Alaska Statewide Invasive Bacterial Disease Surveillance
CDC Arctic Investigations Program

The Pathogen Post

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In This Issue:

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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Welcome to The Pathogen Post

The Centers for Disease Control and Prevention's Arctic Investigations Program in Anchorage, Alaska, maintains a statewide surveillance system for invasive diseases caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, and groups A and B streptococcus. The purpose of this newsletter is to provide feedback to participating laboratories and other health professionals about invasive bacterial disease in Alaska. We hope you find it useful and informative. We welcome your comments and questions; please direct them to the project contacts listed to the left below.

Featured Organism: *Haemophilus influenzae*

One important bacteria that is part of the Alaska Invasive Bacterial Disease Surveillance program is *H. influenzae*. It is found only in humans and can be found in the throat and nasophyarynx of up to 80% of healthy persons. It causes a variety of diseases ranging from upper respiratory tract illnesses (conjunctivitis, sinusitis, and otitis media) to lower respiratory tract disease (epiglotittis, pneumonia and empyema) to invasive disease outside the respiratory tract (meningitis, bacteremia, septic arthritis). Disease rates are highest among the very young and the elderly. The first step of infection by *H. influenzae* is colonization of the respiratory tract, which is facilitated by bacterial adhesions. The bacterial capsule is the primary virulence factor for invasive infections. Six capsular types (a – f) have been identified; type b is the most pathogenic. Antibodies directed against the capsule of *H. influenzae* type b are protective against invasive disease and are produced by immunization with the conjugate vaccines in routine use today.

Hib was the most common cause of childhood meningitis in the U.S. prior to introduction of Hib vaccines in 1991. Since then the incidence of invasive Hib disease has dropped by over 90% and it has become a goal to completely eliminate Hib disease in the U.S.(1). In Alaska, where rates of invasive Hib disease were among the highest in the world, we have seen a 92% decline since vaccine introduction. In the 10 years before Hib vaccine use, Alaska averaged 54 invasive cases per year in children < 5 years old, whereas in the 11 years since then we have averaged only 4 cases per year. Although Hib vaccine has been remarkably effective we did see an increase of invasive disease in Alaska in 1996-7 following a change in Hib vaccine (2). Subsequent investigations revealed that Hib colonization was still common among children in rural communities. These investigations led to changes in the Hib vaccine schedule which stopped the outbreak. This event highlights the need for a strong statewide immunization program and continued monitoring of Hib disease.

Many authorities fear that the decreases in Hib disease would allow some other

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Featured Organism

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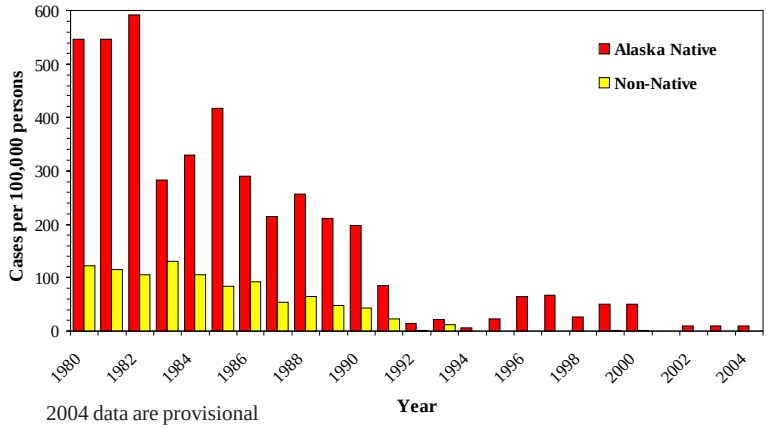
H. influenzae serotype to occupy Hib's ecologic niche and cause increases in disease. Thus far, ongoing monitoring of disease rates have not demonstrated emergence of a new virulent *H. influenzae* strain. In Alaska, we have seen occasional cases of disease due to non-b serotypes but the rates are much lower than what was previously seen for Hib and have not changed substantially since Hib vaccine introduction. Nationally, among the encapsulated strains type f disease is most common (18% of invasive cases) followed by type e (5%), type b (6%) and the remaining types (a, c, d ≤1% each). For all of the serotypes a higher proportion of invasive disease occurs among the elderly, except type a which favors children < 2 years (3). In Alaska in 2003 there were 5 cases of invasive type a disease among 3 infants, two of whom had recurrent disease. *H. influenzae* type a has become an important cause of invasive disease among Navajo children and among indigenous persons in Northern Canada. These cases emphasize the need to continue monitoring and serotyping invasive *H. influenzae* infections.

Invasive Hib Disease, Children <5 Years, Alaska, 1980-2004



2004 data are provisional

1. Healthy People 2010 Objectives: US Department of Health and Human Services; 1998.
2. Galil K SR, Levine O, et al. Reemergence of invasive *Haemophilus influenzae* type b disease in a well-vaccinated population in remote Alaska. Journal of Infectious Diseases 1999;179:101-6.
3. Chang M, Harrison L, Farley M, et al. Charcteristics of invasive *Haemophilus influenzae* disease in the era of Hib vaccine, U. S., 1998-2002. In: Infectious Disease Society of America, Annual Meeting; 2004.
4. Serotyping Discrepancies in *Haemophilus influenzae* Type b Disease - United States, 1998-1999. Morbidity and Mortality Weekly Report 2002;51(32):706.

In Alaska, invasive *H. influenzae* disease is reportable to the Department of Health and Social Services, Section of Epidemiology (800-478-0084). In addition, we request that clinical laboratories send to the CDC Arctic Investigations Program all invasive isolates of *H. influenzae* for confirmation and serotyping. This laboratory-based surveillance is a free service provided by CDC since 1982 and has many benefits. First, we can quickly provide information regarding the serotype of a particular strain using state-of-the-art methods. This information is vital for clinical decisions regarding the need for chemoprophylaxis for contacts of Hib cases. Also, recent studies have demonstrated inaccuracies in serotyping *H. influenzae* isolates by some clinical labs (4). To improve serotyping accuracy we use two methods (see related article). The data collected have allowed us to document the tremendous success of the Hib vaccines and thus support ongoing vaccination efforts. Through this surveillance system we were able to detect the 1996-97 Hib outbreak, determine the cause and provide data that led to changes in statewide vaccine policy and control of the outbreak. None of this would have been possible without the cooperation and dedication of healthcare providers, laboratories and institutions around the state who make up the Alaska Invasive Bacterial Disease Surveillance Network. Thank you for your continued support!

What organism am I?

I appear as a small gram negative coccobacillus or filamentous rod on a gram stain. I require both the hemin and nicotinamide found in chocolate agar for growth, but I may be found satelliting around a Staph colony on a sheep blood agar plate. I am not hemolytic on rabbit or horse blood. I am considered an obligate human parasite of the respiratory tract. I may produce a polysaccharide capsule which enables me to be serotyped by slide agglutination. The occurrence of one of my serotypes at AIP has dramatically declined since the widespread use of a conjugate vaccine. I am sure you recognize me now as one of the important organisms in AIP's surveillance program. You are right! I am *Haemophilus influenzae* (Hi).

We would like to let you know what actually happens to the specimens you send to the AIP Micro Lab. The important work of initially isolating and identifying Hi from sterile sites belongs to you, the frontline clinical labs. Once the isolate arrives here at AIP, either by courier or by mail, it is logged in and restreaked for serotyping, susceptibility testing and

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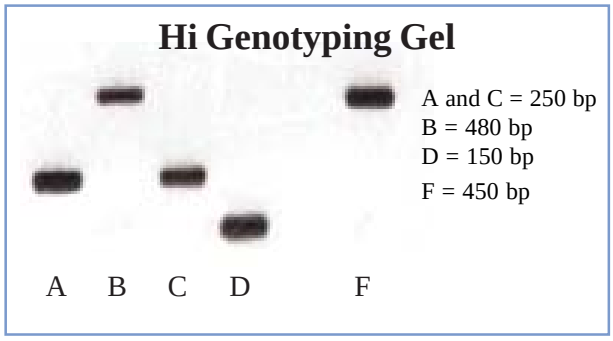
Molecular Minute

One technique we use to characterize Hi is the polymerase chain reaction (PCR), which isolates and amplifies a specific DNA or RNA sequence. PCR is rapid and accurate; results can be obtained in less than 3 hours. All Hi share a similar DNA "cassette locus," which consists of serotype nonspecific regions on each side of a serotype-specific region. We use PCR to target the capsule genes *(Cap)* that encode for a specific genotype which generally corresponds to the six serotypes (a-f) identified by slide agglutination. All encapsulated Hi have an intact *bex*A gene which is responsible for exportation of capsule to the cell surface. PCR genotyping complements the phenotype given by conventional slide agglutination. There are times when standard slide agglutination doesn't provide us an answer such as when a submitted culture doesn't grow or when there is a loss of the intact copy of *bex*A via a recombination event or by laboratory passage. In these instances, the Hi genotype could be determined by PCR.



We use other PCR assays for Hi. We have developed an assay to screen specimens for Hi utilizing the outer membrane porin protein P2 (*omp*P2) along with *bex*A. This assay will differentiate Hi from other bacteria and indicate encapsulation; it is used for confirmation of nontypeable Hi. We have also developed an assay to detect a deletion in the IS*1016*-*bex*A region of Hi serotype A. This deletion may enhance fitness and virulence. There are reports of the *bex*A from invasive nonserotype B Hi strains.

Genetic relatedness of Hi is determined by pulsed-field gel electrophoresis (PFGE). In PFGE, bacterial chromosomal DNA is cut with a specific restriction enzyme. This DNA is then coaxed through a gel using electric fields at varied angles resulting in a specific pattern of "bands." These patterns can then be compared with other Hi to determine relatedness. PFGE is useful for investigations of illness clusters or outbreaks.

These newer molecular techniques allow us to better characterize Hi and enhance our ability to monitor the frequency of Hi serotypes, determine genetic relatedness and detect genetic events that may lead to enhanced virulence.

Carolynn DeByle, Molecular biologist

What Organism am I?

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freezing. We routinely check to be sure the isolate requires both the "X" and "V" factors for growth, and we do a routine gram stain on each isolate. On rare occasions, we have had other organisms sent to us as Hi. It is worth noting that AIP is not set up to identify all organisms and, if special assistance is needed to identify an isolate, the Alaska State Public Health Lab would be better able to assist with the identification. Our program at AIP is focused on the present surveillance organisms and special studies.

Much of the focus of Hi surveillance at AIP has been to monitor serotypes after the introduction of the Hib conjugate vaccine in 1990. Currently, there are six Hi serotypes, a,b,c,d,e, and f. These are based on distinct capsular polysaccharide structures. Serotyping is performed in the micro lab by the slide agglutination method with Difco antisera. If no capsular polysaccharide is present, the isolate is nontypeable by slide agglutination. Known positive and negative controls are run weekly and each culture is screened in saline alone to check for autoagglutination. We have found that this method requires careful attention to technique, practice, and a fresh culture (12-18 hours old). An isolate may slow or stop producing capsule upon repeated passage, so it is best to serotype the isolate as soon as possible from the time of isolation. We are fortunate to have a molecular diagnostics lab which is able to confirm a Hi isolate or serotype by polymerase chain reaction (PCR), if a result is in question. The frequency of serotyping has declined along with the decrease in Hib disease resulting from widespread use of the Hib conjugate vaccine. This has placed an emphasis on the fact that accurate serotyping is needed to monitor the possible emergence of replacement virulent serotypes.

Antibiotic resistance trends are monitored for the Hi invasive isolates by performing susceptibility testing using antibiotic gradient strips, Etests® by AB Biodisk. A direct colony suspension equivalent to a 0.5 MacFarland is prepared in Mueller Hinton Broth from an overnight culture. Haemophilus Test Medium is inoculated with a confluent lawn of growth and then

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What Organism am I?

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the Etests srips are placed on the plate. This is an agar based method which requires the plates to be incubated for 20-24 hours in a 35° C incubator in a 5% CO2 environment. Currently, the antibiotics being tested include ampicillin, ceftriaxone, chloramphenicol, cefuroxime, meropenem, and trimethoprimsulfamethoxazole.

Preservation of specimens is also important in our lab based surveillance of Hi. Each isolate is frozen in skim milk at -80°C and placed in the culture bank thereby creating an ongoing and historical collection of the Hi isolates which have caused invasive disease in Alaska. The culture bank functions as a tool in that it is a depository of information for present and future learning.

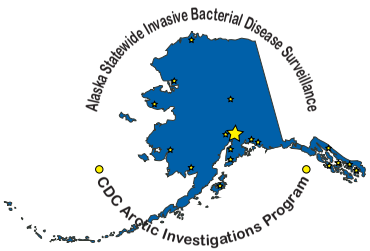
We hope this leaves you with a better understanding of what happens in the AIP Micro lab.

Alisa Reasonover, Microbiologist

Next issue: look for the Featured Laboratory

Did you Know?

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



INVASIVE BACTERIAL DISEASE SURVEILLANCE

Arctic Investigations Program (AIP), Centers for Disease Control and Prevention, 2004 Update

Which *isolates should I send?*

ALL "invasive" isolates of:

- *Haemophilus influenzae*
- *Streptococcus pneumoniae*
- *Neisseria meningitidis*
- Group A *Streptococcus* and
- Group B *Streptococcus*

recovered from normally sterile sites (e.g., blood, CSF, pleural fluid)

Where *should I send them?*

Send isolates to:

Microbiology Laboratory
Centers for Disease Control
Arctic Investigations Program
4055 Tudor Centre Drive
Anchorage, AK 99508

Anchorage labs, if you are not mailing the isolates, please call the AIP Microbiology Lab (729-3444) to discuss.

Who *can I ask about this?*

Surveillance program issues:

Tammy Cottle, Surveillance Coordinator
Phone: (907) 729-3421
Fax: (907) 729-3429
E-mail: tsc3@cdc.gov

Laboratory or shipping issues:

Microbiology Lab: (907) 729-3444 (ask for Alisa or Marcella)

How *should I prepare and mail the isolates?*

Isolates must be shipped in accordance with IATA's packaging guidelines, utilizing the biomailer system.

1. Streak the isolate onto a chocolate agar slant which has been labeled with patient name, DOB, and the specimen collection date. You do not need to incubate the isolate. Cushion the slant(s) with packing material before putting in the secondary container. Complete the information requested on the lab form.
2. Place the chocolate agar slant tube into the plastic container found inside the biomailer box and secure the lid tightly. Attach the completed lab form.
3. Place the plastic container and the completed lab forms in the biomailer box. Seal the box according to the instructions on the lid. Postage is provided; it is not necessary to add postage. Ensure that the package is mailed as soon as possible.

What *supplies do I need?*

You should always have in-date supplies available at your laboratory. Additional supplies will be sent upon request. Call the AIP Microbiology Lab at (907) 729-3444

Supplies include:

- Biomailer boxes including mailing label, infectious materials labels, and postage
- Chocolate agar slants
- Lab forms

Please pull out and post in your lab

Surveillance Organisms Reported 1/1/04-12/31/04

Region	*Streptococcus pneumoniae*	*Haemophilus influenzae**	*Neisseria meningitidis*	Group A Streptococcus	Group B Streptococcus
Bristol Bay	0	0	0	0	0
Interior	9	0	0	0	5
Kotzebue	0	0	0	0	0
North Slope	1	0	0	1	1
Norton Sound	2	0	0	2	0
Southcentral	54	7	5	9	19
Southeast	7	0	0	1	5
YK Delta	12	4	0	4	0

*Serotype b=2, f=4, non-typable=5

Participating Laboratories

Anchorage
Alaska Native Medical Center

Alaska Regional Hospital

Providence Alaska Medical Center

State of Alaska Public Health Laboratory

Barrow
Samuel Simmonds Memorial Hospital

Bethel
Yukon Kuskokwim Delta Regional Hospital

Cordova
Cordova Community Medical Center

Dillingham
Kanakanak Hospital

Elmendorf AFB
Elmendorf AFB Hospital

Fairbanks
Fairbanks Memorial Hospital

Fort Wainwright
Bassett Army Hospital

Homer
South Peninsula Hospital

Juneau
Bartlett Regional Hospital

Ketchikan
Ketchikan General Hospital

Kodiak
Providence Island Medical Center

Kotzebue
Maniilaq Medical Center

Nome
Norton Sound Regional Hospital

Palmer
Valley Hospital

Petersburg
Petersburg Medical Center

Sitka
Sitka Community Hospital

Southeast Regional Health Corp.

Soldotna
Central Peninsula General Hospital

Valdez
Valdez Community Hospital

Wrangell
Wrangell General Hospital