

FEATURE ARTICLE - LYN GILBERT

# Laboratory surveillance of invasive pneumococcal disease

## - essential data to monitor the efficacy of conjugate pneumococcal vaccine.

Before the Federal election both the Opposition Leader and the Health Minister promised to introduce the conjugate pneumococcal vaccine into the routine infant immunisation schedule in 2005. This should significantly reduce the incidence of pneumococcal disease in children under 5 years. Based on "herd immunity" it should also reduce the incidence of throat carriage of *Streptococcus pneumoniae* and disease in people who have not been immunised. However, the long-term efficacy of the vaccine is difficult to predict, because *S. pneumoniae* is a complex and versatile organism, which has the potential to evade the effects of vaccine as it has, to some extent, evaded the effects of antibiotics.

Since it was first described in the 1880s, the pneumococcus (*Streptococcus pneumoniae*) has been recognised as the commonest cause of severe pneumonia, and is responsible for millions of deaths worldwide each year; particularly among infants in developing countries, the elderly and people with underlying diseases. It also causes otitis media in young children, with significant morbidity (including permanent hearing loss) and is now the commonest cause of bacterial meningitis. Invasive pneumococcal disease (IPD), defined by isolation of *S. pneumoniae* from a normally sterile site (usually blood or cerebrospinal fluid) represents a small proportion of cases of pneumococcal disease, but is relatively easily defined and so often used for surveillance purposes.

The polysaccharide capsule of *Streptococcus pneumoniae* protects it from phagocytosis in a non-immune host and so is a major virulence factor. There are at least 90 pneumococcal serotypes, each defined by a different capsular polysaccharide. Antibodies against these polysaccharide antigens protect against infection with the corresponding serotype and, to some extent, others in the same serogroup. Many serotypes can cause disease but a small number of so-called "paediatric" serotypes cause more than 85% of childhood disease, in North America and Europe, and their antigens are included in the conjugate vaccines. Similarly 85% of serotypes

that cause adult pneumococcal disease are represented in the 23-valent polysaccharide vaccine, which has been available for many years. It is not effective in young children and has not been very widely used in adults. A more diverse range of serotypes cause disease in people with the highest risk of pneumococcal disease, including children in developing countries.

The ability of polysaccharide antigens to induce a protective immune response is greatly increased by attaching (conjugating) them to a protein carrier, as in the successful *Haemophilus influenzae* type b (Hib) conjugate vaccines, which have dramatically reduced the incidence of invasive Hib disease and carriage. The number of polysaccharides that can be included in a conjugate vaccine is limited by technical and financial factors but the current pneumococcal conjugate vaccine includes the seven commonest "paediatric" serotypes and newer vaccines will include up to eleven. Antibiotic resistance in *S. pneumoniae* has been increasing worldwide, but is most common among "paediatric" serotypes. For example, intermediate and high level penicillin resistance of *S. pneumoniae*, in Australia, increased from 1% in 1989 to 26% overall in 1997, and 13% in invasive isolates. Widespread childhood vaccination should not only significantly reduce pneumococcal disease in children, but also the overall prevalence of antibiotic resistance in *S. pneumoniae*. Nevertheless, the control of childhood pneumococcal disease will not be straightforward.

Limited data so far indicate that the conjugate vaccines can indeed prevent most invasive and mucosal (e.g. otitis media) disease due to vaccine serotypes. However, there is concern and some evidence that widespread immunisation will lead to an increase in colonization, and potentially disease, due to serotypes not included in the vaccine. Experience in the USA with the 7-valent conjugate vaccine (Wyeth Lederle Vaccine) has confirmed its high protective efficacy against invasive disease due to vaccine serotypes. However, after immunisation, "vaccine" serotypes in the nasopharyngeal flora are sometimes replaced by non-vaccine serotypes.



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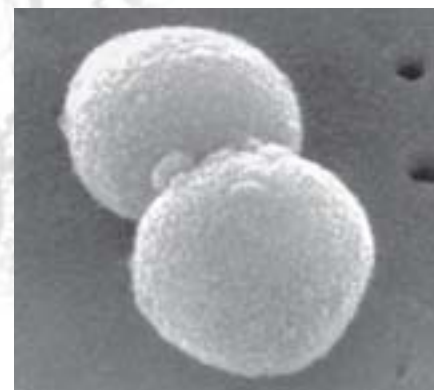
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So far, there has been no reported increase in IPD due to non-vaccine serotypes, but an increase in the proportion of cases of otitis media due to non-vaccine serotypes has been reported, despite a significant overall reduction in pneumococcal otitis media in vaccine recipients.

Comprehensive surveillance of pneumococcal isolates will be essential to monitor the efficacy of vaccination and the possible emergence of unintended changes in epidemiology of pneumococcal disease. First, it will be essential to determine the serotypes causing IPD and the immunisation status of affected patients to identify vaccine failures, if any. Secondly, an increase in the incidence of IPD due to non-vaccine serotypes or antibiotic resistance could indicate replacement of vaccine serotypes, serotype "switching" or emergence of more virulent or antibiotic resistant non-vaccine serotypes.

Until recently, there had been no systematic national surveillance of pneumococcal disease in Australia. However, IPD has recently been made notifiable in Australia. Enhanced surveillance, ahead of the introduction of routine childhood immunisation, includes referral of isolates from cases of IPD to one of three pneumococcal reference laboratories in Australia for serotyping. Antibiotic resistance data are also collected from diagnostic laboratories. Although the numbers are small,

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**Electron micrograph of *Streptococcus pneumoniae* (Photo: CDC)**

## STAFF PROFILE

**Lee Thomas**

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Lee started work in microbiology (ICPMR) in 1991, a year after arriving in Australia from New Zealand where she had worked at Auckland Hospital. Lee's interest in bacterial resistance developed during her time on the blood culture plate reading bench where accurate and speedy detection of resistance has a direct affect on patient outcome. The opportunities to conduct research into new methodologies and present findings at microbiology conferences were part of the supportive educational environment offered to Lee within the microbiology department.

In 2001, the increasing importance of molecular techniques within clinical microbiology motivated Lee to move into the research environment, supervised by Dr Jon Iredell. Three years later, Lee has learnt molecular skills providing her with the knowledge to develop techniques for molecular surveillance and detection of resistance genes.

## Molecular Screening for Antibiotic Resistance

In Australia, approximately 70% of Intensive Care patients are on antibiotic therapy. High levels of antibiotic usage are in turn associated with increased resistance in the microflora, and specific antibiotic selection pressure is directly related to this resistance and even to the acquisition of resistance-conferring genes in outbreak situations

The availability of culture-independent diagnostic tools and accurate definition of the microflora and resistance potential within it, is clearly crucial within ICU and other settings in which critically ill or immuno-compromised patients receive invasive therapy and high dose antibiotics. Insights into these processes also help us understand the spread of resistance genes into the well community, and continually "up the ante" in antibiotic usage and infection control.

The immediacy of the problem is well illustrated by the recent characterisation in our laboratory (Bjorn Espedido) of the first carbapenem-resistant enteric organism in Australia. This is defined by a mobile resistance gene cassette (*imp-4*) which has moved into a highly transferable plasmid, and has been associated with morbidity (highly resistant bacteraemias) and death. The variability of the phenotype has necessitated the use of molecular screening tools to follow the

movement of this resistance gene within the bacterial microflora of the Intensive Care Unit (ICU), where it was first detected. This has allowed us to detect movement into three species of bacteria, and to make same-day detection of the plasmid (and thus the resistance potential) in culture-negative individuals. It was not detected by phenotypic means and thus would not have allowed outbreak management. Such molecular methods will enable us to effectively and quickly manage infection control within ICU and high-dependency wards. This illustration also goes some way to explaining why we have failed to hold back the rising tide of antibiotic resistance to date, and why we need fresh approaches.

We believe that regular molecular surveillance of the microflora, and detection of resistance potential within transmissible elements within the microflora, will assist in the prediction of outbreaks and will aid monitoring of the silent spread of antibiotic resistance. Increasing the emphasis on antibiotic pressure (a current study is being conducted within our Intensive Care Department), means of transmission, silent carriage and transmission, accompanied by the use of simple molecular tools will provide valuable information on the development of antibiotic resistance.

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## Questions or comments?

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## CIDM Public Health – Education Program 2005

The following symposia will be held at Westmead Hospital during 2005:

- **04 March** – Mycobacteria Symposium
- **16 March** – Influenza update (including sample collection workshop)
- **06 May** – The Salmonella Journey
- **19 August** – Parasitology Symposium

If you would like to join our e-list to receive further information about educational events & activities, please email [judithh@icpmr.wsahs.nsw.gov.au](mailto:judithh@icpmr.wsahs.nsw.gov.au)

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there is already evidence of a statistically significant increase in the proportion of isolates of non-vaccine serotypes from Aboriginal children, in whom routine immunization began in 2001.

Continued surveillance of both serotype distribution and antibiotic susceptibility will be required to identify serotype replacement and/or increasing rates of antibiotic resistance among non-vaccine serotypes. Pneumococcal serotyping is currently performed using panels of antisera obtained from the Statens Serum Institute in Denmark. The sera are expensive and the method tedious and somewhat

subjective. At CIDM, we have developed a molecular method for identification of pneumococcal serotypes using a combination of PCR and sequencing targeting the *cps* gene cluster (which is responsible for the synthesis of the polysaccharide antigen). We have modified this method into a multiplex PCR and reverse line blot assay, targeting serotypes in the 23-valent polysaccharide vaccine (which also includes the serotypes in the conjugate vaccines). This can rapidly characterise most invasive serotypes and identify possible vaccine failures.

Our ultimate aim is to develop a large panel of oligonucleotide probes that can be combined on a microarray for rapid, technically simple and

inexpensive serotyping and subtyping of all clinically significant isolates. In addition, it will include probes for virulence, antibiotic susceptibility and housekeeping gene alleles (which are markers of the genetic origin or lineage). This will provide a molecular "fingerprint" of each isolate that will show important variable or phenotypic characteristics – e.g. serotype or antibiotic resistance that can be selected for by environmental pressure - as well as its genetic origins. This comprehensive surveillance will provide early warning of serotype replacement or emergence of novel virulent or antibiotic resistant strains that can evade the protective effects of immunisation.