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Detection of Drug-Resistance in *Streptococcus pneumoniae*

Drug resistance among clinical isolates of Streptococcus pneumoniae *has emerged as a significant national problem in the decade of the 1990s.*[1] *Both penicillin and multidrug resistant strains have increased substantially in prevalence during this period* [2-5] *and have recently included isolates resistant to fluoroquinolones.*[6]

Streptococcus pneumoniae is the most common cause of bacterial meningitis, acute otitis media, sinusitis, and bacterial pneumonia in the US.[1] Because drug resistance can make selection of appropriate antibiotics for therapy difficult, it is important that clinical microbiology laboratories test all clinically significant isolates for antimicrobial susceptibility using methods that can reliably detect resistance.[7] However, a survey conducted by the Texas Department of Health in early 1999 suggests that almost 30% of Texas clinical microbiology laboratories may not be testing all clinically significant isolates (Table 1). The goal of this article is to review the methods that will provide accurate susceptibility testing results with pneumococci and to highlight the drugs that are most important for routine testing in order to encourage good laboratory practices and optimal treatment of pneumococcal disease in Texas.

The National Committee for Clinical Laboratory Standards (NCCLS) has described reference broth microdilution and disk diffusion methods for testing *S. pneumoniae*, as well as relevant quality control and interpretive criteria for both of those methods.[8-10] The NCCLS reference broth microdilution procedure incorporates the use of Mueller-Hinton broth supplemented with 2-5% lysed horse blood, but with otherwise standard inoculum and incubation conditions for 20 to 24 hours.[8] Four commercial microdilution products that closely resemble the NCCLS reference method have received clearance from the US FDA for use in clinical laboratories (Table 2).[11] In addition, the E test has become very popular for testing pneumococci because of the simplicity and convenience of that method.[12] The NCCLS also indicates those drugs that are most important for routine laboratory testing on significant pneumococcal isolates, including those from patients with meningitis or bacteremia (Table 3).[10]

The NCCLS Kirby Bauer disk test utilizes sheep blood supplemented Mueller-Hinton agar and calls for an incubation time of 20 to 24 hours in 5% CO_2.[9] A CO_2 incubation atmosphere is necessary to prevent growth failures with pneumococcal isolates, and it has been incorporated in the standardization of the test, including the development of quality control zone diameter ranges. Unfortunately, the disk diffusion procedure does not provide acceptable accuracy when testing pneumococci with the beta-lactam family of drugs (ie, penicillins and cephalosporins).[9,10,13] Indeed, the routine determination of minimal inhibitory concentrations (MICs) of penicillin and selected extended-spectrum cephalosporins is now recommended for all patients with serious pneumococcal infections.[8-10] The one possible exception to the statement above cautioning against use of the disk test with beta-lactams is the oxacillin disk screening procedure. An oxacillin disk can be used to "screen" for penicillin susceptibility, as indicated by a zone of inhibition of $\geq$ 20 mm. Such strains are uniformly susceptible to penicillin and to other relevant betalactam antibiotics, including amoxicillin and most cephalosporins.[9] However, if the oxacillin zone is < 20 mm, it is necessary to determine a penicillin MIC to clarify whether an isolate is resistant or has intermediate or borderline susceptibility to penicillin.[10] In addition, there is now evidence that patients with pneumonia due to strains with intermediate susceptibility to penicillin or to the extended-spectrum cephalosporins respond satisfactorily to therapy with those

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agents.[14-16] Therefore, it is now important to recognize and accurately report strains in the intermediate category with those drugs.[16]

Table 1. Results of a 1999 Survey of 181 Texas Clinical Microbiology Laboratories Regarding Detection of Drug-Resistant *S. pneumoniae*

Primary Practices Used for Detection of DRSP	No. labs	(%)
Routine testing of all isolates	102	(72)
Testing of only blood and normally sterile fluids	16	(11)
Testing of normally sterile sites and sputum	22	(16)
Drugs Most Often Tested on Isolates from Blood and CSF		
Penicillin	116	(64)
Vancomycin	90	(50)
Cefotaxime	79	(44)
Ceftriaxone	68	(38)
Erythromycin	74	(41)
Trimethoprim/sulfamethoxazole	61	(34)
Clindamycin	51	(28)
Chloramphenicol	44	(24)
Tetracycline	24	(13)
Other drugs	—(<10)	
Drugs Most Often Tested on Isolates from Sputum or Other Respiratory Sources		
Erythromycin	86	(48)
Penicillin (or oxacillin)	81	(45)
Vancomycin	78	(43)
Trimethoprim/sulfamethoxazole	72	(40)
Cefotaxime	63	(35)
Ceftriaxone	60	(33)
Chloramphenicol	48	(27)
Fluoroquinolone	42	(23)
Tetracycline	26	(14)
Clindamycin	21	(12)
Cefuroxime	20	(11)
Other drugs	—(<10)	
Testing not routinely performed	9	(5)
Susceptibility Testing Methods Used for Detecting Penicillin Resistance		
Oxacillin disk	67	(37)
Commercial broth microdilution	38	(21)
E test	26	(14)

Table 2. FDA-cleared Commercial MIC Test Methods for *S. pneumoniae*

Method or Product	Medium	Incubation
Broth microdilution (MicroScan MICroSTREP: MicroTech; Pasco; Sensititre)	Mueller-Hinton broth + 2% lysed horse blood (5×10^5 CFU/ml inoculum)	ambient air, 20-24 h
E test	Mueller-Hinton agar + 5% sheep blood	5% CO_2, 20-24 h

The NCCLS disk diffusion procedure does work well with the non-beta-lactam drugs.[9,13] Because it is a simple and economical approach for routine testing of pneumococci, a convenient approach used by some laboratories is application of selected E test strips (eg, penicillin and cefotaxime or ceftriaxone) along with disks for several non-beta-lactam drugs (eg, erythromycin, trimethoprim/sulfamethoxazole, a quinolone, vancomycin) on the same large Mueller-Hinton sheep blood agar plate.

A partial solution to the problem of escalating drug resistance in pneumococci may be found in the new pneumococcal conjugate vaccine that will soon be approved for use in children younger than one year of age. This vaccine will protect against systemic infections (eg, meningitis and bacteremia) due to the seven pneumococcal serotypes that are most common in childhood infections. The vaccine is also expected to reduce somewhat the incidence of pneumonia and to a lesser degree otitis media in children under the age of five years. It will also be important to continue to administer the current 23-valent pneumococcal vaccine to older children with certain underlying conditions (eg, sickle cell, splenectomy, some cancers) and to all persons older than 65 years of age.

Despite these prevention efforts, it is likely that pneumococcal respiratory infections will continue to be problematic due in part to the widespread prevalence of antimicrobial resistance. Therefore, it is important that clinical laboratories in Texas perform optimal susceptibility testing of pneumococcal isolates

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Table 3. Antimicrobial Agents Recommended for Routine Testing Against *S. pneumoniae* by the NCCLS

Blood[a] and CSF Isolates

Penicillin
Cefotaxime or ceftriaxone
Meropenem
Vancomycin

Isolates from Other Sites

Primary Drugs[b]	Optional Drugs[c]
Penicillin	Cefepime, cefotaxime, or ceftriaxone
Erythromycin	Levofloxacin or ofloxacin
Trimethoprim/sulfamethoxazole	Meropenem
	Tetracycline
	Vancomycin

[a] Other drugs may be also be reported with isolates from patients who do not have meningitis
[b] Includes only drugs in NCCLS Table 1A, Group A[9]
[c] Includes only drugs in NCCLS Table 1A, Group B; some supplemental drugs can be found in Table 1A, Group C[9]

in order to detect resistance, and that physicians strive to preserve our current battery of effective drugs through judicious antibiotic prescribing practices.

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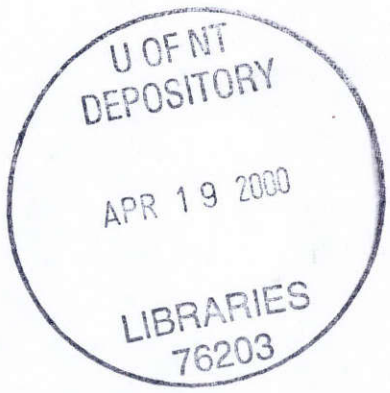

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