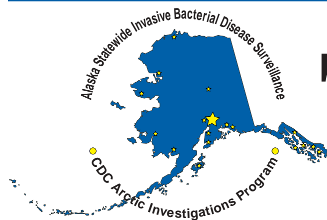

The Pathogen Post

Second/Third Quarter 2005

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Mission:

To prevent infectious disease morbidity and mortality in people of the Arctic and Subarctic, with special emphasis on diseases of high incidence and concern among indigenous people.

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Featured Organism: *Streptococcus pneumoniae*

One of the pathogens monitored by the Alaska Invasive Bacterial Disease Surveillance program is *Streptococcus pneumoniae.* The pneumococcus is a leading cause of disease and death worldwide and is the most common bacterial cause of meningitis, community-acquired pneumonia, sepsis, acute otitis media, and sinusitis. Severe disease rates are highest among the very young and the elderly.

The first step of infection is asymptomatic infection of the respiratory tract, termed colonization. Pneumococci are commonly found in the throat and nasophyarynx of up to 80% of healthy children while approximately one-third of adults are colonized. The bacterial capsular polysaccharide is the primary virulence factor for invasive infections; over 90 capsular types have been identified and they are classified by their reactions with type-specific antisera. Serotype designations include a numeric serogroup (numbers 1 - 48) and an alphabetic designation for those groups with more than one type, e.g. “19A”. Antibodies directed against specific capsular polysaccharides appear in most patients approximately one week after onset of infection and presence of antibody has been shown to be protective against disease. Thus, capsular polysaccharides are the basis for the two vaccine types in routine use today against *S. pneumoniae.*

One of the pneumococcal vaccines in use contains 23 different capsular polysaccharides from the most common invasive serotypes. Known under the brand name *Pneumovax-23®*, this vaccine is recommended in Alaska for all persons 55 and older and for persons 2 – 54 who are at increased risk for invasive pneumococcal disease. It is given as a single dose with a recommendation in Alaska to revaccinate every six years. This vaccine is not effective for children under age 2 years. However, in 2001 young Alaskans began to routinely receive the 7-valent pediatric pneumococcal conjugate vaccine (PCV7, or “Prevnar®”). This vaccine makes use of capsular polysaccharide covalently bonded to a diphtheria protein for the seven serotypes most common in childhood. This conjugate vaccine is able to stimulate the developing immune system of infants much like the earlier conjugate vaccines against Hib. It is given as a 3 dose primary series beginning at age 2 months, followed by a booster dose at 12 -15 months of age.

The 23-valent polysaccharide vaccine has been shown to be effective against invasive disease but controversy exists about its effectiveness for preventing pneumonia. A national health objective for 2010 is to achieve pneumococcal vaccination in 90% of adults aged ≥65 years. The rate of pneumococcal vaccination among persons aged ≥65 years for residents of Alaska was 59% in 2002. Immunization data indicate that vaccination coverage is even lower for persons 55 to 64 years old and for younger persons at risk due to medical conditions. Statewide Alaska invasive disease surveillance recently allowed CDC to detect a regional outbreak of serotype 12F disease in adults and the subsequent investigation indicated 50% of cases had an indication for receipt of vaccine and a vaccine-preventable serotype (ref MMWR, Outbreak of Invasive Pneumococcal Disease in Alaska, 2003-2004, January 28, 2005 / 54(03);72-75.) This event highlights the need to improve vaccine coverage for all persons at risk, whether their indication is due to their age or medical risk factors. One method of improving vaccination rates is through the use of standing orders that allow clinical staff to administer vaccinations

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Featured Organism

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according to an institution- or physician-approved protocol without the need for a physician's examination or direct order. This is now reimbursable under Medicare and Medicaid.

S. pneumoniae has become the most common cause of childhood meningitis in the U.S. since the threat of Hib disease has been reduced through use of vaccine. In Alaska, where rates of invasive pneumococcal disease were among the highest in the world, PCV7 vaccine introduction has resulted in an 85% decline in invasive disease due to vaccine types among children under 2 years old, with similar declines in children 2 – 4 years old. Before PCV7 vaccine use, Alaska averaged 35 invasive pneumococcal cases of serotypes found in the vaccine among children less than 5 years old, whereas in the first 3 years after PCV7 use we have averaged only 6 such cases per year. We have also seen the longstanding health disparity of higher disease rates among Alaska Native children disappear for serotypes covered by the vaccine. Remarkably, we have also seen a decrease in adult disease rates for PCV7-types, indicating an indirect benefit to adults through use of a childhood vaccine. This is thought to result from decreased colonization and transmission from vaccinated children to adults.

> ***PCV7 vaccine introduction has resulted in an 85% decline in invasive disease***

There is some concern that decreases in disease and colonization brought on by use of PCV7 vaccine could allow some pneumococcal serotypes to move into the ecologic niche previously occupied by the vaccine serotypes and thus cause increases in disease. Therefore, ongoing monitoring of disease rates through laboratory surveillance is important to evaluate the ongoing success of PCV7 vaccine and to stay on guard for the emergence of new serotypes. Two serotypes that have shown recent worrisome increases include 19A and 7F. Disease due to serotype 19A has shown increases in other parts of the U.S. and bears careful watching. In Alaska, research is ongoing to see if emerging serotypes have undergone a capsular "switch" under the pressure of vaccine-induced antibody. Other areas of active pneumococcal research at CDC in Alaska include evaluation of the side effects and immunogenic response of second or third doses of polysaccharide vaccine given under current Alaska guidelines, and the use of PCV7 vaccine followed by polysaccharide vaccine to boost antibody response in adults.

The exciting success achieved in preventing pneumococcal disease through use of PCV7 vaccine is understandable only because we have a statewide surveillance system. So, we request that clinical laboratories send us all invasive isolates of *S. pneumoniae* for confirmation and serotyping. This laboratory-based surveillance is a free service provided by CDC since 1986 and has many benefits. First, we can provide information regarding the serotype of a particular strain using state-of-the-art methods (see "What Organism am I?"). This information can be useful for clinics and health corporations interested in monitoring serotype trends and disease rates in their communities. The data collected regarding invasive cases have allowed us to document the tremendous success of the PCV7 vaccine and this has been helpful for supporting ongoing vaccination efforts. Through this surveillance we will be able to detect trends in disease and to identify outbreaks. This will help us to evaluate the effectiveness of vaccine programs and statewide vaccine policy for control of pneumococcal disease. All of this depends on the cooperation and dedication of the healthcare providers, laboratories and institutions around Alaska that are part of the invasive bacterial disease surveillance network.

Thank you for your continued support! ◆

Lab Spotlight: Ketchikan General Hospital

The microbiology lab at Ketchikan General Hospital serves the city of Ketchikan and surrounding communities which comprises a population of approximately 20,000 people. Four personnel staff the lab; Paul Fuang is the microbiology supervisor and is the only staff member to work microbiology full time. The remaining personnel are cross-trained to perform other laboratory functions. The lab processes approximately 10,000 samples yearly and the most common organisms include *E. coli* and *Staphlococcus aureus*. Over 60% of the *S. aureus* samples tested are methicillin-resistant.

Paul says that a factor that makes his job interesting is the influx of tour ships to Ketchikan during the summer. During the five month season, over 800,000 people visit the community bringing a variety of 'foreign' organisms. Six to nine thousand people on four or five ships come into port on a daily basis and some end up at the hospital for various reasons every day. A recent unusual case involved typhoid fever in a chef off one of the cruise ships caused by *Salmonella*.

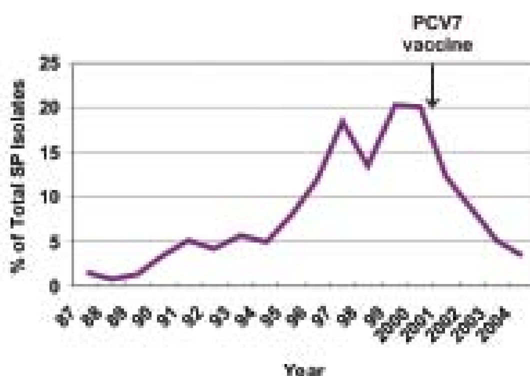
Paul Fuang, Microbiology Supervisor

Molecular Minute

Methods of molecular biology are increasingly used to better understand the epidemiology of infectious diseases. These techniques are particularly important in the search for genetic markers for medically important traits, such as drug resistance and virulence, and for tracking the global pattern of disease distribution and transmission. In the Molecular Diagnostics Lab at AIP, we have used several molecular techniques to characterize invasive and nasopharyngeal isolates of *Streptococcus pneumoniae*.

We use polymerase chain reaction (PCR) to determine the mechanisms of resistance to macrolide antibiotics such as erythromycin and azithromycin. Macrolides are commonly used for empiric treatment of community-acquired pneumonia. Resistance to macrolides has steadily increased and is often coupled with resistance to other first-line antibiotics, which limits treatment options. Macrolide resistance in pneumococci is most often mediated by one of two mechanisms: (1) target-site modification conferred by the *erm*B gene which results in high-level macrolide resistance (MIC of erythromycin, >64 µg/ml) and cross-resistance to lincosamides and streptogramin B drugs (MLS_B phenotype), and (2) active drug efflux mediated by a membrane efflux pump encoded by the *mef*E gene resulting in low- to mid-level resistance (MICs of erythromycin of 1 – 32 µg/ml) and conferring resistance only to macrolides (M phenotype). Prevalence of the respective macrolide resistance mechanisms varies by geographic region. The efflux mechanism accounts for more than two-thirds of macrolide resistant isolates in North America. In Europe and South Africa, target modification is the predominant mechanism. A recent survey of all invasive pneumococcal isolates submitted to AIP since 1986 as part of the invasive bacterial disease surveillance system found that the majority (85.3%) of macrolide resistant pneumococcal isolates carried the *mefE* gene, while 10.7% carried the *erm*B gene. The majority of these isolates were also resistant to other antibiotics. The prevalence of isolates positive for *mefE* steadily increased over time, peaking at 18.3% of all isolates in 2000, then declining to 2.5% of all isolates in 2004 (Fig. 1). This decline is likely due to the dramatic decrease in the number of invasive pneumococcal cases seen within the pediatric population since routine vaccination with PCV7 was instituted in 2001.

Figure 1. Prevalence of macrolide resistant invasive *Streptococcus pneumoniae* isolates from Alaska: 1986 – 2004.



Another exciting area for applied molecular techniques is pneumococcal strain typing. Utilizing techniques such as pulsed-field gel electrophoresis (PFGE) and multilocus strain typing (MLST) we can address two very different kinds of questions: (1) are isolates recovered from a localized outbreak of disease the same or different strains and (2) how are strains causing disease in one geographic area related to those isolated world-wide? At the local level, we recently identified a serotype 12F strain that was responsible for an outbreak of invasive pneumococcal disease in one region of the state from January 2003 – March 2004. PFGE analysis revealed that the serotype 12F isolates from this region were genetically identical but unique when compared with a random sample of 12F isolates collected from other areas of the state (Fig 2). On a more global level, MLST results revealed that this 12F strain was unique when compared with all of the 26 pneumococcal clones recognized by the Pneumococcal Molecular Epidemiology Network (PMEN). The PMEN was established in 1997 with the aim of global surveillance of antibiotic-resistant *Streptococcus pneumoniae* and the standardization of nomenclature and classification of resistant clones. Further information on PMEN can be found at: www.sph.emory.edu/PMEN.

We have also used PFGE to ascertain if the recent statewide increase in invasive pneumococcal disease due to serotype 19A is the result of new strain. PFGE analysis of 80 (n = 45, 1986-00; n = 35, 2001-04) invasive serotype 19A isolates

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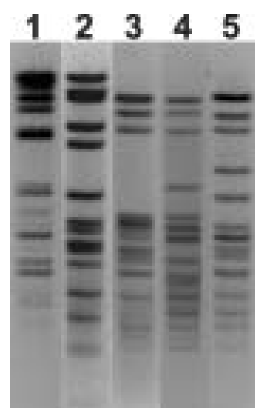
Figure 2. PFGE patterns of invasive and carriage pneumococcal serotype 12F isolates.



Lane 1: Pattern A; outbreak pattern of invasive and carriage isolates from Region A
Lane 2: Pattern F; isolate with same antibiogram as outbreak isolates (1998; different region)
Lane 3: Pattern B; invasive and carriage isolates from other regions of AK and Canada
Lane 4: Pattern C; invasive isolates from other regions and Region A prior to outbreak
Lane 5: Pattern D; invasive isolates from other regions

What organism am I?

I am gram positive, lancet-shaped, non-motile and non-spore forming diplococci. When placed on blood agar, I grow best in a 5% carbon dioxide environment at 37°C. I may look a little green around the edges when grown at 18-24 hours, but that does not mean that I am harmless. My morphology on blood agar can be mucoid and glistening, flat, or round and opaque donut-looking colonies that are usually about 1 mm in diameter. Growth on blood agar is usually inhibited by ethylhydrocupreine (optochin) and I am usually soluble in 2% deoxycholate. I am able to produce a polysaccharide capsule that forms the basis of antigenic serotyping for the 90+ different strains that I have. Does this give a clue to my identification?

...is one of the most common organisms collected from around the state

Streptococcus pneumoniae is one of the most common organisms collected from around the state and is included in our state-wide surveillance program at Arctic Investigations Program. We receive isolates from normally sterile sites on chocolate agar slants from 23 participating laboratories on a daily basis. Upon receipt of a culture, we check for patient identification on the original slant. If a surveillance form is not received with the culture, we fill one out using the identification information given on the slant. A unique culture number and AIP accession number is assigned before the culture is plated onto sheep blood agar.

The *S. pneumoniae* cultures are plated onto 2 sheep blood agar plates (1 plate is used for optochin and bile solubility and the other is used to serotype and freeze). An optochin disk is placed on 1 plate and both plates are put into a bag along with a CO_2 cartridge and placed in a 37° C incubator. After grown for 18-24 hours, the cultures are checked for alpha hemolysis, purity and optochin sensitivity. Bile solubility is done by placing 1-2 drops of 2% deoxycholate reagent directly onto colonies at the outer edge of growth and placed in a 37° C incubator for 10 minutes; then checked for complete clearing of colony growth (lysis). If the *S. pneumoniae* culture is pure, optochin sensitive and bile soluble, it is assigned a rack, layer and freezing position (RLP) in our -80° C culture bank. The culture is frozen in commercially prepared defibrinated sheep blood using 2 ml cryovials labeled with RLP, AIP culture number and date.

Serotyping by the Quellung reaction is performed on each confirmed *S. pneumoniae* isolate. The Quellung reaction is a capsular swelling test that is a result of the interaction between pneumococcal capsular polysaccharide and its homologous antibody. Testing of pneumococcus consists of making a heavy suspension (~1.0 McFarland) in 0.5 mls of phosphate buffered saline. The suspension is mixed with an equal volume of antisera, which is available through Statens Serum Institut. A positive reaction will show agglutination of the pneumococcus with visible capsules when viewed microscopically at 100x. A negative reaction will show no agglutination and no visible capsules.

Serotyping of most cultures is usually performed within a couple of days of receipt, although some results may take longer if serotyping problems are encountered. Problems encountered with serotyping are at times related directly to colony morphology. If two colony types are isolated on one culture received, one colony is usually typable and the other is not. We will attempt to report a final serotype on both colonies but in some cases we end up with a non-typable second colony. Morphologic changes can occur within the first 18-24 hours after initial cultivation at optimal conditions. This is due to the bacteria's enzymatic ability to disrupt and disintegrate its own cell walls. The enzyme responsible for this is autolysin. The physiologic role of autolysin is to cause the culture to undergo characteristic autolysis that kills the entire culture when grown in stationary phase. We have found that in some cases of non-typable pneumos, morphologic changes have already begun to occur by the time we receive them at AIP. Young colonies usually appear round with a plateau-type appearance, as the colony ages, the center starts to collapse as autolysis begins thus giving the colonies a donut-shaped appearance. Morphological changes also noted are original colony types developing a second mutant colony type, therefore generating a new a non-typable *S. pneumoniae*. Such changes may be prevented by limiting the number of passes for each culture before it is received in our laboratory. This is one reason why it is important for participating laboratories to get each *S. pneumoniae* culture to us as soon as possible after initial identification.

All *S. pneumoniae* cultures collected from normally sterile sites are tested for antimicrobial susceptibilities using commercially prepared micro broth dilution trays. Cultures are tested in batches and pulled from our -80°C culture bank freezer. Testing is done according to the Clinical Laboratory Standards Institute (formerly NCCLS). The trays are inoculated with a standardized 0.5 McFarland suspension,

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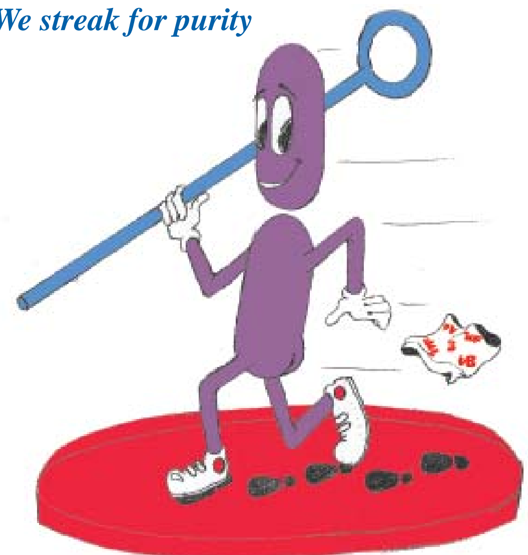
'Strip' Pneumo says:
We streak for purity



Surveillance Organisms Reported 1/1/05-6/30/05

Region	*Streptococcus pneumoniae*	*Haemophilus influenzae**	*Neisseria meningitidis*	Group A Streptococcus	Group B Streptococcus
Bristol Bay	1	0	0	0	0
Interior	7	0	0	1	1
Kotzebue	2	0	0	0	0
North Slope	1	0	0	0	0
Norton Sound	1	1	0	0	0
Southcentral	36	3	0	13	7
Southeast	4	0	0	3	1
YK Delta	12	0	0	4	0

*Serotype b=2, non-typable=2

What organism am I? continued

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and incubated in ambient air for 20 to 24 hours at 35°C. The minimum inhibitory concentration (mic) is read visually using a magnifying mirror and is equal to the lowest concentration of antibiotic which inhibits growth of the organism. The selection of antibiotics for the panel is done in conjunction with CDC's Active Bacterial Core Surveillance Program (ABC's) and is designed to capture trends and changes in the resistance patterns of *S. pneumoniae*.◆

Marcella Harker-Jones,
Medical Technologist

S*treptococcus pneumoniae facts*: Gram positive, lancet-shaped diplococci.

Typical *S. pneumoniae* shows alpha hemolysis which is 'greening' of blood agar due to the reduction of red cell hemoglobin to methemoglobin, but can cause beta hemolysis in anaerobic conditions.

Requires enriched blood media (rabbit, horse, sheep...moose?).

Ethylhydrocupreine (optochin) sensitive (¼'' disk >14 mm, 3/8'' disk >16 mm), complete clearing of colonies in 2% deoxycholate (bile soluble).

Preventive measures such as vaccination reduce pneumococcal disease and death.

Pneumococcal vaccines available: 23-valent polysaccharide and 7-valent conjugate.

Nasopharyngeal colonization occurs in approximately 40% of Alaska's population.

Encapsulation allows bacteria to be more virulent.

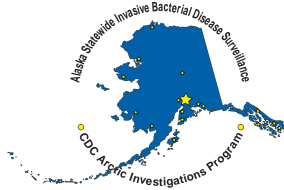
Underlying diseases including alcoholism, anemia, chronic lung disease and heart disease increase the risk of pneumococcal disease.

Morphology helps to deter mine serotype: mucoid (serotype 3), mucoid-sticky (serotype 37), wet (serotype 8).

Otitis media, pneumonia, meningitis, sinusitis, and bacteremia are a few diseases that *S. pneumoniae* causes, mainly affecting the very young and the very old.

Did you Know?

Reports and Forms are available for download on the AIP website
Go to:
www.cdc.gov/ncidod/aip/research/surveillance.html



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Molecular Minute continued

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revealed two major clones representing 91% of the isolates characterized. The number of isolates with pattern A increased from 2 (4.4%) during years 1986 – 2000 to 15 (42.9%) during years 2001 – 04 (Fig. 3). All 19A isolates with pattern A had reduced susceptibility to penicillin and trimethoprim-sulfamethozaxole but were susceptible to all other antibiotics tested. The number of isolates with pattern B decreased from 37 (82.2%) in 1986-00 to 19 (54.3%) in 2001 – 04 (Fig. 3) with some isolates fully susceptible to all antibiotics tested, while others were resistant to multiple antibiotics. This increase of invasive pneumococcal infections caused by serotype 19A after the introduction of the 7-valent pneumococcal conjugate vaccine is of concern and warrants continued surveillance to monitor the emergence of other antibiotic-resistant nonvaccine strains. ◆

Karen Rudolph, PhD., Research Microbiologist

Figure 3. Distribution of PFGE patterns among invasive pneumococcal serotype 19A isolates.