[Frontiers in Bioscience 8, e87-93, January 1, 2003]

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

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 pneumoccocal 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 nasopharnyx 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 saccarides 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

shown to significantly reduce colonization with vaccine serotypes but increase colonization with nonvaccine 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 *Pvu*II was the least discriminating method (57). Automated ribotyping techniques are currently available, and digestion of the pneumococcal DNA with *Hind*III 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.

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

1. Bluestone, C. D., Stephenson, J. S. & L. M. Martin: Ten-year review of otitis media pathogens. *Pediatr. Infect. Dis.* J. 11, S7 S11 (1992)

2. Kilpi, T., Herva, E., Kaijalainen, T., Syrjanen, R. & A. K. Takala: Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. *Pediatr. Infect. Dis. J.* 20, 654-62 (2001)

3. Rosenblut, A., Santolaya, M. E., Gonzalez, P., Corbalan, V., Avendano, L. F. Martinez, M. A. & J. C. Hormazabal: Bacterial and viral etiology of acute otitis media in Chilean children. *Pediatr. Infect. Dis. J.* 20, 501-7 (2001)

4. Overturf, G. D.: American Academy of Pediatrics. Committee on Infectious Diseases. Technical report: prevention of pneumococcal infections, including the use of pneumococcal conjugate and polysaccharide vaccines and antibiotic prophylaxis.*Pediatrics* 106, 367-76 (2000)

5. Doern, G. V., Brueggemann, A. B., Huynh, H., Wingert, E., & P. Rhomberg: Antimicrobial resistance with *Streptococcus pneumoniae* in the United States, 1997-98. *Emerg. Infect. Dis.* 5, 757-765 (1999)

6. Richter, S. S.: The molecular epidemiology of penicillin-resistant *Streptococcus pneumoniae* in the United States, 1994-2000. *Clin. Infect. Dis.* 34,330-9 (2002)

7. Robinson, K. A., Baughman, W., Rothrock, G., Barrett, N. L., Pass, M., Lexau, C., Damaske, B., Stefonek, K., Barnes, B., Patterson, J., Zell, E. R., Schuchat, A., Whitney, C. G. & Active Bacterial Core Surveillance /Emerging Infections Program: Epidemiology of invasive *Streptococcus pneumoniae* infections in the United States, 1995-1998: Opportunities for prevention in the conjugate vaccine era. *JAMA* 285, 1729-35(2001)

8. Schappert, S. M.: Office visits for otitis media: United States, 1975-90. *Vital and Health Statistics of the Centers for Disease Control/National Center for Health Statistics* 214, 1-18 (1992)

9. Paradise, J. L., Rockette, H. E., Colborn, D. K., Bernard, B. S., Smith, C. G., Kurs-Lasky, M. & J. E. Janosky. Otitis media in 2253 Pittsburgh area infants: prevalance and risk factors during the first two years of life. *Pediatrics* 99, 318-333 (1997)

10. Teele, D. W., Klein, J. O. & B. Rosner: Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective cohort study. *J. Infect. Dis.* 160, 83-94 (1989)

11. Murphy, T. F., Bernstein, J. M., Dryja, D. M., Campagnari,A. A. & M. A. Apicella: Outermembrane protein and lipooligosaccharide analysis of paired nasopharyngeal and middle ear isolates in otitis media due to nontypable *Haemophilus influenzae*: pathogenic and epidemiologic observations. *J. Infect. Dis.* 156,723-731 (1987)

12. Chonmaitree, T., & T. Heikkinen: Viruses and acute otitis media. *Pediatr. Infect. Dis. J.* 19, 1005-7 (2000)

13. Vesa, S., Kleemola, M., Blomquist, S., Takala, A., Kilpi, T. &T. Hovi: Epidemiology of documented viral respiratory infections and acute otitis media in a cohort of children followed from two to twenty-four months of age. *Pediatr. Infect. Dis. J.* 20, 574-81(2001)

14. Alho, O. P., Laara, E.& H. Oja: Public health impact of various risk factors for acute otitis media in northern Finland. *Am. J. Epidemiol.* 143, 1149-56 (1996)

15. Duffy, L. C., Faden, H., Wasielewski, R., Wolf, J., & D. Krystofik: Exclusive breastfeeding protects against bacterial colonization and day care exposure to otitis media. *Pediatrics* 100, E7 (1997)

16. Paradise, J. L., Rockette, H. E., Colborn, D. K., Bernard, B. S., Smith, C. G., Kurs-Lasky, M. and J. E. Janosky: Otitis media in 2253 Pittsburgh-area infants: prevalence and risk factors during the first two years of life. *Pediatrics* 99, 318-33 (1997)

17. Uhari, M., Mantysaari, K. & M. Niemela: A meta-analytic review of the risk factors for acute otitis media. *Clin. Infect. Dis.* 22,1079-83 (1996)

18. Heikkinen, T., Ghaffar, F., Okorodudu, A. O. & T. Chonmaitree: Serum interluekin-6 in bacterial and nonbacterial acute otitis media. *Pediatrics* 102, 296-299 (1998)

19. Pitkaranta, A., Virolainen, A., Jero, J., Arruda, E., & F. G. Hayden: Detection of rhinovirus, respiratory syncytial virus, and cornavirus infections in acute otitis media by reverse transcriptase polymerase chain reaction. *Pediatrics* 102, 291-295 (1998)

20. Leibovitz, E., Raiz, S., Piglansky, L., Greenberg, D., Yagupsky, P., Fliss, D. M., Leiberman, A. & R. Dagan: Resistance pattern of middle ear fluid isolates in acute otitis media recently treated with antibiotics. *Pediatr. Infect. Dis. J.* 17, 463-469
(1998)

21. Faden, H., Stanievich, J., Brodsky, L., Bernstein, J., & P. L. Ogra: Changes in the nasopharyngeal flora during otitis media of childhood. *Pediatr. Infect. Dis. J.* 9, 623-6 (1990)

22. Faden, H., Duffy, L., Wasielewski, R., Wolf, J., Krystofik, D., & Y. Tung: Relationship between nasopharyngeal colonization and the development of otitis media in children. *J. Infect. Dis.*175, 1440-1445 (1997)

23. Zenni, M. K., Cheatham, S. H., Thompson, J. M., Reed, G. W., Batson, A. B., Palmer, P. S., Holland, K. L. & K. M. Edwards: *Streptococcus pneumoniae* colonization in the young child: association with otitis media and resistance to penicillin. *J. Pediatrics* 127, 533-537 (1995)

24. Bluestone, C. D., Stool, S. E. and M. A. Kenna (ed.) Otitis media, atelectasis, and Eustachian tube dysfunction. Saunders, Philadelphia (1996)

25. Gray, B. M., Converse, G. M., 3rd & H. C. Dillon, Jr.: Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage, and infection during the first 24 months of life. *J. Infect. Dis.* 142, 923-933 (1980)

26. Raymond, J., Le Thomas, I., Moulin, F., Commeau, A., Gendral, D., & P. Berche: Sequential colonization by *Streptococcus pneumoniae* of healthy children living in an orphanage. *J. Infect. Dis.* 181, 1983-1988 (2000)

27. Smith, T., Lehmann, D., Montgomery, J., Gratten, M., Riley, I. D. & M. P. Alpers: Acquisition and invasiveness of different serotypes of *Streptococcus pneumoniae* in young children. *Epidemiol. Infect.* 111, 27-39 (1993)

28. Bogaert, D., Engelen, M. N., Timmers-Reker, A. M., Elzenaar, K., Peerbooms, G. H., Coutinho, R.A., de Groot, R. & P. W. Hermans: Pneumococcal carriage in children in the Netherlands: a molecular epidemiologic study. *J. Clin. Microbiol.* 39, 3316-3320 (2001)

29. Samore, M. H., Magill, M. K., Alder, S. C., Severina, E., Morrison de Boer, L., Lyon, J. L., Caroll, K., Leary, J., Stone, M. B., Bradford, D., Reading, J., Tomasz, A. & M. A. Sande: High rates of multiple antibiotic resistance in *Streptococcus pneumoniae* from healthy children living in isolated rural communities: association with cephalosporin use and intrafamilial transmission. *Pediatrics* 108, 856-865 (2001)

30. Leino, T., Aukaranen, K., Jokinen, J., Leinonen, M., Tervonen, P., & A. Takala: Pneumococcal carriage in children during their first two years: important role of family exposure. *Pediatr. Infect. Dis. J.* 20, 1022-1027 (2001)

31. Kellner, J. D. & E. L. Ford-Jones: *Streptococcus pneumoniae* carriage in children attending 59 Canadian child care centers. *Arch. Pediatr. Adoles. Med.*153, 495-501 (1999)

32. Reichler, M. R., Allphin, A. A., Breiman, R. F., Schreibner, J. R., Arnold, J. E., McDougal, L. K., Facklam, R. R., Boxerbaum, B., May, D., Walton, R. O. & M. R. Jacobs: The spread of multiply resistant *Streptococcus pneumoniae* at a day care center in Ohio. *J. Infect. Dis.* 166, 1346-1353 (1992)

33. Syrjanen, R. K., Kilpi, T. M., Kaijalainen, T. H., Herva, E. E., & A. K. Takala: Nasopharyngeal carriage of *Streptococcus pneumoniae* in Finnish children younger than 2 years old. *J. Infect. Dis.* 184, 451-9 (2001)

34. Breiman, R. F., Butler, J. C., Tenover, F. C., Elliott, J. A. & R. R. Facklam: Emergence of drug-resistant pneumococcal infections in the United States. *JAMA* 271, 1831-5 (1994)

35. Jacobs, M. R.: Increasing antibiotic resistance among otitis media pathogens and their susceptibility to oral agents based on their pharmacodynamic parameters. *Pediatr. Infect. Dis. J.* 19:S47-S56 (2000)

36. McGee, L.: Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. *J. Clin. Microbiol.* 39, 2565-71 (2001)

37. Arnold, K. E., Leggiadro, R. J., Breiman, R. F., Lipman, H. B., Schwartz, B., Appleton, M. A., Cleveland, K. O., Szeto, H. C., Hill, B. C., Tenover, F. C., Elliott, J. A. & R. R. Facklam: Risk factors for carriage of drug-resistant *Streptococcus pneumoniae* among children in Memphis, Tennessee. *J. Pediatr.* 128, 757-764 (1996)

38. Wald, E. R., Mason, Jr., E. O., Bradley, J. S., Barson, W. J., Kaplan, S. L. & The U. S. P. M. P. S. G.: Acute otitis media caused by *Streptococcus pneumoniae* in children's hospitals between 1994 and 1997. *Pediatr. Infect. Dis. J.* 20, 34-9 (2001)

39. Austin, D. J., Kristinsson, K. G. & R. M. Anderson: The relationship between the volume of antimicrobial

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

40. Rosenfeld, R. M.: What to expect from medical treatment of otitis media. *Pediatr. Infect. Dis. J.* 14, 731-738 (1995)

41. Hirschmann, J. V: Methods for decreasing antibiotic use in otitis media. *Lancet* 352, 672 (1998)

42. Butler, J. C., Breiman, R. F., Lipman, H. B., Hofmann, J. & R. R. Facklam: Serotype distribution of *Streptococcus pneumoniae* infections among preschool children in the United States, 1978-1994: implications for development of a conjugate vaccine. *J. Infect. Dis.* 171, 885-9 (1995)

43. Hausdorff, W. P., Bryant, J., Kloek, C., Paradiso, P. R. & G. R. Siber: The contribution of specific pneumococcal serogroups to different disease manifestations: implications for conjugate vaccine formulation and use, part II. *Clin. Infect. Dis.* 30,122-40 (2000)

44. Eskola, J., Kilpi, T., Palmu, A., Jokinen, J., Haapakoski, J., Herva, E., Takala, A., Kayhty, H., Karma, P., Kohberger, R., Siber, G., Makela, P. H., & Finnish Otitis Media Study Group: Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *New Engl. J. Med.* 344, 403-9 (2001)

45. Black, S., Shinefield, H., Fireman, B., Lewis, E., Ray, P., Hansen, J. R., Elvin, L., Ensor, K. M., Hackell, J., Siber, G., Malinoski, F., Madore, D., Chang, I., Kohberger, R., Watson, W., Austrian, R. & K. Edwards: Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Pediatr. Infect. Dis. J.* 19,187-95 (2000)

46. Lieu, T. A., Ray, G. T., Black, S. B., Butler, J. C., Klein, J. O., Breiman, R. F., Miller, M. A. & H. R. Shinefield: Projected cost-effectiveness of pneumococcal conjugate vaccination of healthy infants and young children. *JAMA* 283, 1460-8 (2000)

47. Lipsitch, M.:Vaccination against colonizing bacteria with multiple serotypes. *Proc Natl Acad Sci USA* 94, 6571-6 (1997)

48. Dagan, R., Melamed, R., Muallen, M., Piglansky, L., Greenberg, D., Abramson, O., Mendelman, P. M., Bohidar, N., & P. Yagupsky: Reduction of nasopharyngeal carriage of pneumococci during the second year of life by a heptavalent conjugate pneumococcal vaccine. *J. Infect. Dis.* 174, 1271-1278 (1996)

49. Mbelle, N., Huebener, R., Wasas, A., Kimura, A., Chang, I., & K. P. Klugman: Immunogenicity and impact on nasopharyngeal carriage of a noavalent pneumococcal conjugate vaccine. *J. Infect. Dis.* 180,1171-1176 (1999)

50. Obaro, S. K., Adegbola, R. A., Banya, W. A. S. & B. M. Greenwood: Carriage of pneumococci after pneumococcal vaccination. *Lancet* 348, 271-272 (1996)

51. Spratt, B. G. & B. M. Greenwood: Prevention of pneumococcal disease by vaccination: does serotype replacement matter? *Lancet* 356:1210-1211(2000)

52. Foxman, B.& L. Riley: Molecular epidemiology: focus on infection. *Am. J. Epidemiol.* 153, 1135-1141(2001)

53. Harrington, S. M.& W. R. Bishai: Molecular epidemiology and infectious diseases, p. 205-230. *In* K. E. Nelson, C. M. Williams, and N. M. H. Graham (ed.), Infectious disease epidemiology: theory and practice. Aspen, Gaithersburg, MD (2001)

54. Lipuma, J. J.: Molecular tools for epidemiologic study of infectious diseases. *Pediatr. Infect. Dis. J.* 17, 667-675 (1998)

55. Dagan, R., Leibovitz, E., Cheletz, G., Leiberman, A., & N. Porat: Antibiotic treatment in acute otitis media promotes superinfection with resistant *Streptococcus pneumoniae* carried before initiation of treatment. *J. Infect. Dis.* 183, 880-886 (2001)

56. Givon-Lavi, N., Dagan, R., Fraser, D., Yagupsky, P., & N. Porat: Marked differences in pneumococcal carriage and resistance patterns between day care centers located within a small area. *Clin. Infect. Dis.* 29, 1274-80 (1999)

57. Hermans, P. W., Sluijter, M., Hoogenboezem, T., Heersma, H., van Belkum, A., & R. de Groot: Comparative study of five different DNA fingerprint techniques for molecular typing of *Streptococcus pneumoniae* strains. *J. Clin. Microbiol.* 33, 1606-1612 (1995)

58. Quale, J. M., Landman, D., Flores, C. & J. Ravishankar: Comparison of automated ribotyping to pulsed-field gel electrophoresis for genetic fingerprinting of *Streptococcus pneumoniae*. *J. Clin. Microbiol.* 39, 4175-4177 (2001)

59. Versalovic, J., Koeuth, T. & J. R. Lupski: Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nuc. Acid. Res.* 19, 6823-31 (1991)

60. Martin, B., Humbert, O., Camera, M., Guenzi, E., Walker, J., Mitchell, T., Andrew, P. W., Prudhomme, M., Alloing, G., Hakenbeck, R., Morrison, D. A., Boulnois, G. J. & J. P. Claverys: A highly conserved repeated DNA element located in the chromosome of *Streptococcus pneumoniae*. *Nuc. Acid. Res.* 20, 3479-3483 (1992)

61. Dunne, W. M. J., Kehl, K. S., Holland-Staley, C. A., Brueggemann, A. B., Pfaller, M. A., & G. V. Doern: Comparison of results generated by serotyping, pulsed-field restriction analysis, ribotyping, and repetitive-sequence PCR used to characterize penicillin-resistant pneumococci from the United States. *J. Clin. Microbiol.* 39, 1791-5 (2001)

62. Greenberg, D., Speert, D. P., Mahenthiralingam, E., Henry, D. A., Campbell, M. E., Scheifele, D. W. & The C. P. S. L. I. M. N.: Emergence of penicillin-nonsusceptible *Streptococcus pneumoniae* invasive clones in Canada. J. Clin. Microbiol. 40, 68-74 (2002)

63. Hsueh, P. R., Teng, L. J., Lee, L. N., Yang, P. C., Ho, S. W. & K. T. Luh: Dissemination of high-level penicillin-, extended-spectrum cephalosporin-, and erythromycin-resistant *Streptococcus pneumoniae* clones in Taiwan. *J. Clin. Microbiol.* 37, 221-4 (1999)

64. Huang, Y. C., Wong, K. S., Hsueh, P. R., & T. Y. Lin. Bacteremic pneumonia caused by penicillin-resistant *Streptococcus pneumoniae* in siblings. *Pediatr. Infect. Dis. J.* 18, 734-5 (1999)

65. Nakashima, T., Fukushima, K., Tahara, M., Sugata, K. I., Ogawa, T., Sugata, A., Gunduz, M., Ueki, Y., Uno, Y., & K. Nishizaki: Random amplified polymorphic DNA analysis applied to acute otitis media caused by penicillin non-susceptible *Streptococcus pneumoniae*. *J. Infect. Chemother.* 7, 239-42 (2001)

66. Enright, M. C.: Multilocus sequence typing. *Trends Microbiol.* 7, 482-7 (1999)

67. Enright, M. C.& B. G. Spratt: A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. *Microbiol.* 144:3049-3060 (1998)

68. Kelly, T., Dillard, J. P. & J. Yother: Effect of genetic switching of capsular type on virulence of *Streptococcus pneumoniae*. *Infect. Immun.* 62, 1813-9 (1994)

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)

70. Brown, E. J.: Interaction of gram-positive organisms with complement. *Curr. Top. Microbiol. Immunol.* 121:159-187 (1985)

71. Magee, A. D. and J. Yother: Requirement for capsule in colonization by *Streptococcus pneumoniae*. *Infect. Immun.* 69, 3755-61 (2001)

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