MOLECULAR EPIDEMIOLOGY OF STREPTOCOCCUS PNEUMONIAE MEDIATED OTITIS MEDIA

Sandi McCoy ¹ and Melinda Pettigrew ²

¹ Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI , ² Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06520-8032

TABLE OF CONTENTS
1. Abstract
2. Introduction
3. Otitis media
4. Pneumococcal carriage
5. Pneumococcal conjugate vaccine
6. Antibiotic resistance
7. Molecular methods
8. Conclusion
9. Acknowledgments
10. References

1. ABSTRACT

Streptococcus pneumoniae is leading cause of bacterial otitis media in young children. Increasing rates of antibiotic resistance and the changing epidemiology of pneumococcal strains dictate the need to develop new methods to study, control, and prevent these important infections. Investigation into the molecular epidemiology of these bacteria will provide important insights into disease; progress in this field is described below.

2. INTRODUCTION

Streptococcus pneumoniae are gram positive diplococci, and are commensals of the human upper respiratory tract that may also cause disease. S. pneumoniae is also the leading cause of bacterial otitis media (OM), accounting for 20-50% of the cases (1, 2, 3). Furthermore, S. pneumoniae strains are also important causes of meningitis, septicemia, and pneumonia in young children. The annual burden of disease in the United States among children younger than 5 years is estimated at 71,000 cases of pneumonia, 17,000 cases of sepsis, 1,400 cases of meningitis and 7 million cases of OM (4). While infections such as pneumonia and meningitis are less common than OM, they are associated with higher mortality rates. Several large studies have focused on the epidemiology of antibiotic resistant isolates or those from severe invasive diseases (5, 6, 7). The molecular epidemiology of pneumococcal OM has not received as much attention and is addressed in this review.

3. OTITIS MEDIA

Otitis media (OM) is an infection of the middle ear resulting in middle ear effusion, fever or irritability, and inflammation of the tympanic membrane. Ear infections are the most frequent reason for physician visits due to illness among infants and young children (8). During their first year of life, 60-80% of children will have had an episode of acute otitis media (AOM) and 17% of children will have had 3 episodes (9, 10). The majority of OM episodes are thought to be bacterial and it is believed that disease occurs when bacteria colonizing the nasopharynx invade the middle ear via the Eustachian tube (11). The transition from colonization of the throat to infection of the middle ear is often preceded by a viral upper respiratory illness (12, 13). Factors such as the presence of parental smoking, lack of breast feeding, attendance in daycare and young age also increase the risk of developing OM (14, 15, 16, 17).

Research suggests that acute AOM caused by S. pneumoniae is clinically different from AOM caused by other pathogens. The inflammatory response in pneumococcal AOM may be more severe than with other bacterial pathogens. Serum interleukin-6 levels in children with pneumococcal AOM were significantly higher than those with AOM caused by H. influenzae or M. catarrhalis (18). S. pneumoniae causes AOM more often without a predisposing viral infection (19), is more prevalent in AOM patients recently treated with antibiotics (20), is more likely to cause bilateral disease and the incidence appears to undergo less seasonal variation (2). The epidemiology of pneumococcal OM is likely to undergo many changes over the upcoming years due to the changing rates of antibiotic resistance and the potential impact of the pneumococcal vaccine on the distribution of pneumococcal strains in circulation.

4. PNEUMOCOCCAL CARRIAGE

Molecular studies of S pneumoniae often focus on carriage of pneumococcal strains. Although a positive nasopharyngeal culture with S. pneumoniae has low predictive power for the presence of the pathogen in the middle ear (21), S. pneumoniae is part of the normal
respiratory flora and the nasopharynx serves as a reservoir for potential middle ear pathogens (22, 23). Carriage studies are often easier to conduct because tympanocentesis is not routinely performed in clinical practice and middle ear isolates may be difficult to obtain. Tympanocentesis may be performed if the child fails to respond to one or more courses of antibiotics, has complications such as mastoiditis or intracranial abscess formation, is immunocompromised, or experiences severe symptoms (24). Middle ear isolates may also be biased towards more pathogenic and/or resistant isolates. Thus, studies of pneumococcal carriage are relevant to disease and may provide information that complements molecular analyses of middle ear isolates.

Carriage studies have shown that most children acquire their first pneumococcal strain between three and six months of age and up to half of all children are colonized by their first birthday (21, 25, 26). Duration of nasopharyngeal carriage is generally short and decreases with increasing age and with each subsequent strain carried. Certain serotypes (types 6, 14, 19 and 23) are associated with a longer duration of carriage, and with fewer total strains carried (25, 26, 27). Carriage rates are higher among children attending day care (28, 29) and when other members of the same household are carriers (25, 29, 30). Carriage rates are lower among children reporting recent antibiotic use (31, 32, 33).

5. ANTIBIOTIC RESISTANCE

The emergence of S. pneumoniae resistant to penicillin and other antibiotics has complicated the prevention and treatment of pneumococcal infections (34). Before 1990, the prevalence of penicillin resistant isolates in the United States was less than 10 percent. By 1997, up to 50 percent of pneumococcal isolates were penicillin-intermediate or penicillin-resistant (35). Penicillin and multidrug resistant pneumococcal populations are highly dynamic and changes are mediated through a combination of factors including the spread of resistant clones and acquisition or loss of resistance genes (36). Resistance to other antibiotics also appear to be increasing, and resistance rates of 14.9% and 23.3% have been reported for ceftriaxone and cefuroxime respectively (5).

Most penicillin resistant isolates are of the same serotypes that are most likely to cause disease (34). Some of the highest rates of penicillin resistance are found among middle ear isolates from children <5 years of age (5). Clinically, antibiotic resistant strains have been associated with more frequent OM episodes and treatment failures (32). Risk factors for the recovery of a penicillin-nonsusceptible isolate from a child include recent antibiotic use, day care attendance, and a history of recurrent OM (32, 37, 38). Of 608 S. pneumoniae samples from 8 children’s hospitals throughout the US, 53.4% of the 403 children who received antibiotics within the 30 days before the OM isolates was recovered had a penicillin non-susceptible strain (38). Of the children who did not receive an antibiotic (197) 20.3% harbored a non-susceptible strain. Children who had a history of recurrent OM (3 or more infections over 6 months) had a higher proportion of nonsusceptible isolates (46% compared to 35%). The investigators also found considerable geographic variation in the resistant rates of penicillin nonsusceptible strains.

Decreasing antibiotic consumption has been shown to reduce the amount of resistant pneumococci circulating in the community (39) and up to 80% of OM cases can resolve without antibiotics (40). Methods proposed to decrease antibiotic treatment of OM include better diagnostic training, avoidance of antibiotics for persistence of middle-ear effusion, abandoning antimicrobial prophylaxis for recurrent OM episodes, and shorter course therapy (41).

6. PNEUMOCOCCAL CONJUGATE VACCINE

Pneumococci have the ability to individually express one of approximately 90 immunologically and structurally distinct capsular polysaccharides. The seven serotypes most commonly associated with invasive disease in young children are 14, 6B, 19F, 18C, 23F, 4 and 9V in order of decreasing frequency (42). Serogroups 19, 6, 23, 14, 3, and 18 were most often found in the middle ear fluid of young children, accounting for over 73% of all isolates (43). A comparison of multiple studies indicated that serogroup 1 was more commonly isolated from blood than from middle ear fluid (Odds Ratio [OR] 3.2, 95% confidence interval [CI] 2.1,4.7). Serogroups 19 and 23 were more commonly isolated from middle ear fluid than from CSF (OR 2.8 95% CI 2.0-3.8) and blood (OR 1.7, 95% CI 1.4, 2.1).

In February 2000, the Food and Drug Administration (FDA) approved the 7-valent pneumococcal conjugate vaccine (pneumococcal-CRM197, Wyeth Lederle Vaccines, Pearl River, N.Y.) for routine use in toddlers and infants. The vaccine contains saccharides of serotypes 4, 6B, 9V, 14, 18C, 19F and 23F coupled to the diphtheria CRM197 protein carrier. While the vaccine is very effective in reducing severe invasive pneumococcal disease, it is less effective in reducing the incidence of OM. A study of 1662 infants enrolled in the Finnish Otitis Media Vaccine Trial found that the vaccine reduced the number of episodes of AOM by any cause by 6%. A 57% reduction in otitis media due to vaccine serotypes was observed, but the number of episodes due to other serotypes increased by 33% (44). The Northern California Kaiser Permanente vaccine trial of 37,868 children reported a 7 percent reduction in OM episodes (45). Serotype 3, which accounts for approximately 8.6% of middle-ear isolates (43), is not included in the vaccine. Although the decrease in AOM of any etiology is modest, on a population level, a 7 percent reduction in episodes of OM could potentially prevent approximately 1 million episodes of OM (46).

Concerns have been raised regarding widespread vaccination and serotype replacement, a process that could occur via an increase in the prevalence of a non-vaccine pneumococcal serotype already present in the population or the appearance and spread of a non-vaccine type that is new to the population (47). Conjugate vaccines have been
Molecular epidemiology of S. Pneumoniae mediated otitis media

shown to significantly reduce colonization with vaccine serotypes but increase colonization with non-vaccine serotypes (48, 49, 50). The implication of serotype replacement on pneumococcal OM prevention is unclear. The increased carriage and transmission of non-vaccine serotypes may not increase OM if non-vaccine types are less capable of invading the middle ear compared to vaccine types. If all pneumococcal strains are equally capable of invading the middle ear, widespread use of the vaccine will have little effect on the overall prevalence of OM (51).

7. MOLECULAR METHODS

Studies of the epidemiology of S. pneumoniae often involve the description of strains using phenotypic methods such as antibiotic resistance profiles and serotyping (2). These methods are invaluable to understanding the epidemiology of S. pneumoniae but may have poor discriminatory power in certain settings. Molecular methods involve the evaluation of the nucleic acid or amino acid content of the strains (52, 53, 54).

Pulsed-field gel electrophoresis (PFGE) is considered one of the most reliable methods to type pneumococcal strains. The technique involves embedding genomic DNA in agarose, digesting the DNA with a rare cutting restriction enzyme, and separating large DNA fragments (10,000 to 5 million base pairs) by electrophoresis on a gel using alternating electric fields, the most popular system being the contour-clamped homogenous electric field (CHEF). The main drawbacks of the techniques are the long time to process samples (2-3 days) and the expense of equipment and reagents (53).

PFGE has been used to show that certain S. pneumoniae geno- types are associated with OM. Penicillin resistant strains collected during a 6 year period incorporating 3 national surveillance studies were studied by PFGE and compared by age, specimen source and geographic region (6). The authors found significant associations between PFGE types and the specimen source. Among middle ear isolates, PFGE types 3 and 10 occurred more commonly than type 2. In comparison, a greater proportion of isolates recovered from the blood were noted to be PFGE type 2 and 5. PFGE has also been used in conjunction with serotype and resistance data to provide a better understanding of OM in the clinical setting. In one prospective study, the investigators showed that some apparent OM treatment failures may represent superinfections that occur when the original middle ear pathogen is eradicated and a new nonsusceptible S. pneumoniae isolate invades the middle ear (55).

Ribotyping involves the use of a probe made from the 16s rRNA gene hybridized to restriction enzyme digested DNA. Ribotyping has been used to study pneumococcal carriage in the day care setting (56). A comparison of five genomic fingerprinting methods indicated that ribotyping with PsiI was the least discriminating method (57). Automated ribotyping techniques are currently available, and digestion of the pneumococcal DNA with HindIII provided comparable results to PFGE (58).

Polymerase chain reaction (PCR) based methods provide a relatively fast way to genotype pneumococcal strains. Repetitive element PCR uses primers targeting repetitive elements distributed throughout the chromosome. The regions between the repeats are amplified and a range of fragment lengths are produced. The enterobacterial repetitive intergenic consensus elements (ERIC) (59) and BOX elements (60) have both been used to type pneumococcal strains (57,61). Arbitrarily primed PCR (AP-PCR) or random amplified polymorphic DNA (RAPD) uses a random, single, short (8-10 bp) primer at a low annealing temperature to produce a genomic fingerprint. RAPD PCR has been used in several studies of invasive and/or drug resistant strains (62, 63, 64). This technique was used to demonstrate that an apparent outbreak of AOM in a Japanese daycare was not due to the dissemination of a single clone (65).

Multilocus sequence typing (MLST) is a relatively new technique based on the principle of multilocus enzyme electrophoresis. Instead of comparing the electrophoretic mobility of housekeeping enzymes, MLST directly compares the nucleotide sequence of ~450 bp fragments of seven housekeeping genes (66, 67). This technique has been used to study the population biology of invasive pneumococcal isolates and has identified sequence types that have an increased capacity to cause invasive disease (67). Benefits of the technique include the reproducibility of the technique, the electronic portability of the sequence data, and the MLST website, which provides a reference database of strains (http://mlst.zoo.ox.ac.uk). To our knowledge, this technique has not been used to study OM isolates but a comparison of a large collection of OM isolates with the MLST database would provide important information regarding the epidemiology of pneumococcal infection.

Molecular typing techniques differ in their cost, time to process samples, reproducibility and discriminatory power. PCR based methods may provide the most rapid results while techniques such as PFGE may have higher discriminatory power (57). The decision to use a particular technique should be made while keeping in mind the resources available and the specific aims of the study. For example, MLST analyzes selectively neutral variation that accumulates slowly in conserved regions of DNA. Changes in PFGE patterns occur in both conserved and variable regions and these changes are thought to accumulate more rapidly. In theory, PFGE would thus be useful in short term studies such as outbreak investigations whereas MLST might be more appropriate for longer term studies (66). The pneumococcal Molecular Epidemiology Network was established in 1997 to support training, provide access to information, to encourage collaboration, standardize the naming of clones and to identify clones using PFGE, BOX-PCR, and MLST (51). While these efforts have focused on major antibiotic resistant clones, they can serve as a model for investigators interested in the molecular epidemiology of pneumococcal OM.
Molecular epidemiology of S. Pneumoniae mediated otitis media

8. CONCLUSION

Studies that combine molecular methods with serotyping, antibiotic resistance profiles, and epidemiologic profiles provide powerful insights into disease. Such studies can potentially be used for surveillance, outbreak investigation, identification of transmission patterns and for identification of molecular determinants of pathogenesis (52). One question of importance is whether any S. pneumoniae strain is capable of causing OM or if specific “virulent” strains are more likely to cause infection. Certain serogroups are more commonly associated with OM; the relationship, however, between the capsule type and virulence is dependent on the genetic background of the strain and virulence is undoubtedly due to genes in addition to capsule (68). Furthermore, the importance of the capsule as a virulence factor has been described in invasive infections (such as bacteremia and meningitis) and is based on the resistance of capsule to opsonophagocytosis (69, 70). While research indicates that the capsule is important for colonization (71), a different mechanism of virulence may be operative in OM infection. Thus, in OM, capsular types may be markers for virulence rather than actual virulence factors. Molecular epidemiologic studies will provide answers to these questions and will undoubtedly play a role in the evaluation of serotype replacement and to monitor the spread antibiotic resistant clones. Future studies should provide insight into the diversity of pneumococcal OM strains and the identification of markers important for pneumococcal OM pathogenesis. These data can then be used to help control and prevent these important infections.

9. ACKNOWLEDGMENTS

The authors would like to thank J.R Gilsdorf and C. F. Marrs for critical review of this manuscript.

10. REFERENCES

Molecular epidemiology of S. Pneumoniae mediated otitis media


Molecular epidemiology of S. Pneumoniae mediated otitis media

consumption in human communities and the frequency of resistance. *Proc Nail Acad Sci USA* 96, 1152-1156 (1999)


Molecular epidemiology of S. Pneumoniae mediated otitis media


69. Avery, O., & R. Dubos: The protective action of a specific enzyme against type III pneumococcus infection in mice. J. Exp. Med. 54, 73-89 (1931)


Key words: Molecular epidemiology, Streptococcus pneumoniae, Review

Send correspondence to: Dr Melinda Pettigrew, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06520-8032, Tel: 203-785-5220, Fax:203-785-6130, E-mail: melinda.pettigrew@yale.edu