



Microbiology Division

Respiratory and Meningeal Pathogens Reference Unit

BACKGROUND

In 2007, RMPRU continued to perform active laboratory-based surveillance for invasive disease throughout South Africa caused by *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*. The unit reported weekly data on these diseases to the Epidemiology Unit, NICD; provided data for presentation at the monthly National Outbreak Response Team (NORT) meeting, national Department of Health, Pretoria and for publication in the quarterly NICD Communicable Diseases Surveillance Bulletin.

Pneumococcal serotyping data from our surveillance programme were shared with the Pneumococcal Vaccines Accelerated Development and Introduction Plan (PneumoADIP) at Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, United States, for the Global Serotype Project. This project requested data from partners worldwide, and will help to inform the decision-making process about pneumococcal vaccine development in the future, and to ensure that these vaccines are relevant throughout the world.

In 2007, data from RMPRU surveillance were also presented and discussed at Department of Health Expanded Programme on Immunisation (EPI) Task Group meetings and National Advisory Group for Immunisation (NAGI) meetings held during the year. In addition, antimicrobial susceptibility data were used to assist in decisions for national treatment guidelines for lower respiratory tract infections.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

MOLECULAR BASIS AND CLONAL NATURE OF INCREASING PNEUMOCOCCAL MACROLIDE RESISTANCE IN SOUTH AFRICA, 2000-2005

The prevalence and molecular epidemiology of pneumococcal macrolide resistance in South Africa was investigated. Minimum inhibitory concentrations and serotypes of pneumococcal isolates causing invasive disease from 2000 through 2005 (n=15 982), were determined. Randomly selected isolates from 2005 (51%, 260/508) had resistance mechanisms determined, and clonality was investigated by pulsed-field gel electrophoresis (PFGE) (n=64) and multilocus

sequence typing (n=7). Macrolide resistance increased from 9% (160/1828) in 2000 to 14% (508/3656) in 2005 (P<0.001). Serotype 14 was the most common macrolide-resistant serotype (40%, 760/1921). The majority of macrolide-resistant isolates (75%, 1437/1921) displayed the macrolide lincosamide streptogramin B (MLSB) phenotype. Of the strains screened genotypically, 57% (147/260) contained erm(B), 27% (71/260) contained mef(A) and 15% (40/260) contained erm(B) and mef(A). One percent (2/260) contained ribosomal mutations. Macrolide-resistant isolates were predominantly penicillin-nonsusceptible and multidrug-resistant. Isolates clustered according to serotype by PFGE, and 22% (14/64), 11% (7/64) and 5% (3/64) of isolates were related to the Taiwan19F-14, England14-9 and Spain9V-3 global clones respectively.

DETECTION OF SEROTYPE 6C AMONG SOUTH AFRICAN SEROTYPE 6A PNEUMOCOCCI CAUSING INVASIVE DISEASE, 2005-2006

A new serotype (6C), indistinguishable from serotype 6A using the conventional Quellung reaction, was recently identified. Serotype 6B in the 7-valent pneumococcal conjugate vaccine cross-protects against 6A. Serotyping was performed on submitted isolates causing invasive pneumococcal disease (IPD) from January 2005 through December 2006 using the Quellung reaction. All serotype 6A isolates (n=606) were screened by PCR for the presence of a 1029-bp sequence that replaces the 1222-bp sequence in the *wciN* region of the capsular locus, differentiating serotype 6C from 6A. Five percent (30/600) were identified as serotype 6C, 13 strains from 2005 and 17 from 2006. The fraction of IPD due to 6C in children in South Africa is small. All 6C isolates identified were susceptible to penicillin.

RISK FACTORS FOR TRIMETHOPRIM-SULFAMETHOXAZOLE RESISTANCE IN *STREPTOCOCCUS PNEUMONIAE* CAUSING INVASIVE DISEASE IN SOUTH AFRICA, 2003-2006

The study design included a descriptive component to investigate the trends in resistance and a cross-sectional study for the risk factor analysis. The study population included laboratory-confirmed cases of invasive pneumococcal disease reported to the national surveillance system. Data were available for approximately 15000 cases for the descriptive component and approximately 7000 cases for the analytic component. The main outcome variable was

infection with an isolate which is non-susceptible to cotrimoxazole while the main exposure variables will include age, gender, race, province, specimen type, prior antibiotic usage, severity of illness, HIV serological status, current antiretroviral therapy, tuberculosis treatment and cotrimoxazole prophylaxis. An initial univariate logistic regression analysis followed by a multivariate logistic regression analysis were performed to assess the possible association between the exposures and outcome. This work will be submitted as part of an MSc (Med) in Epidemiology & Biostatistics thesis.

HIGH LEVELS OF HIV-COINFECTION IN ADULTS WITH INVASIVE PNEUMOCOCCAL DISEASE, SOUTH AFRICA, 2003 TO 2006

Cases with pneumococci isolated from normally sterile sites from January 2003 through December 2006 were reviewed. Enhanced surveillance included clinical data collection and HIV ELISA testing at sentinel sites. Adults were defined as ≥ 15 years. Of 15,179 cases of IPD reported, 94% (14,309) had available age of which 57% (8085) were adults. Median age in adults was 35 years (range 15-105); and enhanced surveillance identified 3787 (47%) of these cases. HIV was known in 61% (2316/3787) of these; 91% (2119/2316) were HIV-seropositive. HIV-seroprevalence was higher in females [1216/1288 (94%) vs. 901/1026 (88%), $p < 0.001$]. HIV-coinfection differed by age group: 192/233 (82%) in 15-24 years; 1570/1659 (95%) in 25-44 years; 333/386 (86%) in 45-64 years; 24/38 (63%) in > 64 years ($p < 0.001$). The majority of IPD in adults was associated with HIV-coinfection with seroprevalence highest in 25-44 years. CFR increased with age, but did not differ by HIV serostatus. Serotype prevalence fluctuated over the 4 years, in a time of minimal vaccine use.

DIFFERENCES IN BLOOD CULTURING PRACTICES IN RURAL AND URBAN AREAS OF SOUTH AFRICA

We aimed to gather detailed data about the number of blood cultures performed at each centre, quality of specimen submission and processing, and to explore clinicians' perceptions of blood culture utility and burden of pneumococcal disease. Our goal was to characterize regional differences in blood culturing practices to assist in the interpretation of existing laboratory-based surveillance for invasive pneumococcal disease on a national level in South Africa. Doctor questionnaires were returned from 20 of 22 hospitals, 341 questionnaires in total. Responses from academic and non-academic hospitals were compared. Preliminary review of responses to questions in the questionnaire for doctors indicated that non-academic hospitals' doctors are less likely to take blood, urine and cerebrospinal fluid (CSF) specimens; while pus, pleural and sputum specimens were reported to be taken equally frequently by both groups of doctors. Approximately 40% of doctors responded

that the microbiology laboratory is not useful in the diagnosis of respiratory tract infections, but this did not differ by type of hospital. Blood cultures are taken less frequently at non-academic hospitals for all syndromes cited. Also to be noted was that only half of the doctors at the academic centers "always or almost always" take blood cultures for respiratory tract infections.

MOLECULAR CHARACTERIZATION OF SERO-GROUP B MENINGOCOCCAL ISOLATES CAUSING INVASIVE DISEASE IN SOUTH AFRICA

Currently there is no licensed vaccine for prevention of bacterial meningitis caused by serogroup B *N. meningitidis*. Outer membrane protein LP2086 has been shown to elicit a good antibody response in animal models and is therefore a promising vaccine candidate. The aim of this study is to investigate the prevalence, distribution and sequence diversity of LP2086 in both serogroup B and other serogroups of meningococci circulating in South Africa. The study is ongoing and is a collaborative study funded by Wyeth Pharmaceuticals.



Happy Skosana and Linda de Gouveia (left); Olga Hattingh and Ruth Mpebe (right),

OTHER HIGHLIGHTS

Dr von Gottberg was invited as a WHO Temporary Advisor to the PneumoADIP (4th annual meeting) and Hib Initiative Surveillance Networks Investigators Meeting at the Nai Lert Park Hotel, Bangkok, Thailand, March 28-30, 2007. In addition to the meeting, she participated in two site visits: to the Ministry of Public Health in Nonthaburi on Tuesday 27 March, and to Sa Kaeo on Saturday 31 March.

Linda de Gouveia was invited by WHO to attend the 6th Annual Inter-country Review and Planning Meeting on Enhanced Surveillance and Response to Meningitis Epidemics in Africa in Ouagadougou, Burkina Faso, 9-10 October 2007.

Dr von Gottberg presented "Meningococcal Disease in South Africa" at "An Experts' Advisory Board Meeting" held by Novartis Vaccines at the Dusit Thani Hotel, Bangkok, Thailand, on November 14, 2007. The

meeting brought together Novartis Vaccines representatives and clinicians, policy makers, and meningococcal reference laboratory heads from around the world to obtain guidance from the advisors on the development and introduction of the pipeline Novartis Vaccines meningococcal vaccines.

INTERNATIONAL TRAINING

Linda de Gouveia (RMPRU) and Vivian Fensham (EQA) coordinated a Hib (*Haemophilus influenzae* serotype b) technical support training course for 10 laboratory technologists, facilitated by Dr. Bekithemba Mhlanga (WHO-AFRO, Harare), at EHNRI (Ethiopian Health and Nutrition Research Institute) Addis Ababa, Ethiopia from 19-23 November 2007.

Dr Anne von Gottberg, Head of RMPRU

COLLABORATIONS

Prof Shabir Madhi (RMPRU, Chris Hani Baragwanath Hospital):

Laboratory quality control and external quality assessment for a colonisation substudy of the clinical PoPS (Prevention of Perinatal Sepsis) trial.

CIPRA-SA II PROJECT 4: pneumococcal serotyping for a colonisation substudy.

PACTG P1041: pneumococcal serotyping for a colonisation substudy.

Dr Werner Albrich (RMPRU, Chris Hani Baragwanath Hospital):

Infant immunization to reduce pneumonia in HIV-positive Women Part II - Prospective: pneumococcal serotyping for invasive and colonising pneumococcal isolates

Validation of genes affecting susceptibility to invasive pneumococcal disease and/or other infectious diseases.

Prof Charles Feldman (Wits University) and Dr Adrian Brink (Ampath Laboratories): Antimicrobial susceptibility of pneumococcal isolates causing

CAPACITY BUILDING

Dr Wolter fulfilled the requirements for the Doctor of Philosophy (PhD) with her thesis entitled “Novel mechanisms of resistance to protein synthesis inhibitors in *Streptococcus pneumoniae*”. The degree was conferred upon her at the graduation ceremony, University of Witwatersrand, 27 November 2007.

Ms Mothibeli fulfilled the requirements for a Master of Science (MSc) (Dissertation) with her dissertation entitled “Molecular epidemiology of South African

serotype 3 and serotype 19A pneumococcal isolates”. The degree was conferred upon her at the graduation ceremony, University of Witwatersrand, 27 November 2007.

Mr Peter Suwirakwenda Nyasulu fulfilled the requirements for a Master of Science (Med) in Epidemiology & Biostatistics (Research Report “Risk factors for mortality in patients with invasive pneumococcal disease in South Africa”). The degree was conferred upon him at the graduation ceremony, University of Witwatersrand, 11 December 2007