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What's new in pneumococcal disease - surveillance using conventional and molecular methods.

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Changes in serotype distribution and antibiotic susceptibility of *Streptococcus pneumoniae* since the introduction of the 7-valent pneumococcal conjugate (PCV-7) vaccine.

The PCV-7 was introduced into the routine childhood immunization schedule in Australia in 2003. As part of the routine national invasive pneumococcal disease (IPD) surveillance programme, the NSW Pneumococcal Reference Laboratory (PRL) at CIDM (one of 3 in Australia) serotypes all isolates from sterile sites (mainly blood and CSF) from patients with IPD in NSW and the ACT. We have also recently undertaken a detailed survey of susceptibility of >2000 invasive pneumococci, collected before between 2002 and 2006, with support from Wyeth Australia Ltd.

After the introduction of PCV-7 in 2003, there was a significant fall (between the periods 2002-4 and 2005-6) in the total number of *S. pneumoniae* invasive isolates referred each year for serotyping to the NSW PRL, reflecting the marked fall in the number of cases notified. Predictably, most of this fall was in the vaccine target age-group (<5 years old) in which there was a highly significant fall in the absolute numbers (from an average of 233.7 per annum in 2002-4 to 98.5 per annum in 2005-6) and proportion of isolates belonging to PCV-7 serotypes (from 88% of isolates in 2002-4, to 57% in 2005-6). The most significant decrease was in serotype 14, which fell from 36% to 19% (p < 0.001) of isolates.

There were smaller falls in the proportion of PCV-7 serotypes in older age-groups (from 67% to 56% in 2002-4 to 2005-6, respectively). These falls were partly offset by absolute increases in numbers and proportions of isolates belonging to non-PCV-7 serotypes, especially in the <5-year age-group; in particular serotype 19A increased from 4% to 13% and serotype 3 from 1% to 6% of isolates in this age-group.

The main driver of changes in antibiotic resistance is changes in the proportions of different serotypes. However, despite significant falls in the proportion of PCV-7 serotypes, which are the most antibiotic resistant, the overall antibiotic resistance rates did not change significantly between the time-periods studied: 20% of isolates overall (25% in the PCV-7 serotype group) were R (minimum inhibitory concentration [MIC] >2µg/mL) or I (MIC 0.12-2 µg/mL) to penicillin and a similar proportion were resistant to erythromycin.

As expected there were major differences in rates of resistance rates between serotypes; 19F and 9V were most likely to be R/I to penicillin - 44% and 71%, respectively in 2002-4; 37% and 56% respectively in 2005-6 (differences not significant). Serotypes 14 and 19F have the highest rates of macrolide resistance (56% and 52%, respectively in 2002-4; 52% and 42% in 2005-6); 14 was the only individual serotype in the penicillin R/I rate rose significantly - from 5% to 11% (p=0.04) - between time periods.

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Serotype distribution among referred isolates from 3 age-groups and between 2 time periods.

Serotype group	<5yrs, 2002-4	<5yrs, 2005-6	p value ¹	5-65 yrs, 2002-4	5-65 yrs, 2005-6	>65 yrs, 2002-4	>65 yrs, 2005-6
PCV7 ²	619 (88%) ³	112 (57%)	<0.001	634 (68%) ³	298 (56%)	433 (66%) ³	216 (57%)
PPV-23v	49 (7%)	53 (27%)	<0.001	245 (26%) ³	188 (35%)	157 (24%)	115 (30%)
PCV-7-related	19 (3%)	12 (6%)	0.03	49 (6%) ³	47 (9%)	65 (10%)	51 (13%)
Non vaccine ²	14 (2%)	20 (10%)	<0.001	-	-	-	-
Grand Total	701	197 (100%)		928 (100%)	533 (100%)	655	382 (100%)
Annual mean	233.7	98.5		309.3	266.5	218.3	191.5

Notes:

1. p values compare proportions of each serotype group isolated from children <5 years old before and after introduction of 7v-PCV.
2. Serotypes in each serotype group are:
PCV-7: serotypes 14, 19F, 6B, 4, 23F, 9V, 18C; **PPV-23** (23-valent pneumococcal polysaccharide vaccine - excluding PCV-7 serotypes): 1, 10A, 15B, 19A, 22F, 3, 7F, 9N, 11A, 12F, 20, 33F, 8, 2, 5, 17F; **PCV-7-related:** 6A, 18B, 9A/L, 18A/F, 19B/C, 23A/B; **non-vaccine serotypes** (all others of a total of 90)
3. Proportion of total in each age-group belonging to relevant serotype group.

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Discovery of a new *Streptococcus pneumoniae* serotype by the CIDM team (in collaboration with a research group, led by Professor Kim Mulholland, undertaking a NIH/NHMRC-funded pneumococcal vaccine trial in Fijian children).

Pneumococcal serogroup 6, classically, comprised two serotypes - 6A and 6B - which produce biochemically similar capsules and have only a single nucleotide difference or polymorphism (SNP) in their capsular gene loci in the gene *wciP*. A new serotype 6C was identified in 2007 among isolates initially identified as 6A by Quellung reaction, based on differential reactions with two monoclonal antibodies (mAbs)¹. The only biochemical difference between 6A and 6C capsules is that a galactose molecule, in 6A, is replaced by glucose in 6C, due to substitution of the gene *wciN* of 6A (*wciN_{6A}*) with a different gene (*wciN_{6C}*)². The importance of serotype 6C is that, unlike 6A, there appears to be less cross-protection against it by the 6B component of PCV-7, which may give it a selective advantage in immunized children³. It seemed plausible that the same substitution could occur in serotype 6B to form another new serotype, with similar limited cross protection. This possibility was recently confirmed experimentally⁴, but this new hypothetical serotype (tentatively designated "6D") had not been identified among pneumococcal clinical isolates

We developed a new serotype-specific PCR assay to differentiate serotypes 6A, 6B and 6C, which targets both *wciN_{beta}* and the *wciP* SNP, which distinguishes serotypes 6A and 6B⁵. Using this assay we studied 98 unique nasopharyngeal serogroup 6 isolates from Fijian children, who were enrolled in a vaccine trial - two thirds of whom had received at least one dose of PCV-7 - and 51 invasive isolates from Australian children.

Two of 22 (9%) Australian and 24 of 64 (38%) Fijian isolates, previously identified as 6A (by conventional serotyping and serotype-specific PCR), contained *wciN_{beta}* and so were designated as 6C. In addition 14 of 34 (41%) Fijian isolates, previously identified as 6B contained *wciN_{beta}* and were designated as a new serotype "6D". Children who had received at least one dose of PCV-7 were significantly more likely to have serotype 6D isolated from their nasopharynges (than those who had not received PCV-7 (11/20; 55% vs 2/14; 14%; p<0.05).

This exciting finding is the first report of naturally occurring *S. pneumoniae* "serotype 6D" anywhere in the world⁶. (It is also the first report of identification of serotype 6C in Australia - we are in the process of studying a larger range of Australian serogroup 6 isolates, to determine the overall proportions of each of the 4 serotypes).

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Staff Profile....

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Peter Howard is the Senior Scientific Officer in charge of the NSW Enteric Reference Laboratory (ERL) of CIDMLS, ICPMR at Westmead Hospital. He is a graduate of Charles Sturt University with a Bachelor of Medical Laboratory Science and has 30 years microbiology experience within the Sydney West Area Health Service.

Peter started working at the Centre for Infectious Diseases and Microbiology (CIDM) 16 years ago and in 1994 he transferred into the Reference Laboratory section of CIDM. During this time he gained extensive experience in enteric pathogen identification. Peter has an interest in infections of Public Health importance and the efficient and rapid transfer of database information, to ensure rapid turn-around-time in investigations and surveillance of enteric related infections.

The ERL receives over 2,500 enteric pathogens from private pathology and public hospital laboratories throughout NSW. Salmonella isolates are the predominant type of organism submitted for typing for epidemiological purposes of contact tracing and food poisoning outbreak investigations. Other enteric pathogens submitted to his laboratory include Shigella, Vibrio, Yersinia enterocolitica and enteropathogenic *E. coli*.

He is also responsible for the daily electronic transfer of the enteric database information to the NSW Department of Health, to enable food poisoning investigations to be undertaken expeditiously when clustering of particular serotypes occur. Peter trains rotating staff within the Reference Laboratory section, and has a keen interest in passing on his knowledge in his field to build a broad skills base within CIDM Reference Laboratories.

On rarer occasions, investigations of food poisoning outbreaks due to other causative microbial agents such as *Clostridium perfringens* are also undertaken by the ERL.

In the future Peter intends to be instrumental in the development of a more efficient method of submission of enteric pathogen information via an electronic web based version of the National Enteric Pathogens Surveillance Scheme (NEPSS) which is currently a manual card system. This would then link all State Enteric Reference Laboratories together nationally and enable efficient entry of additional epidemiological data such as phage typing and MLVA typing.

Upcoming events....

The Dual Use Dilemma in the Life Sciences Seminar 23rd July 2009 Westmead Hospital, Westmead

This interactive seminar will include:

- Informing participants about current international discussions surrounding 'dual use' and 'biosecurity'.
- Generate debate about the merits and pitfalls of proposed policy responses.
- Provide examples of educational programs and oversight measures related to dual use research.

Topics for discussion will include the funding of research, communication of research results, oversight of experiments, the responsibilities of scientists and other biosecurity stakeholders, and examples of national and international governance measures being implemented or considered.

Seating is limited. RSVP by 6 July 2009 to lou_orszulak@wsahs.nsw.gov.au

Infection Control Symposium "Old tricks for new dogs" 14th August 2009 Westmead Hospital, Westmead

Intensive care units (ICUs) are high risk environments for healthcare associated infections (HAIs) and where a co-ordinated HAI control program will achieve the greatest benefits for patients, with flow-on to the whole healthcare system.

Over the past few years there has been an increasing awareness among health administrators in Australia of the need for major improvements in systems for prevention and control of HAIs and emergence of multiresistant organisms (MRO); which are now being translated into national and state-wide programs.

In many areas of HAI and MRO prevention and control, ICUs have led the way and demonstrated the benefits of co-ordinated programs. This symposium will provide a forum for infection control, infectious diseases and intensive care practitioners to exchange information about the current status of HAI/MRO control programs and develop a vision for a co-ordinated ICU-specific program.

Speakers include: Tony Burrell, Tom Solano, Kaye Rolls, Cathryn Murphy, Philip Russo, Peter Collignon, John Ferguson, Rob Cameron, Craig Boutlis, Wendy Yeomens, Tom Gottlieb, Kathy Dempsey, John Gallagher & Lyn Gilbert

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