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Main Articles

A new technique (the NOW test) for the detection of *Streptococcus pneumoniae* in the effusions of otitis media

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Abstract

The presence of *Streptococcus pneumoniae* in chronic otitis media was determined with a new antigen detection kit, the NOW test. The NOW test was originally designed as a urinary antigen test but was adapted to middle-ear effusions for the present study. Middle-ear effusions from 52 children were studied. *Streptococcus pneumoniae* was cultured from 10 per cent of the effusions. The NOW test was positive in 23 per cent of the effusions, 80 per cent of culture positive and 17 per cent of culture negative effusions. The NOW test proved to be rapid, simple, reliable and relatively inexpensive for the detection of pneumococcal antigen in the middle-ear effusions. This test may prove valuable for the management of children with acute otitis media who undergo tympanocentesis.

Key words: Otitis Media; *Streptococcus pneumoniae*; Bacteriological Techniques

Introduction

Otitis media with effusion is part of the normal resolution phase of acute otitis media. Although most effusions disappear quickly, approximately 10 per cent persist beyond two or three months and are considered to be chronic.[1] The pathogenesis of chronic otitis media with effusion (COME) is poorly understood; however, persistent infection or residual bacterial antigens are elieved to play a role in the disease process. Cultures of more than 7 000 chronic middle-ear effusions from 12 studies over the past 20 years have documented the presence of viable strains of *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis* in 11 to 35 per cent of the effusions.[2–13] *Streptococcus pneumoniae* alone was cultured from one to 16 per cent of the fluids.[2–13]

Antigen detection tests have demonstrated the presence of non-viable bacteria in many sterile chronic middle-ear effusions. Countercurrent immunoelectrophoresis and latex agglutination assays were among the tests first employed to detect residual pneumococcal capsular antigen in the effusions.[8,11] These methods significantly raised the sensitivity of pneumococcal detection when compared to culture. Since there are more than 90 types of pneumococcal capsular polysaccharides, antigen detection tests were unable to detect all types. The recent introduction of polymerase chain reaction (PCR) technology for the detection of nucleic acids of bacteria further raised the detection rates of *Streptococcus pneumoniae* and enabled the detection of *Haemophilus influenzae* and *Moraxella catarrhalis* as well as other organisms in middle-ear effusions.[5–7,11] At present, PCR is considered the most sensitive technique available. However, it is expensive and technically difficult, requiring specially trained technicians and sophisticated equipment.

The present study was designed to evaluate a new pneumococcal antigen detection kit, the NOW test. The NOW test was developed to detect pneumococcal antigen in the urine of adults with pneumococcal pneumonia. The test detects the cell wall polysaccharide of *Streptococcus pneumoniae* that is present in all clinical strains of the organism. Thus, unlike earlier antigen detection tests which were limited to the more prevalent capsular polysaccharides of *Streptococcus pneumoniae*, the NOW test was designed to detect all pneumococcal types. The present study successfully adapted the kit to middle-ear effusions and demonstrated the test to be simple, rapid and reliable.

Methods

Population Children with chronic middle-ear effusions, lasting more than three months and scheduled

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TABLE I
BACTERIAL CULTURE RESULTS OF MIDDLE EAR FLUIDS[a]

Sterile	28 (54%)
Pathogens[b] *Streptococcus pneumoniae* 5; *Moraxella catarrhalis* 5; *Haemophilus influenzae* 4	12 (23%)
Non-pathogens[b] *Staphylococcus* 14; diphtheroids 6; *Streptococcus viridans* 3	12 (23%)

[a]One ear fluid test per child
[b]Some mixed cultures

for myringotomy were enrolled in the study through the ENT clinic at the Children's Hospital of Buffalo over a four-month period without restriction to age, sex or history of middle-ear disease.

Procedures Middle-ear effusions were collected at the time of myringotomy with either an Alden-Senturia trap or Jung trap. The fluid was brought to the lab within one hour of collection. The fluid was cultured on sheep blood agar, chocolate agar, and MacConkey agar plates. Bacteria were isolated and identified by standard laboratory techniques. The fluid was also processed for the NOW test.

The NOW test is manufactured by Binax Inc. in Portland, Maine. It is an *in vitro* rapid immunochromatographic assay for the detection of *Streptococcus pneumoniae* antigen in urine specimens from patients with symptoms of pneumonia. The test kit incorporates rabbit anti-*Streptococcus pneumoniae* antibody adsorbed onto a nitrocellulose membrane. If pneumococcal cell wall polysaccharide is in the specimen, an easily discernible pink to purple line appears within 15 minutes on the membrane. A control is included to assure validity of the test. The present study modified the assay; middle-ear effusions were tested in place of urine samples. Preliminary studies were performed with 36 middle-ear effusions that had been stored at −70°C. The NOW test was able to detect the presence of pneumococcal antigen in 15 of 15 culture positive samples. The NOW test was negative in 19 of 21 culture negative samples. We assume that the two 'false positive' test results reflected the presence of non-viable *Streptococcus pneumoniae.*

Results

Population Fifty-two subjects wre enrolled. They ranged in age from three to 147 months with a mean of 42 months. There were 32 males and 20 females. Each child had the diagnosis of chronic otitis media with effusion and was scheduled to undergo a myringotomy. The majority of children underwent tympanostomy tube placement. Twenty-six children had received antibiotics within the previous month. Amoxicillin was the most frequently prescribed antibiotic.

Cultures Bacteria were recovered from 24 (46 per cent) children (Table I). Pathogens were recovered from 23 per cent and non-pathogens from 23 per cent. Among the 12 pathogens, five were *Streptococcus pneumoniae* (8.9 per cent), five were *Moraxella catarrhalis* (8.9 per cent) and four were *Haemophilus influenzae* (7.1 per cent). Fifty-four per cent of the fluid were sterile.

NOW test The rapid detection test was positive in 12 samples (23 per cent). It was positive in four of five (80 per cent) culture-positive samples and eight of 47 (17.1 per cent) culture-negative samples.

Discussion

In the present study, 23 per cent of middle-ear effusions yielded viable pathogens, a rate similar to previously published reports.[2–13] *Streptococcus pneumoniae* grew from 8.9 per cent of the effusions, a rate also comparable to previous reports.[2–13] The NOW test detected pneumococcus in 80 per cent of the culture-positive samples. The NOW test also detected evidence of a prior pneumococcal infection in 17 per cent of the sterile effusions. Thus, the NOW test was positive in 23 per cent of effusions. This rate is close to the rate of 31 per cent recently reported with PCR.[8] In comparative studies, antigen tests increased the detection rate of *Streptococcus pneumoniae* when compared to culture with a few exceptions (Table II).[4,7–11] Although, PCR assays have detected pneumococcal DNA in as many as 46 per cent of chronic middle-ear effusion samples, most PCR assays have yielded lower rates that were similiar to other antigen assays (Table II).

The NOW test was sucessfully adapted for use with middle-ear specimens. It proved to be simple, rapid, and reliable for the detection of *Streptococcus pneumoniae.* It is an improvement over earlier pneumococcal tests because it utilizes a single antigen, the cell wall polysaccharide, rather than a limited number of capsular polysaccharides. The recent increase in penicillin resistance among isolates of *Streptococcus pneumoniae* has complicated the treatment of acute otitis media and fostered the use of tympanocentesis in select cases. A rapid assay such as the NOW test may prove useful in selecting

TABLE II
DETECTION OF S PNEUMONIAE IN EFFUSIONS FROM CHRONIC OTITIS MEDIA WITH VARIOUS ASSAYS

Reference	Year	No. specimens	Culture (%)	Assay, % Positive
Palva *et al.*[10]	1987	108	1	CIE, 15
Miller *et al.*[8]	1990	47	4	LA, 21
Post *et al.*[11]	1995	97	16	PCR, 31
Jero *et al.*[6]	1996	123	11	PCR, 46
Hendolin[5]	1997	25	8	PCR, 8
Matar[7]	1998	47	4	PCR, 8
Faden	Present	52	9	NOW, 23

CIE = countercurrent immunoelectrophoresis; LA = latex agglutination; PCR = polymerase chain reaction; NOW = NOW test.

the most appropriate therapy. The test may also prove useful as a research tool in elucidating the aetiology of chronic middle-ear effusions.

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