Why Have Group A Streptococci Remained Susceptible to Penicillin? Report on a Symposium*

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In spite of 50 years of extensive use of penicillin, group A streptococci remain exquisitely susceptible to this antibiotic. This observation that continuing susceptibility has occurred despite the development of resistance to other antimicrobial agents prompted a day-long meeting at Rockefeller University (New York) in October 1996. Among the most likely explanations for this remarkable state of continued susceptibility to penicillin are that β-lactamase may not be expressed or may be toxic to the organism and/or that low-affinity penicillin-binding proteins either are not expressed or render organisms nonviable. Other potential explanations are that circumstances favorable for the development of resistance have not yet occurred and/or that there are inefficient mechanisms for or barriers to genetic transfer. Recommended future actions include (1) additional laboratory investigations of gene transfer, penicillin-binding proteins, virulence factors, and homeologous recombination and mismatch repair; (2) increased surveillance for the development of penicillin resistance; (3) application of bioinformatics to analyze streptococcal genome sequences; and (4) development of vaccines and novel antimicrobial agents. Thus far the susceptibility of group A streptococci to penicillin has not been a major clinical or epidemiological problem. A similar observation, however, could have been made decades ago about *Streptococcus pneumoniae.* It is therefore vital for the scientific community to closely examine why penicillin has remained uniformly highly active against group A streptococci in order to maintain this desirable state.

In spite of 50 years of extensive and often indiscriminate use of penicillin (sold ''over the counter'' in many countries) for the treatment of infections due to group A *Streptococcus,* the organism continues to remain exquisitely susceptible to this antibiotic [1–3]. Indeed, no clinical isolate resistant to penicillin has been identified to date [2, 3], and a recently completed survey of the susceptibility to penicillin of group A streptococcal strains isolated over a period of over 80 years has revealed no change in the activity of penicillin [4].

This is not to say that resistance to other antibiotics does not occur in group A streptococci. Reports of significant numbers of erythromycin-resistant group A streptococci have appeared in the literature, especially from Japan and Finland [5, 6], as well as reports of resistance to sulfadiazine [7], tetracycline [8], and clindamycin [9]. Resistance to penicillin occurs in other closely related species of gram-positive organisms; penicillin-resistant strains of *Streptococcus pneumoniae* [10] and β-lactamase-producing penicillin-resistant staphylococci [11] are two examples. Perhaps more foreboding has been the recent emergence of penicillin-resistant enterococci [12]. With these examples in mind, the question is whether the exquisite susceptibility to penicillin of group A streptococci will continue.

This remarkable state of continued susceptibility to penicillin of group A streptococci prompted the convening of a day-long symposium on the subject at Rockefeller University (New York) in October 1996. The issues on the agenda included the following. (1) Will this susceptibility continue or is it just a matter of time (an ''accident'' waiting to happen) before certain penicillin-resistant strains will emerge? (2) Are there inefficient mechanisms for genetic transfer of resistance in group A streptococci? (3) Does the organism possess natural barriers to the development of penicillin resistance? (4) Are aberrant penicillin-binding proteins (PBPs) present in group A streptococci and, if so, are they inefficiently expressed or lethal mutations?

While the discussion raised many more questions than answers, it was agreed that continued vigilance on the part of the medical and microbiological community to detect any changes in susceptibility of the organism to the antibiotic was important. Second, better understanding of the organism's growth patterns and biological characteristics, with respect to continued susceptibility to this particular antibiotic, could lead to improved or new methods of bactericidal killing of other related species of microorganisms.

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Topics

Circumstances Favorable for the Development of Resistance Have Not Yet Occurred

Although mutations and the spread of genes conferring antibiotic resistance continue to occur at an alarming rate, resistance does not develop uniformly in all microbial species. Continued susceptibility of *Treponema pallidum* to penicillin indicates that resistance may not invariably occur despite powerful selective pressures that would favor its emergence. There is, however, a time factor to be considered. As has been pointed out [13], penicillin-resistant pneumococcal strains were isolated from treated laboratory animals long before penicillin-resistant clinical isolates were recovered from the human population.

Viewed in this light, it is still possible that penicillin resistance may occur in the future in group A streptococci. The capacity of microorganisms within species [14, 15] and across species [16–18] to share genetic material offers one potential mechanism for the acquisition of penicillin resistance by group A streptococci. Such resistance, were it to develop, could spread rapidly in response to selective pressures (such as exposure to antibiotics) on microbial populations.

Inefficient Mechanisms for Genetic Transfer or Barriers to DNA Uptake and Replication

While there are documented examples of antibiotic-resistant organisms arising through the exchange of genetic material, acquisition of a new resistance trait in a given species of bacteria in nature remains a highly unusual event. In order for this to occur, circumstances favorable for gene transfer must exist. Restriction barriers, disparate ecological niches, differences in cell surface characteristics, presence of nucleases, and susceptibility to bacteriophages vary substantially among bacterial species.

With respect to restriction barriers, group A streptococci produce at least four different types of extracellular DNAse [19, 20] that could limit the opportunity for acquisition of exogenous DNA via transformation and may help prevent the development of antibiotic resistance within this bacterial species. Phage-encoded type II DNA restriction systems, moreover, commonly exist in clinical isolates of group A streptococci (P. Cleary, unpublished observations). Replication or incorporation of unmodified or differentially modified DNA may be eliminated by these restriction enzymes. The combination of extracellular and intracellular nucleases together theoretically might create a formidable barrier.

Unlike pneumococci, group A streptococci are not naturally competent and do not readily take up exogenous DNA. Group A streptococci may lack the genes required for DNA uptake that are found in competent bacterial species [14], although techniques such as electrotransformation have been used to introduce DNA into other normally nontransformable organisms [21]. It should be emphasized, however, that these laboratory techniques have limited clinical relevance at present.

One of the ways for bacteria to circumvent these restriction barriers is the entrance of foreign DNA via bacteriophages. Historically, examples of the introduction of toxins under genetic control of lysogenic phages have been demonstrated for diphtheria [22] and for pyrogenic exotoxin A in group A streptococci [23, 24]. Since many of the group A streptococcal bacteriophages were isolated originally from sewage [25, 26], the recent observation of penicillin-resistant enterococci raises the question of whether these organisms might provide the vehicle by which phage transfer of penicillin resistance to group A streptococci could occur.

This is, in addition, of potential concern since the human vagina may harbor group A streptococci and enterococci [27]. For example, endogenous bacteriophages from an erythromycin-resistant strain of a group A *Streptococcus* were able to transduce antibiotic resistance into a susceptible streptococcal strain [28]. Most transductions to date have been mediated via group A streptococcal phages [29]. Bacteriophage-mediated genetic transfer that occurs within group A streptococci, however, appears to be species-specific. Staphylococcal bacteriophages that transfer β-lactamase genes between staphylococci do not readily infect group A streptococci (V. Fischetti, unpublished observations).

Another mechanism for gene transfer is via plasmids [30], but clinical isolates of group A streptococci uncommonly contain plasmid DNA. In addition, group A streptococci appear to have limited ability to conjugate as compared with the ability of other gram-positive organisms such as enterococci and staphylococci [31]. Yet erythromycin, tetracycline, or kanamycin resistance, encoded by transposable elements, does occur in group A streptococci [32, 33]. Although vancomycin-resistant group A streptococci have not been reported, clinical isolation of a vancomycin-resistant *Streptococcus bovis* due to the introduction of a *vanB* transferable determinant has been noted [34].

β-Lactamase May Not be Expressed or May be Potentially Toxic to Group A Streptococci

There is evidence that genes for resistance to penicillin that originated in staphylococci are now expressed in β-lactamase-producing strains of enterococci [12]. While most β-lactamases in staphylococci are inducible extracellular enzymes, β-lactamase genes in enterococci are usually expressed constitutively at low levels, and the enzyme remains primarily cell-associated [17, 35]. Yet penicillin resistance among the non–group A streptococci results almost entirely from low-affinity PBPs and is not mediated by the expression of β-lactamases [36]. The lack of β-lactamase genes among any streptococci is a notable finding without a clear explanation.

Low-Affinity PBPs Either are Not Expressed or Render Group A Streptococci Nonviable

A common mechanism of penicillin resistance among gram-positive bacteria is the generation of PBPs that have low affinity

for penicillin yet still successfully catalyze the synthesis of the cell-wall peptidoglycan. Group A streptococci and *S. pneumoniae* colonize the same ecological site, the nasopharynx of humans. Most clinical isolates of these species also share a high degree of susceptibility to penicillin, in the MIC range of 5–10 ng of antibiotic per mL.

Similar to pneumococci, group A streptococcal penicillin-tolerant mutants have been isolated in the laboratory. In these bacteria the rate of loss of viability during penicillin treatment was reduced without alteration of the penicillin MIC, in exact analogy to the case of penicillin-tolerant pneumococci [37, 38]. Group A streptococci and *S. pneumoniae* differ strikingly in the lack of penicillin-resistant strains of group A streptococci from clinical specimens, in sharp contrast to the recent extensive spread of a multitude of penicillin-resistant pneumococcal clones.

In this context, a possibly important observation was made in the report by Gutmann and Tomasz and colleagues [39, 40]. Using ethyl methane sulfonate as their mutagen, they were able to create penicillin-resistant laboratory mutants. While these strains were shown to express low-affinity PBPs, they also had severe physiological defects, with extremely poor growth rates and gross morphological abnormalities. Rosendal also noted an apparent decrease in the production of M protein in laboratory-produced penicillin-resistant mutants of group A streptococci [41]. These observations suggest that, at least in the case of the group A *Streptococcus,* the induction of penicillin resistance could lead to such severe changes in the biology of the organism that it would either not reproduce effectively or be rapidly destroyed.

It is therefore highly unlikely that these strains would appear and survive as clinical isolates. The physiological ''fitness'' of clinical strains of penicillin-resistant pneumococci may require the acquisition by these bacteria of some additional genetic elements that would compensate for the potentially defective performance of low-affinity PBPs in cell-wall biosynthesis. It may be relevant that in clinical strains of resistant pneumococci, alterations in the PBP genes are achieved through heterologous recombinational events rather than by point mutations, and the ''mosaic'' PBP genes of clinical strains may contain sequences critical for the physiological ''fitness'' of the bacteria.

Table 1. Future actions concerning the study of group A streptococci.

1. Laboratory investigations
   - A. Mechanisms of gene transfer in group A streptococci, especially bacteriophages
   - B. Role of mutated penicillin-binding proteins (PBPs) in cell-wall synthesis
   - C. Diversity of PBPs in group A streptococci
   - D. Interactions between virulence and antibiotic resistance
   - E. Potential role of homeologous recombination and mismatch repair
2. Increase surveillance for the development of penicillin resistance and efforts to quantify resistance to other antimicrobial agents
3. Apply bioinformatics to compare and interpret multiple streptococcal genome sequences, in order to help elucidate the pathogenesis of invasive disease and rheumatic fever
4. Increase efforts to develop vaccines to prevent pharyngitis and protect against invasive disease
5. Increase efforts to develop new and novel antimicrobial agents with activity against group A streptococci

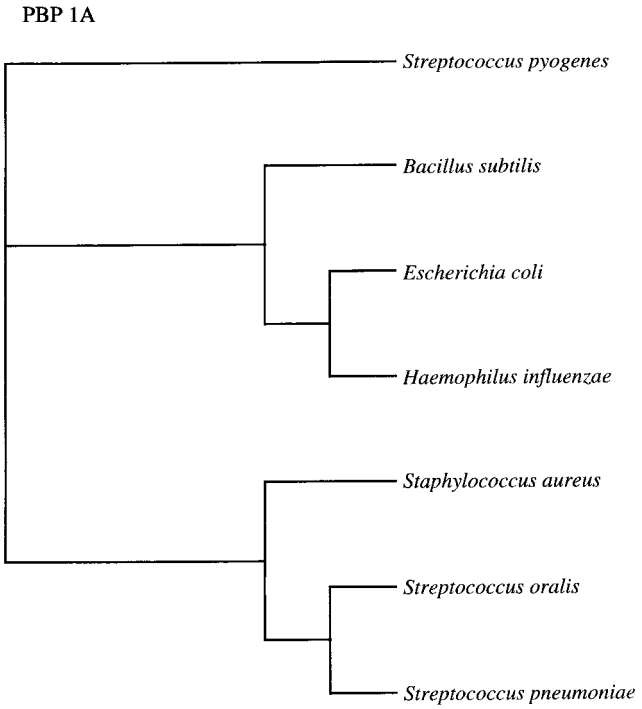

Figure 1. Phylogenetic relationship between *Streptococcus pyogenes* and *Streptococcus pneumoniae* penicillin-binding protein (PBP) 1A.

Discussion

At the end of the meeting, several future directions were enumerated (table 1). The fact that >90% of the sequence of the M1 group A streptococcal genome is now complete (J. Ferretti, unpublished observations) has raised a number of points that are potentially relevant to the questions posed at the symposium. For example, several previously unidentified genes encoding potential virulence factors have been identified, as well as restriction enzymes, resident prophages, and insertion elements. The mobile genetic elements found so far likely contribute to the process of heterogeneity, horizontal gene flow, and evolution of the organism.

The current sequence data may be accessed at the following Internet addresses: **http://www.microbiology.uokhsc.edu** and **http://www.genome.ou.edu.** The data are freely available for examination by the scientific community, but the sequence must be considered as work in progress and will be subject to frequent revision. Only the sequence published at the completion of the project will be considered the final and definitive version.

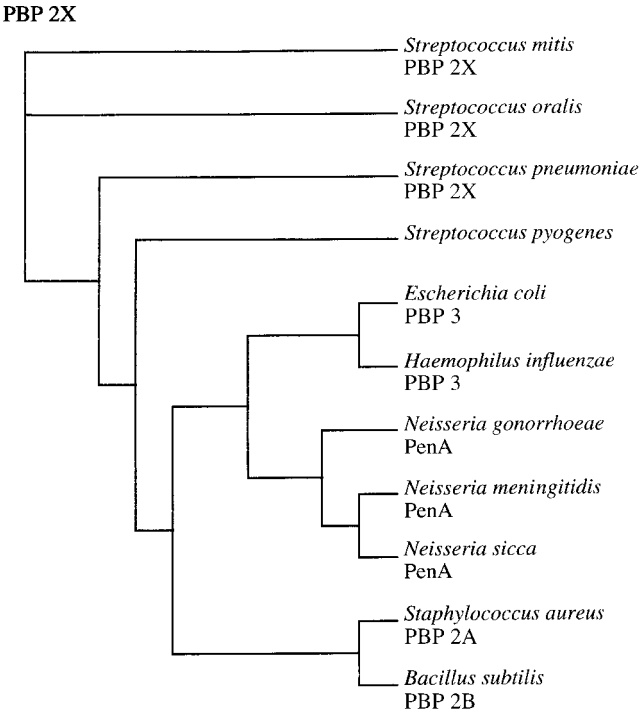

Figure 2. Phylogenetic relationship between *Streptococcus pyogenes* and *Streptococcus pneumoniae* penicillin-binding protein (PBP) 2X.

In addition to the elucidation of metabolic pathways, genes encoding five PBPs have been identified, all of which have considerable sequence similarity to PBPs of other organisms. In resistant isolates of *S. pneumoniae,* there appears to be a wide range of structural diversity of PBPs. Among the six PBPs present in *S. pneumoniae,* three principal PBPs associated with increased resistance to penicillin are PBP 1A, PBP 2X, and PBP 2B. Comparison of the sequences of the two genes encoding PBP 1A and PBP 2X indicate a rather distant phylogenetic relationship between the two organisms (figures 1 and 2). The probability appears to be low, therefore, that group A streptococcal PBPs will become altered in a similar manner as those of *S. pneumoniae.* Nonetheless, it remains possible that other mechanisms can result in alterations to the same type of penicillin-resistant phenotype in the group A streptococci.

The complete sequence of the *Streptococcus pyogenes* genome will provide basic information for answering important questions in a number of additional areas. For example, elucidation of the physiology of the organism and how it responds to various environmental conditions might yield important clues related to virulence factor expression. New and unknown factors involved in virulence and pathogenicity may be discovered, new targets may be identified for drug therapy, and new possibilities for vaccine development may be formulated. All of these advances should allow significant progress to be made in the form of improved treatment or prevention of streptococcal diseases.

Further discussion during the symposium indicated a significant need to define the role of PBPs in cell-wall synthesis and in mechanisms of gene transfer in group A streptococci. Streptococci have a complex symbiotic relationship with bacteriophages [42–46], and it is common to find several distinct temperate phages concealed within the genome of a single organism. The consequences of this relationship and their impact on the evolution of group A streptococcal resistance should be studied.

Even though penicillin resistance in group A streptococci has not yet been recognized, there is a possibility that this event could occur, prompting the need for an effective vaccine and new antibiotic targets. Continued surveillance for the development of penicillin resistance and quantification of resistance to other antimicrobial agents are clearly warranted.

Group A streptococci have remained susceptible to penicillin for the first 50 years of the antibiotic era. There is no guarantee that they will remain susceptible for the next 50 years. In view of the value of this antibiotic for treating infections due to group A streptococci in a cost-effective manner, the scientific community should continue to examine this issue closely. It has important pathogenetic as well as clinical and public health relevance.

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This stamp was issued by Gabon in 1978 to commemorate the 50th anniversary of the discovery of antibiotics by Alexander Fleming. It depicts Fleming, a microscope, various pieces of laboratory equipment, cultures, and the chemical formula of penicillin. (From the medical philateley collection of Dr. J. N. Shanberge, University of Michigan.)