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The Emergence of Penicillin-Resistant *Streptococcus pneumoniae* in Connecticut and an Evaluation of Hospital Laboratory Susceptibility Testing Practices

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INTRODUCTION

Streptococcus pneumoniae, a leading cause of morbidity and mortality in the United States, is the most common cause of community-acquired pneumonia, bacterial meningitis, acute otitis media, and bacteremia.[1] Pneumococcal infections are among the most common reasons for acute-care office visits and hospitalization, and cause an estimated 40,000 deaths annually in the United States.[2,3] Although *S. pneumoniae* was once considered to be uniformly susceptible to penicillin, the prevalence of pneumococcal isolates resistant to penicillin and other antimicrobial agents has increased substantially in the United States since the early 1990s.[1,4-8]

The emergence of drug-resistant *S. pneumoniae* complicates the treatment of pneumococcal infections, which are often treated empirically, without knowing whether the infecting strain is susceptible or resistant to the antibiotic selected. Because geographical variations in the prevalence drug-resistant *S. pneumoniae* have been demonstrated,[4,6,9] information about its regional prevalence is essential for the selection of appropriate antimicrobial therapy. Inappropriate antimicrobial use, such as treating infections with overly broad-spectrum antibiotics, can contribute to the increase of drug-resistant *S. pneumoniae* in a population.[1] There is also the problem of treatment failures occurring when infections caused by resistant strains are treated with ineffective antibiotics.[10]

Surveillance of emerging infections such as drug-resistant *S. pneumoniae* is an integral part of preventing and controlling the spread of disease. Prevalence data obtained

by surveillance programs can identify changing patterns of resistance and be used by public health officials to develop interventions for specific communities or regions.[11] These interventions include issuing guidelines for appropriate antimicrobial treatment, identifying populations at risk for transmission of drug-resistant *S. pneumoniae*, and making recommendations concerning pneumococcal vaccination.[12-14]

Prior to 1993, little was known about the prevalence of drug-resistant *S. pneumoniae* in Connecticut or in other parts of the United States. Working with the Connecticut Department of Public Health (CDPH), I conducted a survey of all hospitals with clinical microbiology laboratories in Connecticut to determine the prevalence of penicillin resistance among isolates of *S. pneumoniae* in 1992-1993.[15] To determine whether the prevalence of resistant *S. pneumoniae* has increased since this first laboratory survey was conducted, active hospital laboratory-based surveillance for invasive *S. pneumoniae* began in March 1995. This was done as part of an Emerging Infections Program (EIP), which is a collaboration between the CDPH and the Centers for Disease Control and Prevention (CDC).

The collection of antimicrobial resistance data by a hospital laboratory-based surveillance program depends on the ability of hospital laboratories to accurately detect resistant isolates. However, there have been few studies to assess if clinical laboratories follow recommended protocols, the specific methods laboratories use for susceptibility testing of pneumococcal isolates, and the accuracy of susceptibility test results. The laboratory survey conducted in 1993 revealed that not all hospital laboratories in

Connecticut were following recommended guidelines for penicillin susceptibility testing of *S. pneumoniae*. A similar survey of acute-care hospital laboratories in Connecticut was conducted in 1995 to determine what methods hospital laboratories were using then to test pneumococcal isolates for antimicrobial susceptibility and what changes had occurred in susceptibility testing practices since 1993.

In this paper I will present background information on the emergence of drug-resistant *S. pneumoniae* and information regarding laboratory methods used to detect antimicrobial susceptibility. I will then present the results of surveillance for penicillin-resistant *S. pneumoniae* in 1992-1993 and 1995-1996, changes in laboratory susceptibility testing practices that occurred between 1993 and 1995, and an assessment of the accuracy of susceptibility test results reported by hospital laboratories in 1995-1996.

BACKGROUND

The History and Emergence of Drug-Resistant *S. pneumoniae*

When penicillin was first introduced in the 1940's it was hailed as a "miracle drug" in the fight against deadly bacterial infections.[16] It is estimated that the introduction of penicillin reduced the mortality due to pneumococcal infections by about 50% for all ages.[17] Alexander Fleming, the discoverer of penicillin, warned in 1945 that misuse of penicillin could lead to the selection and propagation of mutant forms of resistant bacteria.[16] Fleming himself had been able to produce resistant strains of bacteria in his laboratory by growing susceptible strains in increasingly higher amounts of penicillin.[16]

The first clinical isolates of *S. pneumoniae* resistant to penicillin were reported from Australia and New Guinea in the late 1960's.[18,19] In 1977, pneumococcal strains that were highly resistant to penicillin were reported from South Africa,[20] and large increases in the prevalence of resistant *S. pneumoniae* occurred in Spain and Hungary in the 1980's.[21-24] Pneumococcal isolates resistant to penicillin and to other antimicrobial agents have now been detected throughout the world.[25-31]

In the United States, the prevalence of *S. pneumoniae* nonsusceptible to penicillin was relatively low during the 1980's and then increased dramatically during the early 1990's.[31] The results of surveillance studies conducted in the United States that have determined the prevalence of penicillin resistance of *S. pneumoniae* are presented in Table 1. The CDC has conducted national hospital-based surveillance for drug-resistant

S. pneumoniae since 1979 using isolates submitted by hospital laboratories participating in the Pneumococcal Sentinel Surveillance System.[1,4,9] Data from these studies show that the prevalence of isolates nonsusceptible to penicillin increased from 5.0% in 1979-1987, to 6.6% in 1991-1992, to 14.1% in 1993-1994; and the prevalence of isolates highly resistant to penicillin increased from 0.02% to 1.3% to 3.2% during the same time period.[1,4,9] Other national surveys conducted in the United States have also shown a marked increase in the prevalence of penicillin-nonsusceptible *S. pneumoniae*,[6-8,32,33] with figures as high as 27%[8] (Table 1).

Variations in the prevalence of penicillin-nonsusceptible *S. pneumoniae* reported by different surveillance studies could be due to differences in the patient population sampled. Surveillance studies that examined isolates collected from different geographical locations demonstrated that the prevalence of penicillin resistance often varied from one region to another.[4,6,9] Patient's age has also been associated with variations in the prevalence of resistance, with penicillin-nonsusceptible isolates being more common in children than adults.[1,4,6] Epidemiological studies that examined racial differences in prevalence found that whites were more likely to have isolates resistant to penicillin than blacks.[1,35,37,39]

The proportion of isolates tested from different body sites can have an impact on the percentage of isolates identified as resistant. Many studies have shown that isolates obtained from normally sterile sites (*i.e.*, blood and cerebrospinal fluid) have lower rates of resistance than isolates obtained from non-sterile body sites.[8,9,33,37,38] Surveillance

studies that included a relatively large percentage of isolates from non-sterile body sites reported high rates of penicillin-nonsusceptible *S. pneumoniae*[6,8,32] (Table 1).

Pneumococci frequently colonize the upper respiratory tract of many individuals, and drug-resistant strains can be easily transmitted to others. Young children are very susceptible to pneumococcal infection, especially in settings such as day care centers.[40] Another way to assess the prevalence of drug-resistant *S. pneumoniae* is to determine pneumococcal colonization rates in specific populations such as day care centers.[11,41,42] Studies conducted in the United States have reported colonization rates of penicillin-nonsusceptible *S. pneumoniae* in day care centers to be between 21% and 61%.[43-46]

Many surveys have also reported an increase in the prevalence of *S. pneumoniae* resistant to other antibiotics in addition to penicillin. The most recent CDC hospital surveillance study reported that 26% of the isolates tested were nonsusceptible to more than one antimicrobial agent.[1] Strains that are resistant to penicillin are also often resistant to cefotaxime or ceftriaxone, extended-spectrum cephalosporins that are often used to treat patients with meningitis.[1,8,12,39] Other antibiotics that *S. pneumoniae* have been found to be resistant to include erythromycin, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole.[1,4,6,39,47] There have been no reports of *S. pneumoniae* resistant to vancomycin, yet there is concern that vancomycin resistance could develop if this drug is overly prescribed for susceptible pneumococcal infections that could otherwise be treated with a more appropriate antibiotic (*e.g.*, penicillin or a

cephalosporin).[48-50] It is very important to obtain accurate susceptibility data on invasive pneumococcal infections so that appropriate drugs can be selected for treatment.

Antimicrobial Susceptibility Testing of *S. pneumoniae*

National Committee Of Clinical Laboratory Standards

The National Committee of Clinical Laboratory Standards (NCCLS) is a nongovernment organization made up of volunteer members that represent the clinical laboratory testing community.[51] The NCCLS periodically publishes documents called "standards" that describe laboratory procedures and interpretive criteria that are used as guidelines by clinical laboratories. These standards are based on published clinical data and represent a "consensus opinion of good laboratory practice."[51] Laboratories are encouraged to follow standards published by the NCCLS for susceptibility testing of *S. pneumoniae*.[11] The NCCLS cannot approve or endorse commercial products used by clinical laboratories. The Food and Drug Administration (FDA) is responsible for evaluating and approving commercial medical devices used by clinical laboratories, including those used for antimicrobial susceptibility testing.[51,52]

Definitions of Antimicrobial Susceptibility

The NCCLS has defined three interpretive categories that describe the susceptibility of an infecting organism to an antimicrobial drug: 1) *susceptible*, the bacterial infection should respond to therapy when treated with the antimicrobial drug at the recommended

dosage for that type of infection; 2) *intermediate*, the antimicrobial drug may be less effective for treating the infection, but may still be used for treating certain types of infections depending upon the site of infection; and 3) *resistant* (also referred to as highly resistant), the antimicrobial drug does not inhibit the infecting organism when normal dosage schedules are used.[53-55] In addition, the CDC defines isolates that are intermediate or resistant to an antimicrobial agent as *nonsusceptible* to that agent.[56]

Susceptibility Testing Methods

Susceptibility testing methods used to detect penicillin resistance in *S. pneumoniae* include qualitative screening methods to initially identify nonsusceptible isolates and quantitative testing methods to precisely define the level of penicillin susceptibility.

Qualitative susceptibility screening methods. The most frequently used qualitative screening method for penicillin susceptibility is oxacillin disk diffusion. This method has been standardized by the NCCLS.[53] Penicillin disk diffusion is not recommended because it cannot accurately detect penicillin-nonsusceptible isolates.[57] Advantages of the oxacillin disk diffusion screening method include it being relatively inexpensive and simple to perform, and that it does not require any special equipment.[58] *S. pneumoniae* isolates are placed on an agar plate with a paper disk containing 1 μg of oxacillin. Other disks containing additional antibiotics can be placed on the same plate.[54] After 18 hours of incubation, the agar plates are inspected and zones of growth inhibition around each

antibiotic disk are measured to the nearest millimeter. An *S. pneumoniae* isolate with an oxacillin zone of ≥ 20 mm is considered susceptible to penicillin.[51]

The major disadvantage with the oxacillin screening method is that it cannot quantify the level of penicillin resistance of *S. pneumoniae*, nor can it distinguish intermediate strains from strains that are resistant to penicillin.[53,57] Isolates that have oxacillin zones of ≤ 19 mm must be tested by a quantitative susceptibility method to accurately quantify and confirm penicillin resistance.[51] It is very important that isolates found nonsusceptible by oxacillin screening also be tested against an extended-spectrum cephalosporin (cefotaxime or ceftriaxone), since penicillin-nonsusceptible isolates are often nonsusceptible to other β-lactam antibiotics, and cephalosporins are frequently used to treat invasive pneumococcal infections.[11,12,51]

Another qualitative screening method is the breakpoint screening method.[59] This is a variation of the NCCLS broth microdilution method (described below), and can be used by laboratories to initially identify isolates that are nonsusceptible to penicillin before broth microdilution is performed. The NCCLS has determined minimum inhibitory concentration (MIC) breakpoints that are used to categorize an isolate as susceptible, intermediate, or resistant to an antimicrobial drug. The MIC is the lowest concentration of an antibiotic that completely inhibits the visible growth of an organism *in vitro*.[60] An isolate of *S. pneumoniae* with a penicillin MIC of ≤ 0.06 μg/ml is considered susceptible to penicillin.[51] Thus, breakpoint screening is performed by placing *S. pneumoniae* in media containing 0.06 μg/ml of penicillin. If growth is inhibited at this concentration,

then the isolate is considered susceptible to penicillin, and if growth is not inhibited then it is considered penicillin-nonsusceptible. All isolates that are determined to be nonsusceptible by this method must have an exact MIC determined to classify the level of nonsusceptibility.[61]

Quantitative susceptibility testing methods. Quantitative susceptibility testing involves the determination of an antimicrobial MIC. Traditionally this has been accomplished by broth microdilution, a method that has been standardized by the NCCLS.[60] Isolates of *S. pneumoniae* are inoculated into the wells of a microdilution tray (macrodilution is performed in tubes) containing various serial dilutions (*e.g.*, 8 μg/ml, 4 μg/ml, 2 μg/ml) of one or more antimicrobial drugs. After an overnight incubation, an MIC is determined as the concentration of antibiotic for which there is no visible bacterial growth. Laboratories can prepare their own broth microdilution trays as described by the NCCLS,[60] or they can use commercial broth microdilution systems that contain frozen or lyophilized dilutions of various antibiotics. Laboratories often use commercially-prepared broth microdilution trays because the preparation of broth microdilution trays as described by the NCCLS involves the use of lysed horse blood, which is tedious to prepare and not widely available from commercial sources.[62] Although commercial microdilution systems are more convenient to use than the NCCLS broth microdilution method, many systems, especially automated ones, have been found to be unacceptable by researchers that have compared MIC test results obtained by commercial systems with results determined by the NCCLS method.[62-65]

The E test (AB Biodisk, Solna, Sweden), also called agar gradient diffusion, was approved for use by the FDA in 1991.[66] It has become a popular alternative to the broth microdilution method and has been highly recommended in the literature as a method for determining MICs because of its convenience and reliability.[63-65,67-72] However, the NCCLS cannot officially recommend it because it is a commercial product. The E test uses a plastic strip that contains a continuous gradient of antibiotic to be tested. The strip is placed on the surface of an agar plate inoculated with *S. pneumoniae*. Additional strips can be placed on the same plate. After an overnight incubation, an antibiotic gradient is produced resulting in an elliptic zone of growth inhibition. The MIC value is read where the ellipse of inhibition intercepts the test strip.

METHODS

Surveillance of Penicillin-Resistant *S. pneumoniae*

In August 1993, questionnaires were sent to all 44 hospitals with clinical microbiology laboratories in Connecticut. Laboratories that did not respond to the questionnaire were contacted by telephone. To determine the prevalence of penicillin resistance among isolates of *S. pneumoniae*, laboratories were asked to report the number of isolates tested by an MIC method for penicillin susceptibility from July 1992 through June 1993, the body site of these isolates, and the penicillin MIC value (μg/ml).

Active surveillance for invasive *S. pneumoniae* isolates identified by 35 acute-care hospital laboratories in Connecticut was established in March 1995. Hospital laboratories were required to submit all invasive (*i.e.,* obtained from normally sterile sites) pneumococcal isolates to the State Laboratory and to report the results of penicillin susceptibility tests performed on these isolates to the CDPH. Isolates submitted from March 1995 through February 1996 were sent to a CDC-contracted reference laboratory for antimicrobial susceptibility testing by the broth microdilution method described by the NCCLS.[60]

All isolates tested either by the reference laboratory or by a hospital laboratory were categorized into penicillin susceptibility categories based on MIC breakpoints defined by the NCCLS: susceptible (MIC ≤ 0.06 μg/ml), intermediate (MIC 0.1 to 1.0 μg/ml), and resistant (MIC ≥ 2.0 μg/ml). Isolates with MICs of ≥ 0.1 μg/ml were classified as nonsusceptible.[51,56]

Evaluation of Hospital Laboratory Susceptibility Testing Practices

To determine practices used by Connecticut hospital laboratories for penicillin susceptibility testing of *S. pneumoniae*, the laboratory questionnaire sent to hospital laboratories in 1993 asked whether pneumococcal isolates received in their laboratory were tested for penicillin susceptibility, where isolates were tested, what criteria were used to select pneumococcal isolates for susceptibility testing, and what susceptibility methods were used to detect penicillin resistance.

In June 1995, a similar questionnaire was mailed to all 35 acute-care hospital clinical microbiology laboratories in Connecticut. Laboratories that did not respond were contacted by telephone. Additional questions included on the 1995 questionnaire asked what type of test was used for MIC determination and if additional antimicrobial MICs were determined on *S. pneumoniae* isolates identified as oxacillin or penicillin-nonsusceptible. Comparison of susceptibility testing practices between 1993 and 1995 were limited to the 35 acute-care hospital laboratories surveyed in both years.

To determine the accuracy of susceptibility test results reported by hospital laboratories in 1995-1996, categorical susceptibility test results reported by hospital laboratories were compared with those determined by the reference laboratory. Isolates tested by an MIC method were categorized as susceptible (S), intermediate (I), or resistant (R) as described above. Isolates tested by oxacillin disk diffusion were categorized using

interpretive criteria defined by the NCCLS: susceptible (S, oxacillin zone ≥ 20 mm) and nonsusceptible (NS, oxacillin zone ≤ 19 mm).[51]

Comparison of reference laboratory penicillin and hospital laboratory oxacillin categorical results were characterized as follows: concordant (penicillin S by reference laboratory and oxacillin S by hospital laboratory, or penicillin NS by reference laboratory and oxacillin NS by hospital laboratory), false nonsusceptible (penicillin S by reference laboratory and oxacillin NS by hospital laboratory), or false susceptible (penicillin NS by reference laboratory and oxacillin S by hospital laboratory).[57] False nonsusceptible results were assessed among all isolates categorized as susceptible by the reference laboratory, and false susceptible results were assessed among all isolates categorized as nonsusceptible by the reference laboratory.

Comparison of penicillin MIC categorical results determined by the reference laboratory and by hospital laboratories were characterized as follows: concordant (same categorical result by reference laboratory and hospital laboratory), very major error (R by reference laboratory and S by hospital laboratory), major error (S by reference laboratory and R by hospital laboratory), or minor error (S or R by reference laboratory and I by hospital laboratory, or I by reference laboratory and S or R by hospital laboratory).[72] Discordant results were expressed as the percentage of interpretative errors occurring among those isolates at risk for error as follows: very major errors (false susceptibility) were assessed among all isolates classified as resistant by the reference laboratory, major errors

(false nonsusceptibility) were assessed among all isolates classified as susceptible by the reference laboratory, and minor errors were assessed among all isolates tested.[71,73,74]

Data was analyzed using EpiInfo, Version 6 (CDC, Atlanta, Ga). The χ^2 test was used to test the significance of proportions.

RESULTS

Prevalence of Penicillin Resistance in S. pneumoniae

In 1992-1993, 14 hospital laboratories in Connecticut reported penicillin MIC data on 846 pneumococcal isolates from different body sites (Table 2). Hospital laboratories that did not report MIC values either did not perform MIC determinations in their laboratory or did not maintain records of MIC data. Of the 846 isolates, 18 (2.1%) were nonsusceptible to penicillin, including 15 (1.8%) penicillin-intermediate isolates and 3 (0.4%) penicillin-resistant isolates. Of the 846 isolates, 400 (47%) were from normally sterile sites. Of these 400 isolates, 5 (1.3%) were nonsusceptible to penicillin including 4 (1.0%) penicillin-intermediate isolates and 1 (0.3%) penicillin-resistant isolate. There were significantly more middle ear isolates classified as penicillin-nonsusceptible than isolates from other sites ($P < 0.001$). There were no other significant differences in the percentage of isolates classified as penicillin-nonsusceptible from other body sites.

Active hospital-based surveillance in 1995-1996 identified 801 cases of invasive pneumococcal disease. Penicillin MICs were determined by the CDC reference laboratory on 733 isolates from 705 cases. Of the 733 isolates, 119 (16.2%) were classified as nonsusceptible to penicillin, including 52 (7.1%) penicillin-intermediate isolates and 67 (9.1%) penicillin-resistant isolates. This represents a 13-fold increase in invasive pneumococcal isolates nonsusceptible to penicillin in a three year period, and a 37-fold increase in *S. pneumoniae* highly resistant to penicillin.

Ten acute-care hospital laboratories surveyed in both years reported penicillin MICs on invasive pneumococcal isolates identified in their laboratories. The number and percentage of isolates determined by these hospital laboratories as penicillin-nonsusceptible in 1992-1993 and 1995-1996 are presented in Table 3. With the exception of one hospital laboratory that did not report any nonsusceptible isolates in either year, the proportion of isolates identified as penicillin-nonsusceptible increased among all hospital laboratories. Overall the prevalence of isolates determined as nonsusceptible to penicillin by these hospital laboratories increased tenfold, from 1.3% in 1992-1993 to 12.8% in 1995-1996 ($P < 0.001$).

Hospital Laboratory Susceptibility Testing Practices, 1993 and 1995

Penicillin Susceptibility Testing

In 1993 and 1995, all 35 (100%) acute-care hospital microbiology laboratories in Connecticut responded to the laboratory surveys. In both years, 34 (97%) laboratories reported that pneumococcal isolates were routinely tested for penicillin susceptibility either in-house or at a reference laboratory. In 1993, one (3%) laboratory reported that penicillin susceptibility testing was not performed on any pneumococcal isolates. In 1995, one (3%) laboratory reported that isolates were tested only when specifically requested by a physician. Only results from the 34 laboratories that reported having susceptibility tests routinely performed on pneumococcal isolates will be presented.

The majority of hospital laboratories in both years reported testing pneumococcal isolates in-house; 29 (85%) in 1993, and 32 (94%) in 1995. In both years, the 34 hospital laboratories reported that they routinely tested all pneumococcal isolates from invasive sites. However, the number of laboratories testing all pneumococcal isolates from any body site significantly increased from 6 (18%) in 1993 to 23 (68%) in 1995 ($P < 0.001$).

Significant changes in susceptibility testing practices were observed between 1993 and 1995 (Table 4). In both years, the majority of hospital laboratories using a qualitative screening method reported using oxacillin disk diffusion (19/22 in 1993, 27/28 in 1995). The number of laboratories that reported having an initial oxacillin screening test performed on isolates followed by penicillin MIC testing of oxacillin-nonsusceptible isolates increased from 7 (21%) in 1993 to 20 (59%) in 1995 ($P < 0.005$). The total number of laboratories that had penicillin MICs determined on pneumococcal isolates increased from 22 (65%) in 1993 to 31 (91%) in 1995 ($P < 0.05$).

For laboratories that had isolates tested for penicillin susceptibility by a quantitative MIC method in 1995, 19 (61%) of 31 laboratories reported having isolates tested with the E test and 12 (39%) had isolates tested with a broth microdilution method. No laboratories reported using an automated method for MIC testing. The type of MIC testing method used was not asked of hospital laboratories in the 1993 survey.

Accuracy Of Oxacillin Disk Diffusion

A total of 733 pneumococcal isolates were submitted by hospital laboratories and MIC tested at the CDC-contracted reference laboratory. Hospital laboratories reported oxacillin zone diameters on 440 (60%) of these isolates. Categorical oxacillin results were compared with reference laboratory categorical penicillin results (Table 5). Overall, 415 (94%) of the oxacillin categorical results were concordant with the reference laboratory penicillin susceptibility classifications. All isolates categorized as penicillin-resistant by the reference laboratory were correctly identified as nonsusceptible by hospital oxacillin screening. Discordant results by oxacillin screening were 5.7% (21/367) false nonsusceptible, and 5.5% (4/73) false susceptible. Of the 21 false nonsusceptible results, 14 (67%) isolates were further penicillin MIC tested and correctly identified as penicillin-susceptible by hospital laboratories, 3 (14%) were identified as penicillin-intermediate by hospital laboratories, and 4 (19%) were not MIC tested. None of the four isolates falsely classified as susceptible by oxacillin screening were further MIC tested by hospital laboratories. All four of these isolates had reference laboratory penicillin MICs that were at the breakpoint for intermediate resistance (MIC of 0.1 μg/ml).

Accuracy of Penicillin MIC Testing

Connecticut hospital laboratories reported penicillin MIC results on 432 (59%) of the 733 pneumococcal isolates tested by the reference laboratory. Of these 432 isolates, 310 (72%) were tested by the E test and 122 (28%) by broth microdilution. The comparison of

hospital and reference laboratory categorical penicillin MIC results are presented in Table 6. Overall, 383 (89%) of the hospital laboratory MIC results were concordant with the reference laboratory results. There were no very major errors, 0.9% (3/325) major errors, and 10.6% (46/432) minor errors for all isolates tested by an MIC method by hospital laboratories. For isolates tested by hospital laboratories using broth microdilution there were 3.9% (3/76) major errors and 11.5% (14/122) minor errors. The majority of these minor errors (10 of 14) were isolates defined as resistant by the reference laboratory but interpreted as intermediate by hospital laboratories. For isolates that were tested by the E test by hospital laboratories, there were no major errors and 10.3% (32/310) minor errors, including 15 isolates categorized as intermediate by the reference laboratory but susceptible by hospital laboratories, and 12 isolates categorized as resistant by the reference laboratory but intermediate by hospital laboratories.

Susceptibility Testing Against Other Antimicrobial Agents

In 1995, 20 (59%) of the 34 acute-care hospital laboratories reported that they determined MIC values for antimicrobial agents clinically indicated for the treatment of *S. pneumoniae* in addition to penicillin.[51] Fourteen (41%) laboratories indicated that additional antimicrobial MICs were determined on a routine basis for any isolates found to be nonsusceptible by oxacillin screening or penicillin MIC determination. MIC testing against an extended-spectrum cephalosporin (cefotaxime or ceftriaxone) was performed by 14 (41%) laboratories, against chloramphenicol by 10 (29%) laboratories, erythromycin by

10 (29%), trimethoprim-sulfamethoxazole by 9 (26%), vancomycin by 8 (24%), tetracycline by 7 (21%), imipenem by 5 (15%), clindamycin by 1 (3%) and ofloxacin by 1 (3%) laboratory. No laboratories reported MIC testing against rifampin.

DISCUSSION

The prevalence of penicillin-resistant *S. pneumoniae* increased substantially in Connecticut from 1992 to 1996. The comparison of prevalence data from the 1992-1993 survey with the 1995-1996 survey is somewhat limited in that the first survey relied on MIC data determined by hospital laboratories, and less than half of the acute-care hospital laboratories in Connecticut could provide this data in 1993. However, comparison of penicillin susceptibility data from hospital laboratories that determined penicillin MICs in both years also showed that there was a substantial increase in invasive *S. pneumoniae* identified as nonsusceptible to penicillin. Several other studies have also demonstrated a marked increase during this time period in pneumococci resistant to penicillin and other antimicrobial agents.[1,4-8] The proportion of isolates determined as nonsusceptible to penicillin in 1995-1996 in Connecticut was 16%. This is similar to that reported from New York City in 1995,[38] and to two national surveillance studies from 1993-1994.[1,33] The prevalence of isolates (highly) resistant to penicillin in Connecticut during this period was 9%, which is similar to that reported from a national surveillance study from 1994-1995.[6]

The sharp increase in strains resistant to penicillin observed by this study may be due to selective pressure resulting from the widespread use of antibiotics.[1,75] Pneumococci, like all bacteria, are constantly reproducing and chromosomal mutations involving the alteration of penicillin binding proteins allow pneumococci to become less susceptible or totally resistant to penicillin and other β-lactam antibiotics.[76,77] Once a resistant strain develops, genes that encode for antibiotic resistance can be easily transferred from resistant

S. pneumoniae to susceptible *S. pneumoniae*, as well as to and from other bacterial species.[2] In the presence of antibiotics, susceptible pneumococci are killed while resistant strains are selected to survive and multiply.

Studies have demonstrated that prior use of antibiotics is associated with carriage and infection of penicillin-nonsusceptible *S. pneumoniae*.[34,37,43-45,78] Young children, particularly those attending day care, often develop respiratory tract infections and otitis media and frequently receive antibiotics that may select for drug-resistant strains.[2] A survey of antimicrobial prescription practices among office-based physicians in the United States found that from 1980 to 1992, annual visit rates for otitis media among children under 15 years increased significantly.[79] Otitis media was the most common diagnosis for which an antimicrobial drug was prescribed. This survey also found that the use of narrower-spectrum drugs such as penicillin decreased, and the use of broader-spectrum antibiotics such as cephalosporins increased.[79] Inappropriate use of antibiotics such as prescribing antibiotics for viral infections and prescribing overly broad-spectrum antibiotics can lead to the development of resistant strains.[80] A study by Yu *et al* determined that 35% of antibiotic prescriptions for the empirical treatment of patients hospitalized with bacteremia were unacceptable, with physicians often selecting the broadest possible antibiotic coverage to ensure elimination of the infecting organism.[81]

Due to the increasing prevalence of drug-resistant strains of *S. pneumoniae*, clinical microbiology laboratories must be able to accurately determine the antimicrobial susceptibility of *S. pneumoniae*. As part of a national strategy to minimize the impact of

drug-resistant *S. pneumoniae*, the CDC has emphasized the need for clinical laboratories to adhere to interpretive standards published by the NCCLS and to use appropriate methods for the susceptibility testing of *S. pneumoniae*.[11,80] Since there are a variety of methods laboratories can use to test isolates for penicillin susceptibility, it is important to know which methods laboratories are using and the accuracy of hospital laboratory susceptibility test results. There have been many studies published that have assessed the ability of various susceptibility methods to determine the penicillin susceptibility of *S. pneumoniae*; the susceptibility tests were, however, often performed by researchers in a single laboratory using a selected set of pneumococcal isolates.[57,62-65,68-70,82-86] We were able to determine the accuracy of susceptibility testing performed by hospital laboratories by comparing susceptibility data determined by a CDC-contracted reference laboratory with test results determined by hospital laboratories using data on isolates collected as part of an active surveillance program.

Results of the laboratory surveys show that 97% of Connecticut hospital laboratories in both 1993 and 1995 reported routinely having pneumococcal isolates tested for penicillin susceptibility. Our results indicate that there was a significant increase between 1993 and 1995 in the number of hospital laboratories that reported using a combination of oxacillin screening followed by penicillin MIC determination of oxacillin-nonsusceptible isolates. This practice is recommended by the NCCLS.[51]

Oxacillin screening by hospital laboratories accurately identified all penicillin-resistant isolates, but four penicillin-intermediate isolates were misclassified as oxacillin-

susceptible, resulting in 5.5% false susceptibility. However, all four of these isolates had reference penicillin MIC values at the intermediate breakpoint (0.1 μg/ml). The occurrence of these discordant results at the penicillin-intermediate breakpoint lessens their clinical significance. Previous studies conducted in research settings have found few oxacillin false susceptible results.[57,63,82,87-89] Hospital laboratories had 5.7% oxacillin false nonsusceptible results, less than the 11% to 14% observed in other studies.[87-89] The majority of false nonsusceptible isolates were correctly identified as susceptible upon penicillin MIC testing, with only three isolates (14%) still being classified as penicillin-nonsusceptible (intermediate) by the hospital laboratories. Again, these misclassifications probably have limited clinical significance.

The E test was used by more hospital laboratories than broth microdilution for penicillin MIC determination in 1995. This is probably because broth microdilution, as mentioned previously, can be difficult and tedious for laboratories to perform on a routine basis.[2,62] While the use of the E test cannot be officially recommended by the NCCLS (because it is a commercial product), it has been found to be a reliable alternative to broth microdilution for determining penicillin MICs.[63-65,68,71,72] Our study found good agreement between penicillin E test MIC results determined by hospital laboratories and penicillin broth microdilution MIC results determined by the reference laboratory. There were 10.3% minor errors, with no major or very major errors, a result consistent with previous studies that have evaluated the accuracy of the E test.[63,71,72] Intermediate isolates that were misclassified by hospital laboratories as susceptible are of concern because this type of

discordant result could effect the treatment of pneumococcal meningitis. The recommended therapy for pneumococcal meningitis caused by a penicillin-intermediate strain differs from that for a penicillin-susceptible strain.[12]

Hospital laboratories determining MICs by broth microdilution produced slightly more interpretive errors than observed with the E test. There were no very major errors, 3.9% major errors, and 11.5% minor errors noted. Susceptible isolates that were falsely classified as resistant (major errors) are of concern because some patients may have been treated with a broader-spectrum antibiotic than needed. Inappropriate antibiotic use, including the use of overly broad-spectrum antimicrobial drugs can contribute to the increase of drug-resistant *S. pneumoniae*.[1,11] The 1995 laboratory survey did not address the specific methodologies used by hospital laboratories for broth microdilution testing, such as what media was used and whether the microdilution plates were prepared in-house or obtained commercially. Differences in testing methodologies and the use of various commercial testing systems could account for the discrepancies observed between the hospital laboratory broth microdilution results and those of the reference laboratory.[62-65,85]

Only 41% of hospital laboratories in Connecticut reported that they would have a pneumococcal isolate that was oxacillin- or penicillin-nonsusceptible MIC tested against an extended-spectrum cephalosporin (cefotaxime or ceftriaxone). This finding is disappointing since current NCCLS and CDC recommendations are that isolates determined as penicillin-nonsusceptible should be quantitatively susceptibility tested against an extended-spectrum cephalosporin.[11,51,56] This is especially important for patients with pneumococcal

meningitis because treatment failures in meningitis caused by cephalosporin-nonsusceptible *S. pneumoniae* have been reported.[10,90-92] For such patients, the American Academy of Pediatrics currently recommends additional susceptibility testing against vancomycin, meropenem, and rifampin.[12] For nonmeningeal invasive infections caused by penicillin-resistant *S. pneumoniae*, susceptibility testing for vancomycin, rifampin, erythromycin, trimethoprim-sulfamethoxazole, clindamycin, imipenem, meropenem, and chloramphenicol should be considered.[12]

With the increasing prevalence of penicillin-resistant *S. pneumoniae* in Connecticut, it is very important that clinical microbiology laboratories be able to accurately detect penicillin resistance. The study presented here shows that most acute-care hospital laboratories in Connecticut were using appropriate and accurate methods to test pneumococcal isolates for penicillin susceptibility. As recommended by the NCCLS, laboratories that use a qualitative screening method need to confirm nonsusceptibility with a quantitative MIC method, a laboratory practice that was performed by significantly more laboratories in 1995 than in 1993. Susceptibility test results determined by hospital laboratories by oxacillin screening, the E test, and broth microdilution produced relatively few discrepancies. Of concern are susceptibility misclassifications that could result in inappropriate antimicrobial therapy. The changes in laboratory practice observed between 1993 and 1995 indicate that laboratories are adapting to recommendations for susceptibility testing of *S. pneumoniae*, which is critical in this era of increasing antimicrobial resistance. Pneumococcal isolates, especially invasive isolates found to be

penicillin-nonsusceptible, need to be routinely MIC tested against an extended-spectrum cephalosporin. Additional antimicrobial MICs may need to be determined depending on the site of infection and the prevalence of resistance in the local community. This study did not test the proficiency of hospital laboratories to identify pneumococcal strains with known levels of penicillin susceptibility or resistance, and so cannot comment further on specific laboratory practices that may have lead to susceptibility misclassifications.

Penicillin-resistant *S. pneumoniae* will most likely continue to increase in Connecticut and in other parts of the United States. In 1994, the CDC formed a working group to develop a strategy to minimize the impact of drug-resistant *S. pneumoniae*.[11] Surveillance for drug-resistant *S. pneumoniae*, such as done in Connecticut, was listed as the first goal of the plan. Continued surveillance is warranted to detect changing patterns of pneumococcal resistance and to identify populations most as risk for infection with resistant strains of *S. pneumoniae*. Information gained from the surveillance of antimicrobial resistance can be used to increase the awareness of the problem among health care providers and to promote the judicious use of antibiotics.

Part of the CDC's goal to improve and expand the surveillance of drug-resistant *S. pneumoniae* in the United States is to make certain that clinical laboratories are using appropriate and accurate methods for susceptibility testing of pneumococcal isolates.[11] The study presented in this paper addressed this issue and determined that most laboratories in Connecticut are using appropriate methods for penicillin susceptibility testing of *S. pneumoniae*, and should also be encouraged to expand susceptibility testing

with additional antimicrobial agents. These findings will hopefully be published in a clinical laboratory-oriented journal.

TABLE 1—Surveys of Resistance to Penicillin Among Isolates of *S. Pneumoniae*, United States, 1979-1995

Location	Year(s)	Isolates surveyed	No. isolates	Penicillin nonsusceptible[a] I, %	R, %	Total %	Ref.
19 hospitals in 26 states[b]	1979-1987	any site	5469	5.0	0.02	5.0	9
Dallas[c]	1981-1983	sterile site	258	8.1	0	8.1	34
Oklahoma	1989-1990	sterile site	144	6.3	1.4	7.6	35
13 hospitals in 12 states[b]	1991-1992	sterile site	544	5.4	1.3	6.6	4
17 medical centers in U.S.	1991-1992	any site	524	15.2	2.6	17.8	7
19 laboratories in 17 states	1992-1993	resp. tract	799	14.9	7.3	22.2	32
Washington, DC[c]	1992-1993	sterile site	108	8.3	4.6	12.9	36
St. Louis[c]	1992-1993	any site	136	19.9	5.9	25.7	37
New York City[d]	1993	any site	1229	5.7	1.5	7.2	38
12 hospitals in 11 states[b]	1993-1994	sterile site	740	10.8	3.2	14.1	1
33 laboratories in 19 states	1993-1994	any site	1627	10.4	5.7	16.2	33
Atlanta	1994	sterile site	431	17.9	7.4	25.3	39
30 institutions in 23 states	1994-1995	any site	1527	14.1	9.5	23.6	6
New York City[d]	1995	any site	3535	8.8	6.3	15.0	38
24 institutions in 16 states	1995	any site	592	6.1	21.1	27.2	8

[a] I, intermediate; R, resistant (also called highly resistant).

[b] Data from CDC sentinel surveillance.

[c] Isolates from pediatric patients only.

[d] Susceptibility testing performed by hospital laboratories and results reported to health department.

TABLE 2—Prevalence of Penicillin Resistance Among Isolates of *S. pneumoniae* from Different Body Sites, 1992-1993

Site	No. isolates	Nonsusceptible[a]		
		I (%)	R (%)	Total (%)
Blood or cerebrospinal fluid	385[b]	4 (1.0)	1 (0.3)	5 (1.3)
Pleural, peritoneal, pericardial, or joint	15[b]	0	0	0
Middle Ear	10	3 (30)	0	3 (30)
Eye	25	0	0	0
Transtracheal, nasopharyngeal, or sputum	319	7 (2.2)	2 (0.6)	9 (2.8)
Other or unspecified	92	1 (1.1)	0	1 (1.1)
Total	846	15 (1.8)	3 (0.4)	18 (2.1)

[a] I, penicillin-intermediate; R, penicillin-resistant (highly resistant).
[b] from normally sterile body sites.

TABLE 3—Prevalence of Penicillin Resistance Among *S. pneumoniae* Isolates from Normally Sterile Sites MIC Tested by 10 Hospital Laboratories, 1992-1993 and 1995-1996

	1992-1993		1995-1996	
Hospital	No. isolates	NS[a] (%)	No. isolates	NS[a] (%)
A	3	0	11	2 (18.2)
B	52	3 (5.8)	50	9 (18.0)
C	20	0	26	2 (7.7)
D	23	0	21	4 (19.0)***
E	57	1 (1.8)	33	8 (24.2)**
F	38	0	40	0
G	18	1 (5.6)	12	3 (25.0)
H	28	0	33	4 (12.1)
I	21	0	9	5 (55.6)*
J	134	0	93	5 (5.4)***
Total	394	5 (1.3)	328	42 (12.8)*

[a] NS, penicillin-nonsusceptible, includes penicillin-intermediate and penicillin-resistant isolates.

*$P < .001$; **$P < .005$; ***$P < .05$

TABLE 4—Methods Used by 34 Connecticut Hospital Laboratories for Penicillin Susceptibility Testing of *S. pneumoniae*, 1993 and 1995

	No. (%) laboratories	
Method(s)	1993	1995
Oxacillin disk screening only	11 (32)	3 (9)*
Penicillin disk screening only	1 (3)	0
Oxacillin disk screening and MIC testing of NS[a] isolates	7 (21)	20 (59)**
Breakpoint screening[b] and MIC testing of NS[a] isolates	2 (6)	1 (3)
Oxacillin disk screening; MIC testing by request only	1 (3)	4 (12)
MIC testing without an initial screening test	12 (35)	6 (18)

[a] NS, isolates identified as nonsusceptible by a screening test.
[b] 0.06 µg/ml penicillin in Mueller-Hinton broth with 5% lysed horse blood.
* $P < .05$; ** $P < .005$.

TABLE 5—Comparison of Oxacillin Screening by Connecticut Hospital Laboratories and Penicillin MIC Testing by the Reference Laboratory for 440 *S. pneumoniae* Isolates

	Susceptibility classifications of isolates tested by oxacillin screening by hospital laboratories (oxacillin diameter)	
Reference laboratory determinations [a] (No.)	Susceptible (≥ 20 mm)	Nonsusceptible (≤ 19 mm)
Susceptible (367)	**346**	21
Intermediate (31)[b]	4	**27**
Resistant (42)[b]	0	**42**

[a] by the NCCLS broth microdilution method (60).

[b] penicillin-nonsusceptible.

Boldface type indicates concordant results.

TABLE 6—Comparison of Penicillin MIC Testing of *S. pneumoniae* Isolates by Connecticut Hospital Laboratories and by the Reference Laboratory

MIC method used by hospital laboratories (No. isolates tested)	Reference laboratory determinations[a]	(No.)	Penicillin susceptibility classifications of isolates MIC tested by hospital laboratories		
			Susceptible	Intermediate	Resistant
Broth microdilution (n = 122)	Susceptible	(76)	**71**	2	3
	Intermediate	(16)	1	**14**	1
	Resistant	(30)	0	10	**20**
E test (n = 310)	Susceptible	(249)	**245**	4	0
	Intermediate	(27)	15	**11**	1
	Resistant	(34)	0	12	**22**
Total (n = 432)	Susceptible	(325)	**316**	6	3
	Intermediate	(43)	16	**25**	2
	Resistant	(64)	0	22	**42**

[a] by the NCCLS broth microdilution method (60).
Boldface type indicates concordant results.

REFERENCES

1. Butler JC, Hofmann J, Cetron MS, Elliot JA, Facklam RR, Breiman RF. The continued emergence of drug-resistant *Streptococcus pneumoniae* in the United States: an update from the Centers of Disease Control and Prevention's Pneumococcal Sentinel Surveillance System. *J Infect Dis.* 1996;174:986-993.

2. Lonks JR, Medeiros AA. The growing threat of antibiotic-resistant *Streptococcus pneumoniae. Med Clin North Am*. 1995;79:523-535.

3. Centers for Disease Control and Prevention. Update on adult immunization: recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Morb Mortal Wkly Rep*. 1991;40(RR-12):1-94.

4. Breiman RF, Butler JC, Tenover FC, Elliot JA, Facklam RR. Emergence of drug-resistant pneumococcal infections in the United States. *JAMA*. 1994;271:1831-1835.

5. Plouffe JF, Breiman RF, Facklam RR. Bacteremia with *Streptococcus pneumoniae*. *JAMA*. 1996;275:194-198.

6. Doern GV, Brueggemann A, Holley HP, Rauch AM. Antimicrobial resistance of *Streptococcus pneumoniae* recovered from outpatients in the United States during the winter months of 1994 to 1995: results of a 30-center national surveillance study. *Antimicrob Agent Chemother*. 1996;40:1208-1213.

7. Thornsberry CS, Brown SD, Yee YC, Bouchillon SK, Marler JK, Rich T. Increasing penicillin resistance in *Streptococcus pneumoniae* in the U.S. *Infect Med*. 1993;10(Suppl. D):15-24.

8. Thornsberry C, Burton PH, Vanderhoof BH. Activity of penicillin and three third-generation cephalosporins against US isolates of *Streptococcus pneumoniae*: a 1995 surveillance study. *Diagn Microbiol Infect Dis*. 1996;25:89-95.

9. Spika JS, Facklam RR, Plikaytis BD, Oxtoby MJ. Antimicrobial resistance of Streptococcus pneumoniae in the United States, 1979-1987. The Pneumococcal Surveillance Working Group. *J Infect Dis*. 1991;163:1273-1278.

10. Lonks JR, Durkin MR, Meyerhoff AN, Medeiros AA. Meningitis due to ceftriaxone-resistant *Streptococcus pneumoniae*. *N Engl J Med*. 1995;332:893-894.

11. Centers for Disease Control and Prevention. Defining the public health impact of drug-resistant *Streptococcus pneumoniae*: report of a working group. *MMWR Morb Mortal Wkly Rep*. 1996;45(RR-1):1-20.

12. American Academy of Pediatrics. Therapy for children with invasive pneumococcal infections. *Pediatr*. 1997;99:289-299.

13. Simberkoff MS. Drug-resistant pneumococcal infections in the United States, a problem for clinicians, laboratories, and public health. *JAMA*. 1994;271:1875-1876.

14. Centers for Disease Control and Prevention. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep*. 1997;46(RR-8):1-24.

15. Centers for Disease Control and Prevention. Prevalence of penicillin-resistant *Streptococcus pneumoniae* – Connecticut, 1992-1993. *MMWR Morb Mortal Wkly Rep*. 1994;43:216-7,223.

16. Levy SB. *The Antibiotic Paradox: How Miracle Drugs Are Destroying the Miracle*. New York, NY: Plenum Press; 1992.

17. Watson DA, Musher DM, Jacobson JW. A brief history of the pneumococcus: A panoply of scientific discovery. *Clin Infect Dis*. 1993;17:913-924.

18. Hansman D, Bullen MM. A resistant pneumococcus. *Lancet*. 1967;2:264-265.

19. Hansman D, Glasgow H, Sturt J, Devitt L, Douglas R. Increased resistance to penicillin of pneumococci isolated from man. *N Engl J Med*. 1971;284:175-177.

20. Appelbaum PC, Bhamjee A, Scragg JN, Hallet AF, Bowen AJ, Cooper RC. *Streptococcus pneumoniae* resistant to penicillin and chloramphenicol. *Lancet*. 1977;2:995-997.

21. Garcia-Leoni ME, Cercenado E, Rodeno P, Bernaldo de Quiros JCL, Martinez-Hernandez D. Susceptibility of *Streptococcus pneumoniae* to penicillin: a prospective microbiological and clinical study. *Clin Infect Dis*. 1992;14:427-435.

22. Fenoll A, Bourgon CM, Munoz R, Vicioso D, Casal J. Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolates causing systemic infections in Spain, 1979-1989. *Rev Infect Dis*. 1991;13:56-60.

23. Linares J, Pallares R, Alonso T, Perez JL, Ayats J, Gudiol F, Viladrich PF, Martin R. Trends in antimicrobial resistance of clinical isolates of *Streptococcus pneumoniae* in Bellvitge Hospital, Barcelona, Spain (1979-1990). *Clin Infect Dis*. 1992;15:99-105.

24. Marton A, Gulyas M, Munoz R. Extremely high incidence of antibiotic resistance in clinical isolates of *Streptococcus pneumoniae* in Hungary. *J Infect Dis*. 1991;163:542-548.

25. Geslin P, Buu-Hoi A, Frémaux A, Acar JF. Antimicrobial resistance in *Streptococcus pneumoniae*: an epidemiological survey in France, 1970-1990. *Clin Infect Dis*. 1992;15:95-98.

26. Privitera G. Penicillin resistance among Streptococcus pneumoniae in Europe. *Diagn Microbiol Infect Dis*. 1994;19:157-61.

27. Schutze GE, Kaplan SL, Jacobs RF. Resistant pneumococcus: a worldwide problem. *Infect*. 1994;22:233-237.

28. Baquero F. Pneumococcal resistance to β-lactam antibiotics: a global geographic overview. *Microbiol drug resistance*. 1995;1:115-120.

29. Johnson AP, Speller DCE, George RC, Warner M, Domingue G, Efstratiou A. Prevalence of antibiotic resistance and serotypes in pneumococci in England and Wales: results of observational surveys in 1990 and 1995. *British Med J*. 1996; 312:1454-1457.

30. Simor AE, Louis M, Low DE. Canadian national survey of prevalence of antimicrobial resistance among isolates of *Streptococcus pneumoniae*. *Antimicrob Agent Chemother*. 1996;40:2190-2193.

31. Appelbaum PC. Epidemiology and *in vitro* susceptibility of drug-resistant *Streptococcus pneumoniae*. *Pediatr Infect Dis J*. 1996;15:932-939.

32. Barry AL, Pfaller MA, Fuchs PC, Packer RR. In vitro activities of 12 orally administered antimicrobial agents against four species of bacterial respiratory pathogens from U.S. medical centers in 1992 and 1993. *Antimicrob Agent Chemother*. 1994;38:2419-2425.

33. Mason EO, Lamberth L, Lichenstein R, Kaplan SL. Distribution of *Streptococcus pneumoniae* resistant to penicillin in the USA and in-vitro susceptibility to selected oral antibiotics. *J Antimicrob Chemother*. 1995;36:1043-1048.

34. Jackson MA, Shelton S, Nelson JD, McCracken GH. Relatively penicillin-resistant pneumococcal infections in pediatric patients. *Pediatr Infect Dis J*. 1984;3:129-132.

35. Haglund LA, Istre GR, Pickett DA, Welch DF, Fine DP. Invasive pneumococcal disease in central Oklahoma: emergence of high-level penicillin resistance and multiple antibiotic resistance. *J Infect Dis*. 1993;168:1532-1536.

36. Pikis A, Akram S, Donkersloot JA, Campos JM, Rodriguez WJ. Penicillin-resistant pneumococci from pediatric patients in the Washington, DC, Area. *Arch Pediatr Adolesc Med*. 1995;149:30-35.

37. Welby PL, Keller DS, Cromien JL, Tebas P, Storch GA. Resistance to penicillin and non-beta-lactam antibiotics of *Streptococcus pneumoniae* at a children's hospital. *Pediatr Infect Dis J*. 1994;13:281-287.

38. Heffernan R, Henning K, Labowitz A, Hjelte A, Layton M. Laboratory survey of drug-resistant *Streptococcus pneumoniae* in New York City, 1993-1995. *Emerging Infect Dis*. 1997;4(1). URL:http://www.cdc.gov/ncidod/EID/vol4no1/hefferna.htm.

39. Hofmann J, Cetron MS, Farley MM, Baughman WS, Facklam RR, Elliot JA, Deaver KA, Brieman RF. The prevalence of drug-resistant *Streptococcus pneumoniae* in Atlanta. *N Engl J Med*. 1995;333:481-486.

40. McCracken GH Jr. Emergence of resistant Streptococcus pneumoniae: a problem in pediatrics. *Pediatr Infect Dis J*. 1995;14:424-428.

41. Kaplan SL. *Streptococcus pneumoniae*: impact of antibiotic resistance in pediatrics. *Curr Probl Pediatr*. 1997;27:187-195.

42. Chesney PJ. The escalating problem of anitmicrobial resistance in *Streptococcus pneumoniae*. *Amer J Dis Child*. 1992;146:912-916.

43. Reichler MR, Allphin AA, Breiman RF, Schreiber JR, Arnold JE, McDougal LK, Facklam RR, Boxerbaum B, May D, Walton RO, Jacobs MR. The spread of multiply resistant *Streptococcus pneumoniae* at a day care center in Ohio. *J Infect Dis*. 1992; 166:1346-1353.

44. Arnold KE, Leggiadro RJ, Breiman RF, Lipman HB, Schwartz B, Appleton MA, Cleveland KO, Szeto HC, Hill BC, Tenover FC. Risk factors for carriage of drug-resistant *Streptococcus pneumoniae* among children in Memphis, Tennessee. *J Pediatr*. 1996; 128:757-764.

45. Duchin JS, Breiman RF, Diamond A, Lipman HB, Block SL, Hedrick JA, Finger R, Elliot JA. High prevalence of multidrug-reistant *Streptococcus pneumoniae* among children in a rural Kentucky community. *Pediatr Infect Dis J*. 1995;14:745-750

46. Centers for Disease Control and Prevention. Drug-resistant *Streptococcus pneumoniae*--Kentucky and Tennessee, 1993.*MMWR Morb Mortal Wkly Rep*. 1994;43:23-26,31.

47. Estrada B, Steele RW. Penicillin and multiply antibiotic-resistant *Streptococcus pneumoniae*. *Compr Ther*. 1995;21:658-662.

48. Friedland IR. Antibiotic-resistant pneumococci. *J Pediatr*. 1996;128:862.

49. Redondo E. Drug-resistant *Streptococcus pneumoniae*. *N Engl J Med*. 1996;334:53-55.

50. Minton EJ, Macfarlane JT. Antibiotic resistant *Streptococcus pneumoniae*. *Thorax*. 1996;51(Suppl. 2):S45-S50.

51. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial susceptibility testing*. M100-S5. Villanova, PA: National Committee for Clinical Laboratory Standards; 1994.

52. Tomfohrde KM. The FDA review criteria for assessment of antimicrobial susceptibility devices- too much or not enough regulation? *Adv Exp Med Biol*. 1994;349:135-143.

53. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disk susceptibility tests*, 5th ed. Approved standard M2-A5. Villanova, PA: National Committee for Clinical Laboratory Standards; 1993.

54. Jorgensen JH. Antimicrobial susceptibility testing of bacteria that grow aerobically. *Infect Dis Clin North Am*. 1993;7:393-409.

55. Sahm DF, O'Brien TF. Detection and surveillance of antimicrobial resistance. *Trends Microbiol*. 1994;2:366-371.

56. Centers for Disease Control and Prevention. Case definitions for infectious conditons under public health surveillance. *MMWR Morb Mortal Wkly Rep*. 1997;46:(RR-10):32-33.

57. Swenson JM, Hill BC, Thornsberry C. Screening pneumococci for penicillin resistance. *J Clin Microbiol*. 1986;24:749-752.

58. Jorgensen JH. Selection criteria for an antimicrobial susceptibility testing system. *J Clin Microbiol*. 1993;31:2841-2844.

59. Woods GL, Washington JA. Antibacterial susceptibility tests: dilution and disk diffusion methods. In Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. *Manual of clinical microbiology*, 6th ed. Washington, DC: American Society for Microbiology; 1995.

60. National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, 3rd ed. Approved standard M7-A3. Villanova, PA: National Committee for Clinical Laboratory Standards; 1993.

61. Bartlett RC. When we should be testing, how often and what to report. *Adv Exp Med Biol*. 1994;349:35-60.

62. Nolte FS, Metchock B, Williams T Diem L, Bressler A, Tenover FC. Detection of penicillin-resistant *Streptococcus pneumoniae* with commercially available broth microdilution panels. *J Clin Microbiol*. 1995;33:1804-1806.

63. Kiska DL, Kerr A, Jones MC, Chazotte NN, Eskridge B, Miller S, Jordan M, Sheaffer C, Gilligan PH. Comparison of antimicrobial susceptibility methods for detection of penicillin-resistant Streptococcus pneumoniae. *J Clin Microbiol*. 1995;33:229-232.

64. Krisher KK, Linscott A. Comparison of three commercial MIC systems, E test, Fastidious Antimicrobial Susceptibility panel, and FOX Fastidious panel, for confirmation of penicillin and cephalosporin resistance in *Streptococcus pneumoniae*. *J Clin Microbiol*. 1994;32:2242-2245.

65. Tenover FC, Baker CN, Swenson JM. Evaluation of commercial methods for determining antimicrobial susceptibility of *Streptococcus pneumoniae*. *J Clin Microbiol*. 1996;34:10-14.

66. Thornsberry C. E-Test symposium. Foreword. *Diagn Microbiol Infect Dis*. 1992;15:457-458.

67. Appelbaum PC. Antibiotic-resistant pneumococci-- facts and fiction. *J Chemother*. 1994;6(S4):7-16.

68. Macias EA, Mason EO Jr, Ocera HY, LaRocco MT. Comparison of E test with standard broth microdilution for determining antibiotic susceptibilities of penicillin-resistant strains of *Streptococcus pneumoniae*. *J Clin Microbiol*. 1994;32:430-432.

69. Jacobs MR, Bajaksouzian S, Appelbaum PC, Bolmstrom A. Evaluation of the E-test for susceptibility testing of pneumococci. *Diagn Microbiol Infect Dis*. 1992;15:473-478.

70. Clark RB, Giger O, Mortensen JE. Comparison of susceptibility test methods to detect penicillin-resistant *Streptococcus pneumoniae*. *Diagn Microbiol Infect Dis*. 1993;17:213-217.

71. Jorgensen JH, Ferraro MJ, McElmeel ML, Spargo J, Swenson JM, Tenover FC. Detection of penicillin and extended-spectrum cephalosporin resistance among *Streptococcus pneumoniae* clinical isolates by use of the E test. *J Clin Microbiol*. 1994; 32:159-163.

72. Skulnick M, Small GW, Lo P, Patel MP, Porter CR, Low DE, Matsumura S, Mazzulli T. Evaluation of accuracy and reproducibility of E test for susceptibility testing of *Streptococcus pneumoniae* to penicillin, cefotaxime, and ceftriaxone. *J Clin Microbiol*. 1995;33:2334-2337.

73. Murray PR, Niles AC, Heeren RL. Comparison of a highly automated 5-h susceptibility testing system, the Cobas-Bact, with two reference methods: Kirby-Bauer disk diffusion and broth microdilution. *J Clin Microbiol*. 1987;25:2372-2377.

74. Bradford PA, Sanders CC. Use of a predictor panel for development of a new disk for diffusion tests with cefoperazone-sulbactam. *Antimicrob. Agent Chemother*. 1992;36:394-400.

75. Klugman KP. Pneumococcal resistance to antibiotics.*Clin Microbiol Rev*. 1990;3:171-196.

76. Friedland IR, McCracken GH. Management of infections caused by antibiotic-resistant *Streptococcus pneumoniae*. *N Engl J Med*. 1994;331:377-382.

77. Spratt BG. Resistance to antibiotics mediated by target alterations. *Science*. 1994; 264:388-393.

78. Arason, VA. Kristinsson KG, Sigurdsson JA, Stefansdottir G, Molstad S, Gudmundsson S. Do antimicrobials increase the carriage rate of penicillin resistant

pneumococci in children? Cross sectional prevalence study. *British Med J*. 1996;313:387-392.

79. McCaig LF, Hughs JM. Trends in antimicrobial drug prescribing among office-based physicians in the United States. *JAMA*. 1995;273:214-219.

80. Jernigan DB, Cetron MS, Breiman RF. Minimizing the impact of drug-resistant *Streptococcus pneumoniae* (DRSP). A strategy from the DRSP Working Group. *JAMA*. 1996;275:206-209.

81. Yu VL, Stoehr GP, Starling RC, Shogan JE. Empiric antibiotic selection by physicians: evaluation of reasoning strategies. *Am J Med Sci*. 1991;301:165-172.

82. Barry AL, Brown SD, Fuchs PC. In vitro activity of ceftizoxime, ceftazidime, cefuroxime, ceftriaxone, cefotaxime, and penicillin against *Streptococcus pneumoniae* as determined by three quantitative methods. *Eur J Clin Microbiol Infect Dis*. 1996;15:344-346.

83. Poulsen RL, Knudsen JD, Petersen MB, Fuursted K, Espersen F, Frimodt-Moller N. Detection of penicillin resistance in *Streptococcus pneumoniae* by diffusion tests. *APMIS*. 1996; 104:549-556.

84. Thorvilson J, Kohner P, Henry N, Cockerill F. Comparison of agar dilution, broth dilution, disk diffusion, and the E-test for susceptibility testing of penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae*. *Eur J Clin Microbiol*. 1997;16:391-394.

85. Marshall KJ, Musher DM, Watson D, Mason EO Jr. Testing of Streptococcus pneumoniae for resistance to penicillin. J Clin Microbiol. 1993;31:1246-1250.

86. Ngui-Yen JH, Bryce EA, Porter C, Smith JA. Evaluation of the E test by using selected gram-positive bacteria. *J Clin Microbiol*. 1992;30:2150-2152.

87. Doern GV, Brueggemann AB, Pierce G. Assessment of the oxacillin disk screening test for determining penicillin resistance in *Streptococcus pneumoniae*. *Eur J Clin Microbiol Infect Dis*. 1977;16:311-314.

88. Jorgensen JH, Swenson JM, Tenover FC, Ferraro MJ, Hindler JA, Murray PR. Development of interpretive criteria and quality control limits for broth microdilution and disk diffusion antimicrobial susceptibility testing of *Streptococcus pneumoniae*. *J Clin Microbiol*. 1994;32:2448-2459.

89. Yee YC, Thornsberry C, Brown SD, Bouchillon SK, Marler JK, Rich T. A comparative study of the in-vitro activity of cefepime and other antimicrobial agents against penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae*. *J Antimicrob Chemother*. 1993;32(Suppl.B):13-19.

90. Bradley JS, Conner JD. Ceftriaxone failure in menigitis caused by *Streptococcus pneumoniae* with reduced susceptibility to beta-lactam antibiotics. *Pediatr Infec Dis J*. 1991;10:871-873.

91. John CC. Treatment failure with use of a third-generation cephalosporin for penicillin-resistant pneumococcal meningitis: case report and review. *Clin Infect Dis*. 1994;18:188-193.

92. Sloas, MM, Barrett FF, Chesney PJ, English BK, Hill BC, Tenover FC, and. Leggiadro RJ. Cephalosporin treatment failure in penicillin- and cephalosporin-resistant *Streptococcus pneumoniae* meningitis. *Pediatr Infect Dis J*. 1992;11:662-666.