, erythromycin, and chloramphenicol resistance. PCR- or hybridization-based methods can detect resistance to tetracycline, erythromycin, kanamycin, and chloramphenicol because the presence of gene sequences encoding resistance is almost always associated with a resistance phenotype. Such methods are more rapid than culture-based methods, but they require pure cultures to diagnose pneumococcal infection because of the wide dissemination of these resistance genes among different bacterial species. For tetracycline resistance, tetM- and tetO-specific probes or primers can be used (67, 94). For resistance to erythromycin, ermAM- or mefE-specific probes or primers can be used, with the former used to predict clindamycin and streptogramin B resistance (84). DNA-based detection of chloramphenicol and kanamycin resistances would rely on cat and apha3 gene probes or primers, respectively (21, 81, 87). Alternatively, Tn1545- and Tn5253-specific sequences are usually associated with specific patterns of resistance (6, 21), so single hybridization probes or primer sets can often be used to deduce multiple resistances.

Detecting Carriage of Multiple Strains

Early investigations of colonization by multiple pneumococcal strains attempted to amplify potential minority clones by inoculating specimens into mice. A current standard approach to identifying individuals who carry multiple pneumococcal strains with different resistance profiles is to characterize multiple colonies from a single nasopharyngeal specimen. However, if one strain predominates and other strains are present at very low densities, representing, for example, less than 1% of the total pneumococcal population, the number of colonies that need to be characterized from a single nasopharyngeal swab becomes unwieldy by conventional procedures. A pioneering study of multiple colonization by S. pneumoniae and H. influenzae in children in Papua New Guinea attempted to circumvent this problem by plating nasal specimens directly onto large (11-cm diameter) plates and serotyping 50 suspect pneumococcal colonies per specimen (43).

To assess whether an individual carries a minority resistant pneumococcal population, an antibiotic selective plating approach can be a useful alternative. At the initial pneumococcal isolation step, in addition to plating the nasopharyngeal specimen on a medium favorable for pneumococcal growth (typically a blood agar medium supplemented with gentamicin to prevent growth of gram-negative bacteria), the specimen can also be plated on a medium additionally supplemented with the antibiotic of interest, for example, penicillin. Individuals are identified as carrying a minority resistant strain if the isolates obtained from the nonselective plate are susceptible to penicillin and the isolate from the selective plate is confirmed by the MIC as being penicillin resistant. If the nasopharyngeal specimen is initially stored in an appropriate transport medium, selective plating can be reserved for individuals identified as carrying a dominant sensitive population.

A powerful approach would be to combine replica plating with colony DNA hybridization to detect minority populations containing specific resistance genes. In particular, this holds promise for gene targets such as ermAM, tetM, mefE, and cat, which are not found in sensitive pneumococcal populations.

EPIDEMIOLOGY OF PNEUMOCOCCAL RESISTANCE

Association between Recent Antibiotic Use and Carriage of Resistant Pneumococci

Although the genetic and environmental mechanisms that result in pneumococcal resistance are complex, the primary risk factor for the carriage and spread of resistant pneumococci is clear. Epidemiologic studies have repeatedly identified recent antibiotic use as the strongest risk factor for the carriage and spread of resistant pneumococci, at both the community (2, 7, 57) and individual (30, 35, 62) levels.

Moreover, among patients with invasive disease, recent antibiotic use correlates with an increased risk of infection with nonsusceptible (intermediate and resistant) pneumococci (35). This suggests not only that recent antimicrobial use increases the risk that an individual will carry, and therefore potentially transmit, resistant pneumococci, but that among infected individuals, it also increases the risk that individuals will develop invasive pneumococcal illness caused by resistant strains.

Additional risk factors for resistant pneumococcal carriage identified by observational studies include young age (with highest carriage among individuals <1 year old) and attendance at day care centers (3, 30, 33, 49, 58). Human immuno-
deficiency virus (HIV) infection may also be a risk factor in some populations, for example, urban areas of South Africa (54).

**Mechanisms Responsible for Association between Antibiotic Use and Carriage**

The biological mechanisms resulting in the association between recent antibiotic use and carriage of resistant *S. pneumoniae* have not been well studied. Possible mechanisms fall into three broad categories: the unmasking of a resistant pneumococcal clone that was present at a low density in the nasopharynx prior to antimicrobial therapy; replacement of sensitive pneumococci with resistant pneumococci acquired from the community during or after antimicrobial therapy; and within-host evolution of a resistant pneumococcal population in response to the selective pressure of antimicrobial therapy. Distinguishing features of these alternative mechanisms are noted in more detail in Table 3. A better understanding of which of these mechanisms generate the observed association will provide an important framework for developing interventions to prevent the spread of resistance. For example, if replacement of sensitive strains with resistant strains from the community is the primary mechanism, then antibiotics that clear the nasopharynx may increase the risk of resistant carriage more than antibiotics that do not. In contrast, if unmasking is the primary mechanism, antibiotics that clear the nasopharynx of both sensitive and resistant strains may be the most effective treatment. The type of treatment least likely to select for resistance may vary with the prevalence of resistant strains in the community. This prevalence influences both the risk of carrying multiple pneumococcal strains and the risk of acquiring resistant strains during and after treatment.

Longitudinal studies of nasopharyngeal carriage before and after the initiation of antibiotic treatment provide data that can help to distinguish between alternative mechanisms for the increased risk of carrying resistant pneumococci following antimicrobial treatment (Table 3). Two recent studies of nasopharyngeal carriage among children undergoing antibiotic therapy suggest that initially sensitive strains very rarely acquire resistance during antimicrobial therapy (19, 28). There is some evidence, however, that selection for genetic alterations increasing resistance may increase the MICs for initially non-susceptible strains. The most common mechanism behind the association of antibiotic use and carriage of resistance, however, appeared to be either unmasking of a minority subpopulation of resistant strains or acquisition of new resistant strains during or after antimicrobial therapy (19, 28). A study that directly evaluated evidence for the unmasking hypothesis found an elevated rate of transition from carriage of susceptible to nonsusceptible strains in children undergoing antibiotic treatment but not in a control group, but the rate of transition from no pneumococci to carriage of nonsusceptible pneumococci was not elevated (39). This suggests that unmasking of resistant strains played a role in generating the association between recent antibiotic use and carriage of resistant strains. Although the sensitivity of the screening method for minority resistant clones in this study was low, 3.6% of children with sensitive pneumococci were found to carry minority resistant populations before treatment, and in one case DNA fingerprinting was able to document that the minority clone present at baseline was the
resistant clone that predominated in the nasopharynx post-treatment (39).

JUDICIOUS ANTIBIOTIC USE PROGRAMS

Rationale

The identification of recent antibiotic use as the primary risk factor for carriage of resistant pneumococci has led to the development of control and prevention programs that promote more judicious antibiotic use. Inappropriate prescription of antibiotics contributes to a large proportion of unnecessary antibiotic use in both developed and developing countries. For example, studies of antibiotic prescribing in the United States have documented that antibiotics are often prescribed for the treatment of viral upper respiratory infections (42). There is evidence from several countries that rates of antibiotic use correlate directly with the prevalence of resistant pneumococcal strains (2, 7, 57). For example, a cross-sectional study of antimicrobial consumption and the carriage of penicillin-resistant pneumococci in children in five different communities in Iceland found that children in communities with higher levels of antimicrobial consumption were at higher risk for nasopharyngeal carriage of resistant pneumococci (2). Similarly, a longitudinal analysis of sentinel surveillance for invasive pneumococcal disease in the United States found that increases in the prevalence of penicillin-resistant pneumococci in the early 1990s correlated with increased prescription rates for certain β-lactam agents, while a fairly constant prevalence of tetracycline-resistant pneumococci was associated with slight decreases in the prescription rate for tetracycline (12).

Will a decline in antibiotic use resulting from judicious antibiotic use campaigns necessarily lead to declines in the prevalence of resistant pneumococci? If resistant strains are rare enough in the population that they may be lost through stochastic changes or if resistant strains have a fitness disadvantage relative to sensitive strains in the absence of antibiotics, reducing antibiotic selective pressure is expected to lead to a reduced prevalence of resistant strains. Few studies have directly addressed fitness disadvantages (for example, growth rate or competition disadvantages) associated with resistance in pneumococci. For the case of penicillin-resistant pneumococci, which result from alterations in essential cell wall synthesis enzymes called PBPs, it would seem likely that alterations in these enzymes result in growth deficiencies. However, resistant clinical isolates with PBP alterations not only maintain their resistance during extensive laboratory passaging in the absence of drug selection, but appear to be unaffected in general physiology (51). To address these and other issues of pneumococcal resistance, empiric observations of the impact of decreasing antibiotic selection pressure on the prevalence of resistant strains are necessary.

Impact on Resistance

Countrywide initiatives to reduce antibiotic resistance by reducing antibiotic use were attempted in Finland for the case of macrolide-resistant group A streptococci (GAS) and in Iceland for the case of penicillin-resistant S. pneumoniae. GAS have some features in common with S. pneumoniae, including a carriage state and a similar genetic mechanism of macrolide resistance. In Finland, in response to a dramatic rise in the prevalence of erythromycin-resistant GAS from 5% in 1988 to 13% in 1990, national guidelines recommending reductions in the outpatient use of macrolide antibiotics were issued in 1991. Total macrolide consumption in outpatient therapy declined from 2.4 daily doses per 1,000 inhabitants in 1991 to a level of 1.4 in 1992; the daily doses per 1,000 inhabitants remained between 1.3 and 1.7 during the next 5 years (82). From 1992 to 1996 the prevalence of erythromycin-resistant GAS isolates also decreased, from 16.5% in 1992 to 8.6% in 1996. This decline was evident after 1993, approximately 2 years after the release of national guidelines. Thus, a reduction in antibiotic use was temporally associated with a subsequent decline in the prevalence of resistant GAS strains. Although the prevalence of erythromycin-resistant GAS declined, it remained close to 9%; moreover, there was an increase in the consumption of azithromycin and roxithromycin that correlated with the decline in erythromycin use.

In Iceland, the incidence of penicillin-nonsusceptible pneumococci among patients with invasive pneumococcal disease increased from 0% in 1988 to 16.3% in 1992 (K. G. Kristinsson, M. A. Hualmarsdottir, and T. Gudnason, 35th Intersci. Conf. Antimicrob. Agents Chemother. 1995, abstr. C9). A majority of penicillin-nonsusceptible strains were also resistant to macrolide antibiotics and to trimethoprim-sulfamethoxazole. The authorities responded to this rapid increase in nonsusceptible pneumococci by launching a public health campaign against the overuse of antibiotics that was aimed both at the public and at physicians. Additionally, children undergoing antibiotic therapy and known carriers of nonsusceptible pneumococci with colds or coughs were discouraged from going to day care centers. The number of daily doses of antibiotics in Iceland decreased from 23.6 per 1,000 inhabitants in 1990 to 21.4 in 1994, due largely to decreased use of macrolides and trimethoprim-sulfamethoxazole (Kristinsson et al., abstr.). Furthermore, in contrast to the steady increases of previous years, the incidence of penicillin-nonsusceptible invasive pneumococcal isolates declined from a peak of 19.8% in 1993 to 18.6% in 1994; among pneumococcal carriers, the proportion of healthy children attending day care who were colonized by a nonsusceptible strain also decreased from 20% in 1992 to 16% in 1995 (Kristinsson et al., abstr.). Thus, these data suggest that decreased antibiotic use reversed the trend of an increasing incidence of nonsusceptible strains in Iceland, although the reductions in both antibiotic consumption and the incidence of resistant pneumococci were not as striking as those observed for GAS in Finland.

Two recent controlled studies assessed the impact of well-defined judicious antibiotic use interventions on antibiotic prescribing practices and the incidence of resistant pneumococcal strains. The impact on antibiotic prescription rates of an intervention targeting four adult primary care practices in Denver, Colorado, was evaluated using a prospective, nonrandomized controlled trial design (42). The intervention, which was aimed at reducing antibiotic prescribing for outpatient adults diagnosed with uncomplicated acute bronchitis, consisted of household and office-based patient educational materials, clinician education, practice profiling, and academic detailing. During the 1-year study period, practices that received the full intervention showed a significant decline in antibiotic prescription rates, from 74 to 48%, whereas no significant decline was detected for control sites (42). Practices which received only a limited intervention, which consisted solely of office-based educational materials, also did not show significant declines. While this study did not address the impact of reduced prescription rates on the incidence of resistant pneumococci, it does suggest that active education of clinicians about the importance of judicious antibiotic use can lead to reduced rates of antibiotic prescription for specified conditions.

**TABLE 4. Methods for preventing the development and transmission of resistance due to antimicrobial therapy**

<table>
<thead>
<tr>
<th>Method</th>
<th>Carrier state uncommon&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Carrier state common&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avoid unnecessary antibiotics</td>
<td>Minor concern</td>
<td>Major concern</td>
</tr>
<tr>
<td>(e.g., for viral infections)</td>
<td>Longer treatment preferred over short course to ensure that pathogen has been eradicated; failure to eradicate results in clinical treatment failure</td>
<td>Short-course therapy may be advantageous because the time period during which an individual may acquire a resistant strain is reduced; eradication of pathogen from site of carriage not a practical therapeutic goal</td>
</tr>
<tr>
<td>Modify treatment duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use high dosages</td>
<td>High dosages can eradicate both intermediate- and high-level-resistant pathogens, facilitating eradication and reducing opportunity for resistant mutants to proliferate</td>
<td>Impact on bacterial carriage unknown; eradication from site of carriage not a practical therapeutic goal, even for high-dose therapy</td>
</tr>
<tr>
<td>Treat with drugs that minimize selection for resistance</td>
<td>Likely to have only minor impact; drugs which do not exert strong selective pressure typically less efficient at eradication</td>
<td>Likely to have only minor impact; may reduce probability of unmasking of minority resistant strains or acquisition of resistant strains from the community</td>
</tr>
<tr>
<td>Treat with multiple drugs</td>
<td>Resistance often due to point mutations, insertions, deletions; likelihood of a single genetic event conferring resistance to multiple drugs is the product of separate likelihoods</td>
<td>Impact unknown; may not be effective against pneumococci because de novo resistance due to point mutations alone is rare except for some forms of trimethoprim-sulfamethoxazole and low-level fluoroquinolone resistance</td>
</tr>
<tr>
<td>Cycling drugs</td>
<td>Impact unclear; may be useful for hospital-acquired infections</td>
<td>Unlikely to be effective because the majority of infections are community acquired and because pathogens are often exposed to drugs used for treatment of unrelated conditions</td>
</tr>
<tr>
<td>Encourage adherence to treatment regimen</td>
<td>Erratic treatment allows pathogen to proliferate at subtherapeutic MICs, increasing the opportunity for resistant mutants to amplify</td>
<td>Unclear whether poor compliance increases or decreases the risk of carrying resistant pneumococci</td>
</tr>
</tbody>
</table>

<sup>a</sup> Carrier state refers to pathogen populations commonly present asymptomatically in the nasopharynx, vagina, or intestinal tract serving as primary reservoirs for disease transmission. While diseases such as AIDS and tuberculosis have latent periods, these periods are associated with low levels of pathogen replication and are thus not associated with high risk of acquiring resistance or transmitting the disease. Examples of pathogens not generally known to be carried nonsymptomatically include *Plasmodium falciparum*, *Shigella* spp., and *M. tuberculosis*. The goal for these pathogens is to prevent the development of resistance during treatment.

<sup>b</sup> Examples of pathogens known to be carried nonsymptomatically include *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and GAS. The goal for these pathogens is to prevent the acquisition or amplification of resistant strains at the site of carriage during treatment.

---

abstr. 67) was designed to evaluate both antibiotic prescribing rates and the prevalence of nonsusceptible pneumococci in villages receiving the intervention versus control villages. The intervention focused on otitis media and consisted of an educational program for village health aides and their consultants, workshops on the appropriate diagnosis of otitis media, and information sheets to providers and village residents. Preliminary results suggest that respiratory health care visits for children <5 years of age decreased by 23% and antimicrobial prescriptions per respiratory visit decreased by 22% in the intervention region, while no declines were evident in control villages that did not receive the intervention (78). Additionally, although the overall prevalence of pneumococcal carriers did not decrease in the intervention area, the proportion of all pneumococcal isolates that were nonsusceptible to penicillin (MIC, ≥0.12 μg/ml) declined by 28% in the intervention region but did not decline significantly in the control regions (78).

Thus, studies from the village to country level demonstrate that judicious antibiotic use interventions can result in reduced antibiotic prescriptions and also lead to decreases in pneumococcal resistance. Declines in penicillin-resistant pneumococci in Iceland and in Alaskan villages receiving interventions suggest that judicious-use campaigns may have the potential to limit the spread of resistant pneumococci. Further controlled studies of interventions designed to look at the prevalence of resistant pneumococci as an outcome are needed to address the effectiveness of this class of interventions. Questions of importance include the time required for clinically relevant declines in resistance and whether declines in resistance are unique to a particular set of circumstances (e.g., small communities with centralized health care such as in Alaska) or can be expected as a general result when antibiotic use is decreased.

**MODIFICATIONS OF ANTIBIOTIC REGIMENS**

Even antibiotics used in appropriate situations will continue to exert a selective pressure for resistance. When antibiotics are indicated, it is therefore important that they be prescribed in a way that minimizes the risk of acquiring and transmitting resistance. How to do so has not been investigated explicitly for pneumococcal infections. However, a number of treatment modifications have been proposed (Table 4). These include modifying the duration of therapy, increasing treatment dosage, choosing drugs which are less effective at selecting for resistance, treating with multiple drugs, and cycling of drugs at the hospital or community level. A number of these mechanisms have proved successful at limiting the spread of resistance in pathogens such as HIV and mycobacteria, in which drug resistance is also a problem. However, the appropriateness of these modifications for pathogens that have a carrier state has not been explored. A summary of the rationale for each of these modifications and the likelihood that they will help limit the spread of resistant pneumococci are shown in Table 4.
Shorter Duration of Therapy

For pathogens without a carrier state, the goal of antibiotic therapy is to eradicate the pathogen. Thus, treatment must last long enough to ensure that the pathogen is eradicated. In contrast, for pathogens such as \textit{S. pneumoniae} which are commonly carried asymptomatically, eradication of the pathogen from the site of carriage is not always possible. Consequently, shorter durations of therapy have been proposed as a means of resolving clinical symptoms and at the same time reducing an individual’s exposure to antibiotics. Moreover, shorter courses may reduce the total amount of time that pathogens are exposed to MICs below levels that inhibit resistant strains but above levels that inhibit sensitive strains. Such conditions exert strong selective pressure for resistant strains.

A number of recent studies have demonstrated that shorter-course antimicrobial therapy for acute otitis media is as effective clinically as the standard 10-day course of amoxicillin for many patients (79). The World Health Organization currently recommends 5 days of treatment with trimethoprim-sulfamethoxazole or amoxicillin for treatment of acute respiratory infections in developing countries. Additionally, the recent introduction of drugs such as azithromycin and ceftriaxone, which can be administered in short courses, has led to an increasing popularity of short-course treatments for pneumococcal infections.

In some cases, short-course therapies reduce a patient’s exposure to antibiotics. This may reduce the risk of carrying resistant pneumococci following antibiotic treatment, depending on how quickly unmasking or replacement occurs. However, although duration may be shorter with some treatments, for example, a single dose of ceftriaxone, antibiotic concentrations may still remain above the MIC for long periods following treatment (24). In such cases, a shorter treatment duration does not result in reduced exposure to antibiotics.

Consistent with the hypothesis that shorter treatment durations decrease the probability of carrying a dominant resistant population, an observational study of 941 children 3 to 6 years old in France identified a low daily dose of \textit{\beta}-lactam antibiotic combined with a long duration of treatment (\textless 5 days) as risk factors for pharyngeal carriage of resistant strains (45). However, a recent multicenter trial of 5 versus 10 days of amoxicillin-clavulanate therapy for acute otitis media for young children (20) found that the overall risk of a child’s carrying penicillin-nonsusceptible \textit{S. pneumoniae} did not increase after antibiotic treatment in either treatment group. Because this study was a randomized, double-blind trial with sufficient power to detect other differences between treatment groups, these results may reflect the impact of treatment duration more clearly than those from the observational, retrospective study. Moreover, a study investigating changes in the nasopharyngeal flora 3 to 4 days after the initiation of antibiotic therapy found that substantial changes in the \textit{S. pneumoniae} population had already occurred by that time (28). Together, these observations suggest that shorter-course therapy may not reduce the risk of carrying resistant pneumococci and that significant changes in pneumococcal populations occur within the first days of antimicrobial therapy. Further controlled studies designed to assess the risk of carrying resistant pneumococci after different durations of antimicrobial therapy are needed to directly address this question. The challenge in such studies is obtaining a sufficient sample size of children posttreatment who still carry pneumococci.

Higher-Dose Therapy

Higher-dose therapy has been suggested to reduce the risk of acquiring a dominant resistant pathogen population, regardless of whether a pathogen has a carrier state (Table 4). The theory behind this modification is to achieve high enough drug concentrations at the site of infection to eradicate both sensitive and resistant strains. In vivo studies of respiratory pathogens using animal models (22, 23) have identified specific pharmacokinetic and pharmacodynamic parameters that maximize the bactericidal activity of antibiotics. For \textit{\beta}-lactam drugs, macrolides, and trimethoprim-sulfamethoxazole, the duration of time that serum levels remain above the MIC has been identified as the important determinant of bacteriologic efficacy. For such drugs, bactericidal efficacies of 85 to 100% were generally achieved if the drug concentration exceeded the MIC for at least 40% of the dosing interval (23). Studies of bacteriologic efficacy in children with acute otitis media have confirmed that bacteriologic cure is associated with dosing regimens in which \textit{\beta}-lactam concentrations exceeded the MIC for 40% or more of the dosing interval (23). In contrast, for quinolones, aminoglycosides, azalides, and azithromycin, the area under the serum concentration-versus-time curve in relationship to the MIC is the most important parameter (23, 25). For these drugs, bactericidal efficacy increases with drug concentration.

This pharmacological framework suggests that if serum antibiotic concentrations can be maintained above the MIC for at least 40% of the dosing interval, even strains with high-level resistance to \textit{\beta}-lactam or macrolide antibiotics will be eradi-cated from the site of infection. This has led to the hypothesis that high-dose therapy may also eradicate resistant pneumococci from the nasopharynx, thus reducing the risk of resistant pneumococcal carriage following therapy. Direct investigations of antibiotic concentrations required to eradicat pneumococci from the nasopharynx have not been performed, although there is evidence that carriage rates are reduced significantly following antibiotic treatment.

Even if high-dose therapy is more likely to eliminate nasopharyngeal carriage of \textit{S. pneumoniae} resistant to the drug used for treatment, this will not prevent acquisition of resistant strains from the community. In fact, if, as some studies suggest, there is a “niche” for pneumococci in the nasopharynx that opens when pneumococci or other members of the nasopharyngeal flora are cleared by antimicrobial therapy (11), high-dose therapy may increase the likelihood of recolonization by a resistant strain in communities where the prevalence of resistance is high. Again, these important questions can be best resolved by clinical trials in which participants are randomized to receive either low or high doses of a given antibiotic and nasopharyngeal carriage is carefully and sequentially monitored.

Choosing Drugs That Minimize Selection for Resistance

Another proposed treatment modification to limit the spread of resistance is to use drugs that minimize selection for resistance. This modification was initially proposed for pathogens for which within-host evolution of resistance in response to treatment is a primary concern. In contrast, for pneumococci, the principal concern is not to avoid within-host evolution of the pathogen but to minimize the opportunities for unmasking of minority resistant strains or acquisition of resistant strains from the community. While transformation is the predominant mechanism for resistance to \textit{\beta}-lactam antibiotics in clinical isolates of \textit{S. pneumoniae}, antibiotic-resistant point mutants are readily select-
able in the laboratory. There is also laboratory evidence that drugs differ in their activity against *S. pneumoniae* and in their potential to select for resistance and cross-resistance. For example, an in vitro study of *S. pneumoniae* (14) found that aminopenicillins selected for resistance to themselves and to cephalosporins, while cephalosporins, with the exception of cefixime, were shown to select only for resistance to themselves. This is consistent with observations that cephalosporin resistance is conferred by alterations to the PBP genes *pbp2a* and *pbp2x*, while high-level penicillin resistance also requires alterations in those two genes and in the *pbp2b* gene (47, 71). The relevance of these laboratory findings to clinical situations is unclear, since point mutations do not appear to play a similar role in vivo in bacterial populations.

In the clinical context, studies of nasopharyngeal carriage of *S. pneumoniae* before and after antibiotic treatment show clearly that some drugs eliminate *S. pneumoniae* from the nasopharynx more effectively than others. A study of the effects of cefpodoxime-proxetil and amoxicillin-clavulanate treatment on nasopharyngeal carriage of *S. pneumoniae* in children in France (19) found that the amoxicillin-clavulanate treatment led to a significantly greater decline in the number of children carrying *S. pneumoniae* posttreatment (a 64% decline in the amoxicillin-clavulanate group versus a 41% decline in the cefpodoxime-proxetil group). Similarly, a study comparing the effects of cefuroxime-axetil and cefaclor on nasopharyngeal carriage during the first days following treatment initiation found that the decline in the prevalence of *S. pneumoniae* carriage in the cefuroxime-axetil group at day 4 or 5 following treatment initiation was three times greater than in the cefaclor group (28).

These studies, however, were not designed to assess whether the drugs with lower activity against *S. pneumoniae* were also associated with a decreased risk of carrying a resistant strain posttreatment. Recent investigations of the impact of azithromycin on streptococcal carriage have shown that azithromycin is extremely effective at reducing nasopharyngeal carriage of *S. pneumoniae*. However, two studies of mass treatment with azithromycin found that this resulted in an increased prevalence of macrolide-resistant *S. pneumoniae* 1 month after treatment (63, 70).

Thus, available evidence, along with the fact that pneumococcal resistance has been observed for all of the major drug classes used to treat pneumococcal infections, suggests that trying to minimize selection for resistant pneumococci by choosing alternative drugs for treatment is likely to play only a minor role in minimizing the spread of resistant pneumococci.

**Treatment with Multiple Drugs**

Treatment of infections with multiple drugs has been a cornerstone of minimizing the spread of resistance in both HIV and *Mycobacterium tuberculosis*. For people infected with these pathogens, treatment durations are often long (many years for HIV and 6 to 12 months for *M. tuberculosis*) and within-host evolution of resistance is common. Because the probability that a strain will acquire resistance to two or more drugs is the product of the probabilities that a strain will acquire resistance to each individual drug, simultaneous treatment with multiple drugs is effective at preventing infections with resistant strains. Although treatment of pneumococcal infections with multiple drugs has been suggested to improve clinical efficacy in some circumstances (16, 32), there is currently no evidence to suggest that it is an effective intervention to limit the development of resistant pneumococci. Because treatment durations for persons infected with pneumococci are typically much shorter than for persons with tuberculosis and HIV, and because for most antibiotic classes acquisition of resistance is rarely due to single point mutations, treatment with multiple drugs is unlikely to be a useful approach to prevent the acquisition of pneumococcal resistance. If the unmasking of minority resistant strains is a primary mechanism of acquiring dominant resistant populations, treatment with multiple drugs might in some cases reduce the potential for unmasking to occur. However, if acquisition of resistant strains from the community is the primary mode of acquiring a dominant resistant pneumococcal population, treatment with multiple drugs may have the adverse effect of exerting selection pressure for acquisition of strains with multiple resistance. The risk of this will depend on the prevalence of multidrug resistance in the community.

**Cycling of Drugs**

Many hospitals have implemented formulary control of drugs administered and have scheduled cyclical changes of antibiotics at the hospital level in an effort to reduce the incidence of multiresistant nosocomial infections (68). While formulary control of antibiotics can lead to more judicious antibiotic use and can successfully limit the use of antibiotics such as vancomycin (83), evidence that “cycling” of antibiotics (periodically substituting a new member of a drug family or a new drug family for one currently in use) reduces the incidence of resistant infections remains unclear (68). Moreover, it is possible that some forms of cycling can increase the risk of acquiring a multiresistant infection (9). Multicenter studies that control for confounding factors will prove useful in assessing the impact of such strategies (68).

While formulary control may help limit the spread of resistant nosocomial infections, the majority of pneumococcal infections are community acquired, and except in small, isolated communities served by a limited number of health facilities, such control of antibiotics is not feasible. If cycling is found to be useful for preventing resistance in common nosocomial infections, it is possible that a similar strategy could minimize the development of multiresistant pneumococcal disease in patients in long-term care facilities, for example, the elderly and persons with AIDS.

**Improved Compliance with Treatment Regimens**

Directly observed therapy for individuals with tuberculosis has been an important intervention to minimize the risk of developing a resistant infection. Would a similar emphasis on improved compliance with treatments for pneumococcal infections help minimize the spread of resistant pneumococci? Increased compliance may improve treatment outcome by increasing the time that antibiotic concentrations are maintained above the MIC (23). Whether improved patient compliance also reduces the patient’s risk of carrying resistant pneumococci is less clear. Missing antibiotic doses may increase the time during which the concentration of antibiotic in the nasopharynx is in the dangerous zone between the MICs for sensitive and resistant strains, increasing the selective pressure for resistance. Depending on the pattern of how doses are missed, however, the concentration of antibiotic may also drop below the MIC, reducing the selective pressure for either unmasking or replacement. Moreover, because the etiologic agent causing respiratory infections is rarely identified and antibiotics are often prescribed for nonbacterial infections, a general public health campaign to increase compliance with antibiotics prescribed for respiratory symptoms might paradoxically contribute to the inappropriate use of antibiotics. Thus, for the case of suspected pneumococcal infections, it is unclear how the tra-
ditional message that patients should finish their entire course of medication affects pneumococcal resistance.

Noncompliance may be more important in developing countries, where antibiotics are often available without a prescription. In some countries this may contribute to misuse and overuse of antibiotics; for example, surveillance in a rural area of Bangladesh showed that 48% of the antibiotics purchased for adults were purchased in quantities that were often less than a single day’s dose (50). However, the availability of antibiotics without a prescription does not always result in antibiotic use without a physician’s advice. Data from a peri-urban community in Mexico suggest that the majority of antibiotics were prescribed by a physician (13); a study of antibiotic use in Manila, the Philippines, suggests that while two thirds of antimicrobial agents were purchased without a prescription, over half of these purchases were made upon the recommendation of a physician (60).

**ALTERNATIVES TO ANTIBIOTICS**

**Pneumococcal Vaccines**

A polysaccharide vaccine that is currently available can protect individuals >2 years of age against invasive pneumococcal infections caused by 23 common *S. pneumoniae* serotypes. The efficacy of this vaccine varies depending on a person’s age and immune status; in case-control studies, the effectiveness of the vaccine against invasive disease has ranged from 56 to 81% (15). Strongest recommendations for vaccination are for persons over 65 years of age and those aged 2 to 64 years with the following risk factors: chronic cardiovascular disease, chronic pulmonary disease, diabetes mellitus, or functional asplenia (15).

However, the polysaccharide vaccine suffers from the following limitations: it does not induce an effective immune response in children less than 2 years old, an age group with a high burden of disease; it does not protect against nonbacterial pneumococcal disease or common upper respiratory diseases (e.g., acute otitis media and sinusitis); it does not protect against carriage of pneumococci; and declining antibody levels following vaccination suggest that vaccine-induced immunity may wane over a 5- to 10-year period. The fact that the vaccine is not indicated for children <2 years of age and is primarily recommended for the elderly prevents it from being an effective intervention against pneumococcal disease in most developing countries. Because children are a large reservoir of resistant pneumococci, and because the vaccine has no effect on pneumococcal carriage, the polysaccharide vaccine has little potential to control the spread of resistant pneumococci.

Pneumococcal conjugate vaccines, in which purified polysaccharides of the epidemiologically most important serotypes are conjugated to a carrier protein, have recently been developed and tested in clinical trials. A 7-valent formulation received Food and Drug Administration licensure in February 2000, and several other formulations are also undergoing clinical trials. Preliminary data suggest that these vaccines are highly effective for children <2 years of age. No significant safety problems associated with vaccination have occurred in phase I to III trials conducted since 1992 (38). Clinical trials show that the vaccine is more than 95% effective at preventing invasive disease due to serotypes included in the vaccine (61; S. Black, H. Shinesfield, P. Ray, et al., 38th Intersci. Conf. Antimicrob. Agents Chemother. 1998, abstr. LB-9). Furthermore, the vaccine has some efficacy against noninvasive disease (38).

The vaccine’s ability to prevent invasive pneumococcal disease may also present an opportunity to reduce empiric use of antibiotics for young children with fever of unknown origin. Concern about invasive pneumococcal disease among young children who present with fever of unknown source is a common reason for antibiotic prescription for this age group. The importance of such empiric therapy has been questioned in the United States now that the incidence of invasive disease caused by *H. influenzae* type b has declined dramatically (66). Such concerns may be even less well founded among children who also received appropriate vaccination with the pneumococcal conjugate vaccine, and thus antibiotic use in this age group may be reduced in settings with high vaccination coverage.

Preliminary data also suggest that the pneumococcal conjugate vaccine reduces carriage of vaccine serotypes (31, 74), similar to an unexpected benefit of the conjugate *H. influenzae* type b vaccine (69). This effect has been demonstrated in infants (31) as well as toddlers (30), and it raises the possibility that the conjugate vaccine may be effective at preventing colonization with vaccine-related serotypes, reducing the opportunity for transmission of these serotypes within communities. Because the serotypes in current formulations of the vaccine include pediatric serotypes that are commonly associated with antibiotic resistance, vaccination of infants may reduce the circulation of antibiotic-resistant strains (31). Additionally, if the conjugate vaccine is effective at preventing invasive disease in children, and in particular if it reduces the rate of otitis media, antimicrobial therapy for pneumococcal infections is likely to decline, thus reducing the selective pressure for resistance. There is the possibility, however, that the vaccine prevents carriage of vaccine-included serotypes only. Evidence from a vaccine trial in the Gambia (74) and a recent study of day care attendees in Israel (R. Dagan, N. Givon, P. Yagupsky, et al., 38th Intersci. Conf. Antimicrob. Agents Chemother., 1998, abstr. G-52) suggest that carriage of nonvaccine serotypes increases following vaccination, although a previous study in Israel did not find evidence of increased carriage of nonvaccine serotypes (29). The impact of mass vaccination on carriage of nonvaccine serotypes remains to be seen, as well as whether widespread use of the conjugate vaccine increases the selective pressure for serotype switching or the acquisition of antibiotic resistance in strains not covered by the vaccine.

**Xylitol Sugar**

Xylitol is a sugar that has been used as a sweetening substitute for sucrose because it has a preventive effect against dental caries. Recent evidence suggests that in addition to inhibiting growth of *Streptococcus mutans*, the primary cause of dental caries, this sugar can inhibit the growth of *S. pneumoniae* and possibly also of other bacterial colonizers of the nasopharynx such as *H. influenzae* (90, 91).

A controlled trial of healthy children attending day care centers in Finland randomized the children to control groups that received syrup, chewing gum, and lozenges sweetened with sucrose and a low concentration of xylitol or to treatment groups that received a high daily dose of xylitol in the form of syrup, chewing gum, or lozenges (91). The children were then monitored for a 3-month period, and information on respiratory infections, acute otitis media, and antibiotic therapy was collected. While the xylitol exposure did not reduce the overall number of respiratory infections in treatment groups relative to their respective control groups, children who regularly received xylitol in the form of syrup or chewing gum had a significant (30 to 40%) reduction in the occurrence of acute otitis media, and all treatment groups received fewer antibiotic prescriptions than did controls (91).

Prophylactic use of xylitol may thus protect against acute
otitis media, particularly for children with high exposure to *S. pneumoniae*, such as those attending day care centers. Because acute otitis media is the primary reason for prescription of antibiotic therapy in developed countries, reducing the incidence of acute otitis media could greatly reduce antibiotic use in these settings. In the above study, however, xylitol in all groups was administered five times per day; such frequent dosing may be difficult to achieve, and studies of the minimum dosing required to get a significant protective effect would be useful.

**MATHEMATICAL MODELS: A TOOL FOR EVALUATING INTERVENTIONS**

Mathematical models of disease transmission dynamics have proved useful in evaluating the impact of interventions such as vaccination programs, chemoprophylaxis, and the introduction of new disease treatments. Recently, models of the transmission dynamics and population genetics of antimicrobial resistance also have been developed to address the increasing spread of drug-resistant pathogens (4, 5, 9, 64). These models highlight key parameters that may influence the spread of resistance and can be used to explore the impact of alternative interventions to reduce the spread.

Few models have taken into account the specific biologic features of pneumococci that distinguish them from other pathogens. Nonetheless, some general conclusions can be drawn from models of commensal pathogens and pathogens with a carrier state, such as pneumococci. Consistent with classical population genetics of a trait under constant, strong selective pressure, the incidence of resistance typically increases sigmoidally. Thus, levels of resistance are initially low and fairly constant and then go through a period of rapid increase before approaching another plateau at close to 100%. Models also typically show that resistance emerges much more quickly than it decays and that to achieve significant declines in resistance, significant reductions in drug use are required.

Key parameters that might influence how quickly resistance will decline if antibiotic use is decreased typically include the rate at which bacteria are acquired from the environment, the average number of treatment courses per year that a host receives, and the growth rate differential of resistant bacteria compared with sensitive bacteria.

Mathematical models can also be useful in understanding the impact of the introduction of pneumococcal vaccine on the epidemiology of antibiotic-resistant pneumococci. A model exploring the impact of vaccination against multiple serotypes on the nasopharyngeal carriage of specific serotypes within a community predicts that the introduction of serotype-specific vaccines will reduce competition for colonization of the nasopharynx among nonvaccine serotypes, leading to an increase in carriage of non-vaccine serotypes (65). For vaccines containing more than two serotypes, this effect may be greater than reductions in carriage of vaccine serotypes. The model additionally predicts that the vaccine coverage level required to eliminate a vaccine serotype is higher for multivalent vaccines than for vaccines targeted at a specific serotype.

Because multiple and often interrelated factors influence the spread of resistant *S. pneumoniae*, mathematical models with assumptions tailored more specifically to address the spread of resistance in pneumococci will be a useful complement to empirical studies of the efficacy of interventions. Additionally, the introduction of the conjugate pneumococcal vaccine or other interventions to reduce antibiotic resistance provides opportunities to test empirically the predictions of mathematical models.

**CONCLUSIONS AND FUTURE RESEARCH NEEDS**

Interventions to reduce the spread of pneumococcal resistance are at an early stage of implementation and evaluation. The unique biology of pneumococci, in particular the prevalence of asymptomatic carriers capable of transmitting resistant strains, and the fact that for a majority of drug classes de novo resistance due to single point mutations is rare distinguish pneumococci from many other pathogens for which drug resistance is a problem. Consequently, a number of interventions that have proved successful at limiting the development of resistance in other pathogens may not be effective at preventing the spread of resistant pneumococci.

Encouraging more judicious antibiotic use is the most promising intervention that has been implemented to date. Recent studies now provide evidence that judicious antibiotic use campaigns in the United States and in Europe can lead to declines in antibiotic prescribing; some have also shown concomitant declines in pneumococcal resistance. Further observations of the effect of reduced antibiotic use on the prevalence of resistant pneumococci are needed to assess the extent to which these results can be generalized. A key element of such studies is identifying appropriate control populations so that observed trends can be ascribed more readily to interventions.

Studies of the impact of judicious-use campaigns in developing countries will also prove an important complement to studies in industrialized countries. In some developing settings, underuse of antibiotics due to limited access to health care may contribute more to morbidity and mortality than overuse or misuse. Additionally, when antibiotics are available without a prescription and when few people can afford more than a single day’s dose, interventions targeting inappropriate use need to be developed. Because physicians often influence a patient's decision to take antibiotics, even in these settings, education targeted at physicians may still be an important component of such campaigns.

Finally, targeting judicious-use campaigns at drug classes for which resistance is not already prevalent is likely to have a much greater impact at preventing the spread of resistance than programs targeted at drugs for which resistance is already an important problem. In many developed countries, for example, fluoroquinolone use is not recommended for children, and pneumococcal resistance to quinolones is rare but increasing (17). Focusing on judicious use of this drug class before it becomes widespread, particularly in children, in whom the incidence of pneumococcal carriage is high, should be a priority. Similarly, in many developing countries resistance to more expensive drug classes such as macrolides is still very rare. Campaigns stressing judicious use of these drugs as they become more widespread may allow resistance to these drug classes to be curbed before it becomes a problem.

Modifications of antibiotic treatment regimens, for example, shorter-duration or higher-dose therapy, may reduce the risk that individuals undergoing antibiotic therapy carry resistant pneumococci posttreatment. Clinical trials designed to assess the impact of such therapies on resistant carriage are needed. Further research into the impact of nonadherence to prescribed regimens on pneumococcal resistance is also needed. Some types of nonadherence, for example, stopping a course of treatment a few days early, can be studied by comparing shorter and longer durations of therapy. Other forms, such as taking only a single day’s dose, are more difficult to study because they cannot ethically be investigated in a clinical trial format.

Other modifications that have had a large impact on minimizing the development of resistance by HIV and *M. tuberculosis*, for example, simultaneous treatment with multiple drugs,
ACKNOWLEDGMENT

We acknowledge Benjamin Schwartz for useful discussion and suggestions.

REFERENCES

8. Reference deleted.
10. Reference deleted.
27. Reference deleted.