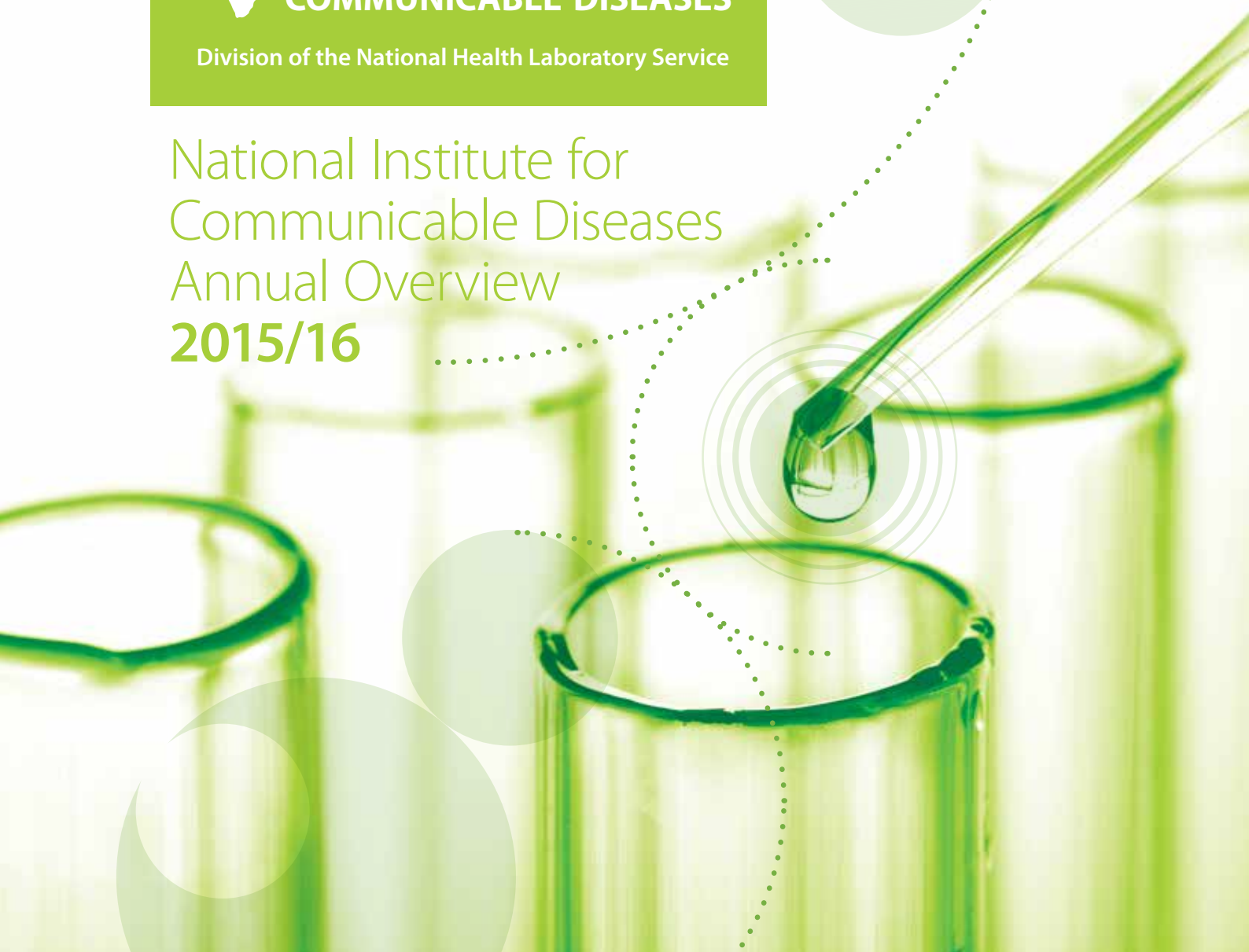
NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

Division of the National Health Laboratory Service

National Institute for Communicable Diseases Annual Overview **2015/16**



NATIONAL HEALTH
LABORATORY SERVICE

The NHLS is a proud recipient of the 2015 European Quality Award.

National Institute for Communicable Diseases

The National Institute for Communicable Diseases (NICD) is responsible for surveillance of communicable diseases and is a vital resource for knowledge and expertise in communicable disease intelligence in South Africa.

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Contents

List of Abbreviations

ACILT	African Centre for Integrated Laboratory Training
AFENET	African Field Epidemiology Network
AFP	Acute Flaccid Paralysis
AFRICHOL	African Cholera Surveillance Network
AMRRL	Antimicrobial Resistance Reference Laboratory
ART	Antiretroviral Therapy
ASR	Age-Standardised Incidence Rate
BDQ	Bedaquiline
bNAb	Broadly Neutralising Antibody
BV	Bacterial Vaginosis
cART	Combination Antiretroviral Therapy
CDC	Centres for Disease Control and Prevention
CDW	Corporate Data Warehouse
CED	Centre for Enteric Diseases
CEZD	Centre for Emerging and Zoonotic Diseases
CIN	Cervical Intraepithelial Neoplasia
CLSI	Clinical Laboratory and Standards Institute
COTHI	Centre for Opportunistic, Tropical and Hospital infections
CrAg	Cryptococcal Antigen
CRDM	Centre for Respiratory Diseases and Meningitis
CRE	Carbapenem Resistant *Enterobacteriaceae*
CRS	Congenital Rubella Syndrome
CS	Cleavage Site
CT	Chlamydia Trachomatis
CTB	Centre for Tuberculosis
DALY	Disability-Adjusted Life Year
DBS	Dried Blood Spots
DoH	Department of Health
DRS	Drug Resistance Survey
DST	Department of Science and Technology
DST	Drug-Susceptibility Testing
DTM&H	Diploma in Tropical Medicine and Hygiene
DTRA	Defence Threat Reduction Agency
EID	Early Infant Diagnosis
ELDS-net	European Legionnaire's Diseases Surveillance Network
EML	Electron Microscope Laboratory
EML	Ebola Mobile Laboratory
EOC	Emergency Operations Centre
EPI	Expanded Programme of Immunisation
ERC	European Research Council
ESBL	Extended Spectrum Beta-Lactamases
ESC	Extended-Spectrum Cephalosporins
ESKAPE	Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas and ESBL

EVD	Ebola Virus Disease
FELTP	Field Epidemiology and Laboratory Training Programme
FETP	Field Epidemiology Training Programme
GERMS-SA	Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa
GHSA	Global Health Security Agenda
GPEI	Global Polio Eradication Initiative
GUD	Genital Ulcer Disease
GUS	Genital Ulceration Syndrome
HAstV	Human Astrovirus
HBV	Hepatitis B Virus
hc2	Hybrid Capture
HCV	Hepatitis C Virus
HEU	HIV-Exposed Uninfected
HIPPS	HIV Incidence Provincial Surveillance System
HIV	Human Immunodeficiency Virus
HIVDR	HIV Drug Resistance
HPV	Human Papillomavirus
HR	High-risk
HSRC	Human Sciences Research Council
HSV	Herpes Simplex Virus
HUU	HIV-Unexposed and Uninfected
HVTN	HIV Vaccine Trials Network
IAEA	International Atomic Energy Agency
IARC	International Agency for Research on Cancer
IgG	Immunoglobin G
IHR	International Health Regulations
ILI	Influenza-Like Illness
IMD	Invasive Meningococcal Disease
IMR	Isoniazid Mono-Resistance
IPD	Invasive Pneumococcal Disease
IPV	Inactivated Polio Vaccine
IQC	Internal Quality Control
IQR	Interquartile Range
JCS	Johannesburg Cancer Case Control Study
KAP	Knowledge, Attitudes and Practices
KPIS	Key Population Implementation Science
KS	Kaposi's Sarcoma
LARS	Laboratory-Based Antimicrobial Resistance Surveillance
LGV	Lymphogranuloma Venereum
LPV	Lopinavir
LRTI	Lower Respiratory Tract Infection
MDR	Multi-Drug Resistant
Men C	Meningococcal Serogroup C
Men W	Meningococcal Serogroup W
MG	Mycoplasma Genitalium

MGIT	Mycobacteria Growth Indicator Tube
MIC	Minimal Inhibitory Concentration
MIRU	Mycobacterial Interspersed Repetitive Unit
MLST	Multi-Locus Sequence Typing
MLVA	Multiple-Locus Variable number tandem repeat Analysis
MNORT	Multisectoral National Outbreak Response Team
MRC	Medical Research Council
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSM	Men who have sex with men
MUS	Male Urethritis Syndrome
NAPHISA	National Public Health Institute of South Africa
NCC	National Certification Committees
NCR	National Cancer Registry
NEC	Necrotising Enterocolitis
Necsa	South African Nuclear Energy Corporation
NG	Neisseria Gonorrhoeae
NGS	Next Generation Sequencing
NIBSC	National Institute for Biological Standards and Controls
NIC	National Influenza Centre
NICD	National Institute for Communicable Diseases
NICU	Neonatal Intensive Care Unit
NMC	Notifiable Medical Conditions
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NPSP	National Pneumonia Surveillance Programme
NRF	National Research Foundation
NTBRL	National TB Reference Laboratory
NTP	National TB Programme
OPV	Oral Polio Vaccine
OR	Odds Ratio
ORU	Outbreak Response Unit
PCP	*Pneumocystis Jirovecii* Pneumonia
PCR	Polymerase Chain Reaction
PCV	Pneumococcal Conjugate Vaccine
PEF	Polio Essential Facility
PEPFAR	President's Emergency Plan for AIDS Relief
PET	Provincial Epidemiology Team
PFGE	Pulsed Field Gel Electrophoresis
PHC	Primary Healthcare
PHC	Primary Healthcare Centres
PHEIC	Public Health Emergency of International Concern
PI	Protease Inhibitor
PMS	Post Marketing Surveillance
PMTCT	Prevention of Mother-to-Child Transmission
POCT	Point of Care Testing
PT	Proficiency Testing

PTB	Pulmonary Tuberculosis
RAPIDD	Research and Policy for Infectious Disease Dynamics
RED	Reaching Every District
RFLP	Restriction Fragment Length Polymorphism
RMPRU	Respiratory and Meningeal Pathogens Research Unit
RNA	Ribonucleic Acid
RR	Rifampicin Resistant
RSV	Respiratory Syncytial Virus
RTV	Ritonavir
RVF	Rift Valley Fever
RVFV	RVF Virus
SACIDS	Southern African Centre for Infectious Disease Surveillance
SAFETP	South African Field Epidemiology Training Programme
SaNTHNet	South African National Travel Health Network
SARGDDC	South African Regional Global Disease Detection Centre
SARI	Severe Acute Respiratory Infections
SARI	Severe Acute Respiratory Illness
SASTM	South African Society of Travel Medicine
SASTM	South African Society of Travel Medicine
SIDA	Swedish International Development Cooperation Agency
SNP	Single Nucleotide Polymorphism
SRI	Severe Respiratory Illness
ST	Sequence Type
STI	Sexually Transmitted Infection
TAC	TaqMan® Array Card
TB	Tuberculosis
TEPHINET	Training Programme in Epidemiology and Public Health Interventions Network
TV	Trichomonas Vaginalis
UCSF	University of California, San Francisco
UNICEF	United Nations Children's Emergency Fund
USAID	United States Agency for International Development
VDPV	Vaccine Derived Poliovirus
VDS	Venereal Disease – Syphilis
VNTR	Variable Number Tandem Repeat
WGS	Whole Genome Sequencing
WHO	World Health Organization
XDR	Extensively Drug Resistant

NICD Director's Overview

Executive Director: Prof. Shabir A Madhi

All too often, the need for robust surveillance systems to inform health policy and mitigate the effects of communicable disease outbreaks is overlooked in resource-constrained countries. Rather, Health Departments tend to focus on addressing the more immediate curative healthcare needs of the population. It is, however, essential to realise that unless accurate data inform health policy, the successes and challenges faced by the healthcare system can be misinformed and scarce resources misallocated. In 2015/16, the National Institute for Communicable Diseases (NICD) further strengthened its efforts at delivering on its mandate to conduct surveillance of communicable diseases in South Africa. This was in part facilitated by the commitment of the Department of Health (DoH) to promote communicable disease surveillance in South Africa, resulting in the NICD now being directly funded by a grant from the DoH. Public confidence in the NICD is increasing and is manifested by numerous enquiries received on a daily basis from the general public and healthcare workers, and frequent engagements with the media as a trusted source of information when faced with any communicable disease threat. Also, notably, the NICD was commended by all political parties at the National Health Portfolio Meeting in Parliament in 2015, for its service to South Africa and beyond.

The NICD has continued to evolve over the past five years in its transformation, from a predominantly laboratory-focused surveillance institution and research entity to one in which active surveillance on key communicable threats forms the cornerstone of its activities. This has included decentralisation of its engagement with provincial health authorities, through the placement of an NICD epidemiologist in the majority of the provinces in South Africa. These epidemiologists, working closely with the Centres at the NICD and their provincial counterparts in the Provincial Health Departments, have focused on strengthening the Provincial Health Departments' responses to the control of tuberculosis (TB), which is *the* most important communicable disease challenge in South Africa. Furthermore, the dedicated provincial epidemiologists have been pivotal in enabling more seamless interfacing between the NICD Outbreak Response Unit and the Provincial Communicable Disease directorates, ensuring prompt interventions and early containment of potential communicable disease outbreaks, such as diphtheria in KwaZulu-Natal, and the typhoid scare in Gauteng.

In addressing the challenge of TB in South Africa, the Centre for Tuberculosis (CTB) also reported for the first time in the history of the country on the national burden of microbiologically-confirmed TB in South Africa from 2004 until 2012. These data were published in the prestigious *Lancet Infectious Diseases Journal***.** Although the data showed a decline in the incidence of TB, which was temporally associated with an expansion of the anti-retroviral treatment programme in South Africa, the incidence of TB still remains among the highest in the world, with an overall incidence of 774 per 100,000; including provinces such as KwaZulu-Natal and the Northern Cape that report incidence figures of > 1,100 per 100,000. The surveillance further unmasked the under-reporting of TB in the country, with approximately 25%-30% of individuals with microbiologically-confirmed TB not documented to have initiated anti-TB treatment. The CTB also concluded a national survey on TB drug resistance in 2015, which screened approximately 200,000 presumed TB cases. The results of this study will be instrumental in informing future TB treatment strategies in South Africa. The CTB was acknowledged for its expertise in laboratory-based TB surveillance by being awarded the status of a designated World Health Organization Supranational Reference Laboratory in 2016.

On the HIV surveillance front, the Centre for HIV and STI at the NICD has continued, in collaboration with the World Bank, the NDoH and HE^{2}RO, to track adherence to antiretroviral therapy through use of the NHLS Corporate Data Warehouse. The centre has remained at the cutting edge of research on the HIV vaccine development front, which has included further characterisation of broadly-neutralising antibodies against HIV, either as potential monoclonal antibodies or identifying future vaccine epitopes for the development of an HIV vaccine aimed at the control and prevention of HIV. The findings of this study were published in leading international journals, including *Nature Medicine.* In acknowledgement of this research, Prof. Lynn Morris was awarded the Medical Research Council Gold Scientific Achievement Award in 2015.

During 2015, the Centre for Enteric Diseases (CED) and Centre for Respiratory Diseases and Meningitis (CRDM) expanded their activities to include country-wide sentinel site active surveillance on pathogen-specific causes of diarrheal and pneumonia disease. This has become all the more important, with these two syndromes now being the leading causes of under-five mortality among children aged 1–59 months of age in South Africa, following the great achievements in the prevention of mother-to-child

transmission of HIV in South Africa over the past few years. However, the challenge of the persistently high prevalence of maternal HIV infection in South Africa remains a major threat to children, with the CRDM having reported a much higher incidence of bacterial and respiratory viral hospitalisation among HIV-exposed-uninfected children compared to their HIV-unexposed counterparts. These findings have important policy implications for South Africa in planning further reductions in under-five mortality rates.

The role of the NICD beyond the borders of South Africa is evident from it being a host to seven WHO reference laboratory facilities for the African region. Furthermore, the support which the NICD lends to other African countries is manifest in the extended role of the Centre for Emerging and Zoonotic Diseases (CEZD) in the post-Ebola epidemic aftermath, continuing its support of Sierra Leonean scientists in diagnostic capacity. CEZD was at the forefront of identifying the first laboratory confirmed cases of the yellow fever outbreak experienced in Angola since January 2016 – one of the largest yellow fever outbreaks in Africa for decades. In support of the DoH's aim to eradicate malaria from South Arica, the COTHI has continued to support the malaria programme in surveillance on resistance acquired by the major malaria vector *Anopheles arabiensis* in northern KwaZulu-Natal Province. This has guided policy on the need for a focus on insecticide resistance management, and alternative control techniques targeting outdoor-resting mosquitoes.

To further strengthen its surveillance activities, the NICD completed the commissioning of a Central Sequencing Facility with state-of-the-art equipment during 2015. The development of this local expertise at the NICD and in South Africa enables research and surveillance activities that depend on Next Generation Sequencing solutions, including those related to addressing TB and HIV drug resistance, HIV antibody research, vaccine epitope identification and outbreak responses.

NICD activities during 2015/16 place it on a firm footing to continue promoting the health and well-being of South Africans, through surveillance of current and any imminent communicable disease threats in the country. This, however, would not have been achievable without the remarkable dedication of the staff who works at this institute, as has been witnessed during the first five years of my tenure as the Director of the NICD. With the imminent establishment of a National Public Health Institute of South Africa (NAPHISA), which will be mandated to establish robust communicable disease and non-communicable disease surveillance in South Africa, the NICD will likely provide a solid foundation to launch this much needed structure to further secure the health and well-being of South Africans.

CENTRE FOR ENTERIC DISEASES

Centre Co-Head: Dr Karen Keddy

Centre Co-Head: Dr Nicola Page

BACKGROUND

The Centre for Enteric Diseases (CED) of the National Institute for Communicable Diseases (NICD) provides information on the surveillance of pathogens associated with enteric diseases in children younger than five years of age in South Africa. The data is used for the Primary Health Care (PHC) Services programme, Communicable Disease Control sub-programme. The centre contributes to efforts to reduce the under-five mortality rate by providing data for improved decision making to combat the number of deaths due to diarrhoea in these children. The CED also provides information on food- and waterborne outbreaks and provides the expertise to strengthen outbreak preparedness and response to public health emergencies in line with international health regulations. The centre focuses on surveillance of pathogens associated with diarrhoea and enteric fevers, including typhoid fever and timeously identifying the potential causes of outbreaks due to these pathogens. Centre staff members provide policy advice and technical support to government and contribute to the training of medical professionals including medical scientists, medical technologists, epidemiologists, public health workers, nurses and registrars.

SURVEILLANCE AND DIAGNOSTIC SERVICES

The 2015 diarrhoea season was less severe compared to 2013 and 2014; with fewer rotavirus cases noted. The centre expanded the diarrhoeal sentinel surveillance network to include sites in the Eastern Cape and North West provinces.

Laboratory testing and support was provided for 13 outbreaks, including notifiable diseases.

RESEARCH PROJECTS

Post-marketing intussusception monitoring after introduction of oral rotavirus vaccine in South Africa

NICD researchers: *Prof. N Page, Ms S Nadan, Mr R Netshikweta, Ms T Kruger*

Principle investigators: *Prof. S Madhi, Dr M Groome (DST/NRF: Vaccine Preventable Diseases, University of the Witwatersrand. Respiratory and Meningeal Pathogens Research Unit (RMPRU)*

Funding source: *Bill and Melinda Gates Foundation*

Intussusception is a rare intestinal blockage associated with a human-simian rotavirus reassortant vaccine formulation. While current rotavirus vaccines did not demonstrate an increased risk of intussusception during large scale vaccine trials, recent studies have indicated a low-level risk of intussusception after vaccine administration. There is currently no data on intussusception risk in African settings. Active surveillance for intussusception cases has been implemented in seven South African cities. Since the start of the study, 377 stools have been collected (207 cases and 171 controls). Human adenovirus (n=105), norovirus genogroup II (n=26), astrovirus (n=22); bocavirus (n=15); rotavirus (n=14), sapovirus (n=10) and norovirus GI (n=8) have been detected. The study will continue for the next two years.

Reanalysis of stool specimens from Venda, South Africa, collected as part of the MAL-ED study, using TaqMan® Array Cards for the detection of multiple enteric pathogens

NICD researchers: *Prof. N Page, Ms S Nadan*

Principle investigator: *Dr Eric Houpt (University of Virginia); Dr Amidou Samie (University of Venda)*

Funding source: *Bill and Melinda Gates Foundation*

TaqMan® Array Card (TAC) technology has been adapted to simultaneously screen stool specimens for a variety of viral, bacterial and parasitic enteric pathogens. A total of 5 094 specimens from Venda, South Africa will be screened over an 18 month period with the University of Venda performing nucleic acid extractions and CED performing the TAC reactions. Thus far, the assay has been validated, extraction problems solved and results generated for 77 specimens.

Laboratory testing for Phase I/II, descending age, double-blinded, randomised, placebo-controlled dose escalation study to examine the safety, reactogenicity, tolerability and immunogenicity of the P2-VP8 subunit parenteral rotavirus vaccine in healthy toddlers and infants

NICD researchers: *Prof. NA Page, Ms S Nadan*

Principle investigator: *Dr M Groome (DST/NRF: Vaccine Preventable Diseases, University of the Witwatersrand. Respiratory and Meningeal Pathogens Research Unit (RMPRU)*

Funding source: *PATH Vaccine Solutions*

The P2-VP8 vaccine was administered intramuscularly to three different toddler and infant cohorts at increasing concentrations. The two highest doses tolerated were assessed in an expanded infant cohort. All infants receive Rotarix® after the third study injection, and a stool sample was obtained following the first Rotarix® dose to assess for shedding. The impact of the injectable vaccine on faecal shedding of Rotarix® during the week after the first Rotarix® dose was evaluated, as a surrogate test of concept for efficacy. Stool specimens were analysed using Rotavirus EIA and real-time RT-PCR with standards to determine relative viral concentration. The vaccine was well-tolerated at all dose levels in toddlers and infants and a reduction of Rotarix® shedding was noted in vaccine recipients.

The epidemiology and molecular characterisation of human astroviruses in selected areas of South Africa

NICD researchers: *Ms S Nadan*

Principle investigator: *Prof. NA Page*

Funding source: *Poliomyelitis Research Foundation, NHLS Research Trust*

Between 2009 and 2014 human astroviruses (HAstVs) were detected in 6.8% (437/6389) of stool specimens collected. A total of 404 specimens had sufficient clinical material for further characterisation. Of these, 14.1% (57/404) have been characterised as classic type HAstVs; 15.6% (63/404) have been partially characterised; and 70.3% (284/404) indicate recombinant or novel genotypes. Genotype 1 was detected most frequently in the classic HAstVs (7.4%), followed by type 5 (3.5%) and type 2 (2.2%) with types 6 and 8 detected in less than 1%. Classic HAstV types 3, 4 or 7 have not been detected. Whole genome sequence analysis will be used to confirm the recombinant or novel strains. To date, the method has successfully identified known classic strains.

PulseNet and whole-genome sequencing implementation for public health surveillance

NICD researchers: *AM Smith, N Tau, SL Smouse, KH Keddy*

Collaborator: *PulseNet USA*

Current methodologies will be supplemented by the implementation of whole-genome sequencing (WGS) analysis of enteric pathogens, thereby enhancing surveillance activities and molecular epidemiological analysis of enteric infections. PulseNet is in the process of implementing WGS analysis; harmonisation/standardisation of WGS data analysis is envisaged. WGS shows promise to transform public health microbiology of enteric pathogens and investigation of foodborne and waterborne infections. WGS represents a single, rapid and cost-effective approach to identify and characterise bacteria. It has the potential to replace all microbiological phenotypic and genotypic methodologies currently used in a typical public health laboratory. WGS data can be interrogated to provide vast amounts of information concerning bacterial pathogens – information with huge benefits for public health.

Whole-genome sequencing analysis of a cluster of *Listeria monocytogenes* isolated in South Africa

NICD researchers: *AM Smith, N Tau, SL Smouse, KH Keddy*

Collaborators: *P Naicker, C Bamford (University of Cape Town)*

Funding source: *Global Disease Detection, grant 1U19GH000571-02*

There is growing concern about the increasing prevalence of *Listeria monocytogenes* associated with foodborne outbreaks. During September 2015, an increased number of human cases of *L. monocytogenes* were reported from hospitals in the Western Cape Province of South Africa. Six isolates were investigated by whole-genome sequencing (WGS) analysis. Phylogenetic analysis including single nucleotide polymorphism (SNP) analysis and multi-locus sequence typing (MLST) determined that the six isolates were not a single strain, but that we were in fact dealing with three different strains. The first strain was represented by three isolates and showed MLST sequence type (ST) 6; the second strain was represented by two isolates showing MLST ST 876; the third strain was represented by a single isolate showing ST1.

Whole-genome sequencing analysis of *Vibrio cholerae* O1 isolated in sub-Saharan Africa

NICD researchers: *AM Smith, KH Keddy, H Ismail, N Tau, SL Smouse*

Collaborators: *MA Mengel, B-M Njanpop-Lafourcade (AMP, France), Africhol collaborators*

Funding source: *Global Disease Detection, grant 1U19GH000571-02*

The African Cholera Surveillance Network (AFRICHOL) project is concerned with *Vibrio cholerae* O1 surveillance, epidemiology, and disease burden in sub-Saharan Africa. The CED provides laboratory support for phenotypic and genotypic characterisation of isolates. A selection of isolates from countries has been submitted to the CED. Part of the project is whole-genome sequencing (WGS) analysis of isolates to be performed by the Sanger Institute, UK. The CED has isolated genomic DNA from isolates and these have been shipped to the Sanger Institute. This includes DNA from a total of 357 isolates: 131 from Togo, 98 from DRC, 44 from Guinea, 41 from Ivory Coast, 16 from Mozambique, 15 from Zimbabwe and 12 from Uganda.

Development and evaluation of a multiple-locus variable-number tandem-repeats analysis assay for subtyping *Salmonella* Typhi strains from Sub-Saharan Africa

NICD researchers/investigators: *NP Tau, AM Smith, KH Keddy*

Funding source: *Global Disease Detection, grant 1U19GH000571-02*

The study aim was to develop a relevant, highly reproducible multiple-locus variable number tandem repeat analysis (MLVA) assay consisting of five variable number tandem repeats (VNTR) markers to analyse representative *Salmonella* Typhi strains from sub-Saharan Africa. Thirteen previously published polymorphic VNTR loci were evaluated using a selection of 50 *Salmonella* Typhi strains. The five VNTR loci that showed good allele variation were selected and combined in a single multiplex polymerase chain reaction (PCR) assay for MLVA analysis of *Salmonella* Typhi strains. The MLVA assay characterised isolates into 47 MLVA types and showed high discriminatory capacity (Simpson diversity index) (D) 0.998 (95% confidence interval (CI) 0.995 – 1.000). This MLVA assay is a highly discriminatory molecular epidemiological tool, suitable for epidemiological analysis of *Salmonella* Typhi isolates from sub-Saharan Africa.

Molecular Epidemiology of *Salmonella* Enteritidis from human isolates in South Africa

NICD Researchers: *M Muvhali, AM Smith and KH Keddy*

Multiple-locus variable number tandem repeat analysis (MLVA) was used to subtype *Salmonella* Enteritidis isolates for the years 2013–2015 from Gauteng and Western Cape. One thousand two-hundred and twenty-one isolates were subtyped. MLVA profile numbers were created based on the different MLVA patterns obtained from subtyping. Three MLVA profiles predominated and accounted for 73% of all isolates. The largest MLVA profile (profile number 28) accounted for 54% of all isolates and was the most prevalent amongst Gauteng isolates (519/661; 79%). The second largest profile (profile number 7) (11%) was predominant amongst isolates from the Western Cape (87/135; 64%). The third most common MLVA profile (profile number 22) accounted for 8% of the total isolates and was predominant in Gauteng (77/101; 76%).

Characterisation of *Campylobacter* isolates from a South African population

NICD researchers: *MS Thobela, AM Smith, KH Keddy*

The molecular epidemiological pattern of *Campylobacter* infections in Southern Africa was studied in the years Jan 2014 – Dec 2015. *Campylobacter* was detected in 801/848 (94%) of the total received isolates. *Campylobacter jejuni* accounted for the majority of reported cases (78%) in contrast to *Campylobacter coli* (11%). Cases of coinfection and *non-C. jejuni/C. coli* species of *Campylobacter* were identified, accounting for 1.9% and 2.7% respectively. There is a preponderance of males (55%) among infected persons. *Campylobacter* infections were highest in children of between 0-4 years of age (33%). Isolates showed low prevalence of resistance to erythromycin 10% and azithromycin 14% and high resistance was seen in ciprofloxacin (53%) and tetracycline (53%).

TEACHING AND TRAINING

Postgraduate level

- CED trained registrars as part of the NICD registrars training course in the identification and epidemiology of enteric bacteria and viruses
- Medical intern scientists were trained as part of NICD training courses in bacteriology and virology disciplines
- Dr Keddy and Prof. Page delivered lectures at the Annual African Vaccinology Course on 11 November 2015.

International guest researchers

- Bidjada Bawimodom (Togo) 11 April–15 June 2015
- Emilia Lyonga (Cameroon) 11 June–6 December 2015
- Jill Falman (USA) 22–23 June 2015

International courses

- NA Page attended the Wellcome Trust Advanced Course in Computational Molecular evolution, Hinxton, UK, 13–24 April 2015
- AM Smith attended the Next Generation Sequencing and Bioinformatics Training Course, Los Alamos National Laboratory, USA, 1–5 June 2015

PROFESSIONAL DEVELOPMENT

- Postgraduate candidates enrolled at CED: 3 PhD, 2 MSc, 1 BSc (Hons)
- Postgraduate candidates graduated from CED: 1 PhD, 1 MPH

HONOURS

- Nicola Page was appointed as an Extraordinary Professor in the Department of Medical Virology, University of Pretoria
- KH Keddy was selected for the ICEID Leaders Programme (International Conference on Emerging Infectious Diseases, Atlanta Georgia, USA 2015

RESEARCH OUTPUT

Publications

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Conference presentations

Type	Number
International congresses	10
National congresses	2
Local congresses e.g. university academic days	7

CENTRE FOR EMERGING AND ZOONOTIC DISEASES

Centre Head: Prof. Janusz Paweska

BACKGROUND

In the era of increasing emergence of zoonotic pathogens, the Centre for Emerging and Zoonotic Diseases (CEZD) provides strategic capacity for laboratory investigation of outbreaks associated with high consequence zoonotic pathogens. This includes a range of bacterial and viral microbes; continual improvement of the NICD's capability to diagnose and investigate emerging and zoonotic diseases; and surveillance of selected zoonotic pathogens in South Africa, especially those causing viral haemorrhagic fevers.

Infections caused by high consequence zoonotic pathogens are often fatal, and mostly untreatable, e.g. Ebola virus disease (EVD). Why "high consequence"? Outbreaks of viral haemorrhagic fevers tend to be geographically confined and affect a relatively lower number of people compared to more common infections (less than 30 000 EVD cases were reported during the 2013–2016 EVD outbreaks in West Africa, while more than 20 million cases of measles or up to half a million flu-related deaths occur per year). However, the concomitant acute public health response required to contain an EVD outbreak is usually inversely proportional to the size of the outbreak. The World Bank ascribed losses of over US$2.2 billion to the EVD outbreak in Guinea, Liberia and Sierra Leone for 2015. Whilst billions were spent by countries around the globe to keep the EVD outbreak at bay, another US$162 billion is being invested by the World Bank for recovery efforts in the wake of the outbreak. Thousands of healthcare, laboratory and relief workers, and other professionals from countries around the world, descended on the towns and cities of Guinea, Liberia and Sierra Leone in a struggle of more than two years to contain the EVD epidemic.

One of the major lessons learned for the Ebola West African epidemic was that countries with weak health systems cannot withstand the sudden emergence of deadly pathogens. Under the weight of the EVD epidemic, health systems in Guinea, Liberia and Sierra Leone collapsed. People stopped receiving, or stopped seeking, healthcare for other more common disease conditions that cause more deaths annually than EVD. The severity of EVD, compounded by fear both within and outside the affected countries, caused schools, universities, markets, businesses, airline and shipping routes, and borders to close, further deepening the setback to struggling economies. What began as a health crisis snowballed into a humanitarian, social, economic and security crisis. The Ebola crisis underscored a point often made by the World Health Organization: "fair and inclusive health systems are the bedrock of social stability, resilience and economic health".

Another example is the emergence of the mosquito-transmitted Zika virus in the Americas. More than 23 South American countries have been reporting locally acquired Zika virus disease cases since 2014 with hundreds-of-thousands of suspected cases. Again, the economic impact on the affected countries is estimated to be in the range of billions of US dollars, ascribed to loss of tourism, productivity and foreign investment. The social toll of the outbreak will only be fully understood in the years to come since the virus is associated with birth malformities and microcephaly.

SURVEILLANCE AND DIAGNOSTIC SERVICES

The laboratories of CEZD provide comprehensive capacity for the diagnosis and research of viral haemorrhagic fevers, arthropod-borne diseases, human rabies, anthrax, plague, leptospirosis and others. A range of these diagnostic tests are accredited by SANAS to the ISO 15189 standard, whilst the international recognition of the centre is founded in its status as World Health Organization (WHO) Reference Centre for Plague and as a WHO Collaborating Centre for Reference and Research on Viral Haemorrhagic Fevers and Arboviruses.

The resources available to the centre include biosafety level 3 facilities and the only positive pressure facility, biosafety level 4 facility on the African continent. These facilities represent a strategic resource for the investigation, handling and storage of the most deadly pathogens known to science. The centre manages research and diagnostic laboratories to enable molecular and serological testing as well as culturing of these pathogens.

Early in 2016, the CEZD identified the yellow fever outbreak in Angola, which emerged after an apparent quiescence of 36 years in that country, and resulted in one of the biggest outbreaks of yellow fever reported in the past ten years. With the identification of the outbreak, rapid and intense public health intervention ensued, including vaccination of hundreds of thousands of Angolan citizens.

In February 2016, the WHO declared a Public Health Emergency of International Concern in response to the increase in cases of microcephaly and neurological disorders reported in Brazil, associated with the sharp increase in the number of Zika virus disease cases in the country. The outbreak of Zika virus has spread to 23 countries in Latin America and it is feared that travellers may return to South Africa with the disease. The CEZD has established a repertoire of testing to investigate Zika cases, including molecular and serological assays.

Through its routine diagnostic service the CEZD contributes valuable surveillances data on the occurrence of zoonotic diseases to the National Department of Health and other stakeholders. In addition to routine diagnostic services, the CEZD investigates patients presenting with febrile diseases of unknown aetiology as part of the GERM-SA surveillance programme. These patients reside and work at the so-called human-wildlife-livestock interface and a number of zoonotic microbes are considered to be the cause of their illnesses.

Apart from surveillance involving human subjects, the CEZD conducts surveillance for plague in susceptible rodent populations in the City of Johannesburg and the Nelson Mandela Bay Municipality (Coega area) in order to alert public health authorities to the possibility of increased human plague risk. More than 980 rodents were tested during the period of which one, trapped in Mayibuye (CoJ Region A), tested positive for plague antibodies. Surveillance was increased in this area and flea/rodent control activities were undertaken.

The CEZD operates an Electron Microscope Laboratory (EML) which continues to function as a core facility for transmission electron microscopy services to the NICD, with the bulk of the >500 ultrastructurally characterised isolates being the newly described Mahlapitsi virus, *Neisseria meningitidis* and *Streptococcus pneumoniae* (Fig 1. a, b, c). The EML provided valuable diagnostic screening of unknown pathogens, and confirmatory identification of mycoplasma infections and selected viruses was performed (Fig 1. d, e, f).

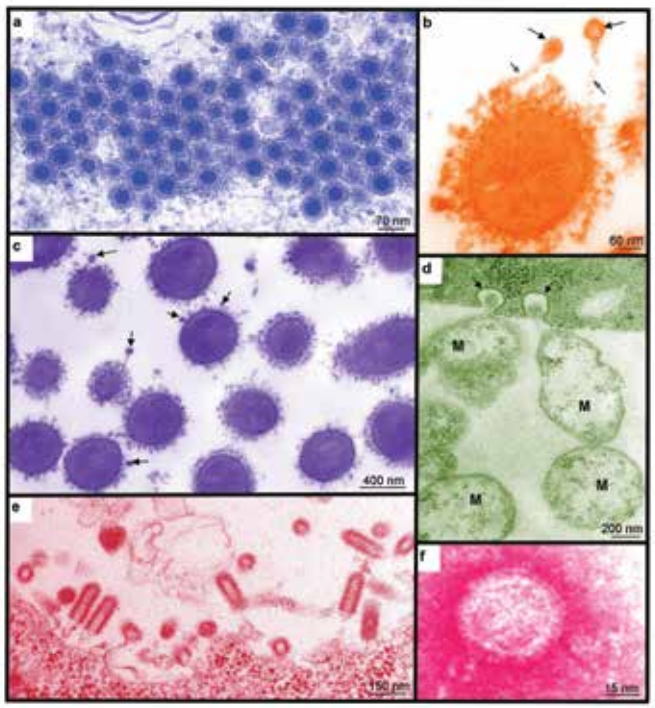

Figure 1: Artificially coloured transmission electron micrographs of: (a) Crystalline cytoplasmic inclusion body in Vero 6 cell infected with Mahlapitsi virus (*Orthoreovirus*); (b) Two Siphoviridae phages (nucleocapsid heads - solid arrows, siphonous tails penetrating outer cell layer – open arrows) infecting a *Streptococcus pneumoniae* cell; (c) Invasive *Streptococcus pneumoniae* isolate with cells fringed with fimbriae and associated extracellular polysaccharides, apparently as a result of a bacteriophage (arrows) infection; (d) Mycoplasma (M) infection of Hep-2 cells (arrows indicate point of entry through plasmalemma); (e) Budding of bullet-shaped, enveloped Rhabdoviridae virions from infected Vero cells; (f) Negatively-stained *Flavivirus* virion, the smallest enveloped RNA virus. The genus includes Zika virus, Yellow Fever virus, Dengue virus, West Nile virus and the mammalian tick-borne virus group, among others.

RESEARCH PROJECTS

Rift Valley fever during the inter-epidemic period

NICD investigators: ***Prof. JT Paweska**, Mrs V Dermaux-Msimang, Mr A Kemp, Dr P Jansen van Vuren, Dr J Rossouw*

Collaborators: *Dr Billy Karesh, Dr Melinda Rostal (EcoHealth Alliance)*

Funding source: *EcoHealth Alliance*

Outbreaks of Rift Valley Fever (RVF) are associated with persistent high rainfalls leading to massive flooding and the resultant emergence of large numbers of mosquito vectors transmitting the RVF virus (RVFV) to susceptible vertebrate species. Outbreaks of RVF have devastating economic effects on countries for which animal trade constitutes the main source of national revenue. The propensity of the virus to spread into new territories and re-emerge in traditional endemic regions to cause large outbreaks in human and animal populations, presents a formidable challenge for public and veterinary health authorities. South Africa has experienced multiple RVF outbreaks, with the three major outbreaks reported in 1950–1951, 1974–1976 and 2010–2011. A large-scale, long-term, and multidisciplinary project has been undertaken on RVF in South Africa to investigate the epidemiology and ecology of the disease, in order to better understand the maintenance of the RVFV during inter-epidemic periods, as well as the abiotic and biological factors leading to re-emergence of the RVF outbreaks. Some of the specific objectives of the project include investigation of the effects of climate and weather; links between vegetation and soil type on RVFV vector mosquito species' maintenance and breeding habitats; determination of the seroprevalence of RVFV antibodies in farm, abattoir workers and veterinarians; as well as understanding the dynamics of the immune status in domestic and wild ruminants. Data generated by this project will assist in the development of a predictive model for RVF epidemics and aid cost-effective vaccination of animals against RVF.

This is a One Health collaborative project between US and SA medical and veterinary governmental, academic, research and private institutions, including the National Institute of Communicable Diseases and EcoHealth Alliance, Free State Department of Economic Development, Tourism & Environmental Affairs, University Space Research Association, NASA, South African National Parks, University of Pretoria, the University of the Free State, ExecuVet veterinary consultants and RSA Department of Defence. The project is funded by the Defence Threat Reduction Agency (DTRA).

Figure 2: CEZD epidemiologist, Veerle Dermaux-Msimang interviewing a farm worker in the Free State Province.

Evaluation of the Egyptian Fruit Bat as a reservoir host for Ebola virus

NICD investigators: ***Prof. JT Paweska****, Ms N Storm, Mrs AA Grobbelaar, Mr A Kemp, Dr P Jansen van Vuren*

Collaborators: *Professor W Markotter (University of Pretoria)*

Funding source: *Poliomyelitis Research Foundation*

Bats are thought to play a role as reservoir hosts for ebola viruses based on detection of antibodies and viral RNA in certain species. The Egyptian Fruit Bat is regarded as a reservoir host of the related Marburg virus and serological evidence points to this bat's involvement in Ebola virus ecology. To evaluate the Egyptian Fruit Bat's role in Ebola virus transmission, an experimental infection study was conducted. The results showed, contrary to findings in a similar study with Marburg virus, that Ebola virus did not replicate in 16 various tissues tested, but bats developed a relatively strong immune response following inoculation. The lack of virus replication following experimental inoculation indicates that the Egyptian Fruit Bat does not play a role as a reservoir host for Ebola virus. This is further supported by the lack of virus transmission from inoculated bats to in-contact control bats. Up to now there is no conclusive evidence as to the wildlife reservoir host for Ebola virus, and the findings from this study serve as caution that serological data alone cannot be used to infer reservoir status in certain bat species, but merely indicate that they might share an ecological niche with the true reservoir host.

Figure 3: Prof. Janusz Paweska and Mr Justice Kgatitsoe sampling an Egyptian Fruit Bat during the Ebola-reservoir study conducted in the maximum security facility of the NICD.

Evaluation of an Ebola point-of-care diagnostic assay

NICD investigators: *Dr P Jansen van Vuren, Mrs AA Grobbelaar, Ms N Storm,* ***Prof. JT Paweska***

Collaborators: *Prof. Ian Sanne (Clinical HIV Research Unit, Wits Health Consortium); Mr Ousman Conteh, Mr Kelfala Konneh and Dr Abdul Kamara (Ministry of Health and Sanitation, Sierra Leone)*

Funding source: *The study was supported by Cepheid Inc.*

The unprecedented outbreak of Ebola virus disease (EVD) in West Africa, from 2013 to 2016, has highlighted the need for improved rapid diagnostic assays. Timely laboratory testing of suspected viral haemorrhagic fever cases is critical for patient management, reducing risk of infection, and for limiting virus spread. In this project a point-of-care molecular assay, based on the Ebola GeneXpert® platform, was evaluated using clinical material from suspected EVD cases and compared to established molecular assays and virus isolation. The assay was shown to be as sensitive and specific as currently accepted assays, but with the advantage of shortened turnaround time and automation. The Ebola assay on the GeneXpert® platform is easy to operate and could potentially be deployed within Ebola treatment centres in future to enable rapid generation of laboratory screening results, to be interpreted along with patient clinical and exposure history, and confirmed in a reference laboratory.

Figure 4: CEZD Scientist, Antoinette Grobbelaar performing the Ebola GeneXpert® assay on samples from suspected Ebola virus patients from Sierra Leone.

Ebola diagnostics in Sierra Leone

Provision of rapid and more widely accessible diagnostic capacity in West African countries affected by the EVD epidemic was one of the priorities to combat the Ebola crisis. A bottleneck in rapid testing for Ebola virus infection left patients stranded in Ebola holding centres for days and thus contributed to raising fears about seeking treatment. Rapid and accurate laboratory confirmation of EVD suspected cases was paramount in the control of the EVD epidemic and in minimising its further geographic spread. In response to the public health emergency caused by the EVD outbreak in West Africa, under the leadership of CEZD, the NICD established Ebola mobile laboratory (EML) diagnostic capacity in Freetown in the second half of August 2014 as a part of the WHO-Global Outbreak Alert and Response Network to the Ebola epidemic in West Africa. The western urban area of Sierra Leone, where the NICD teams worked, remained a hotspot of EVD epidemic for months. During the EVD crisis in the capital of Sierra Leone, for weeks the NICD EML was the only Ebola diagnostic capacity to respond to overwhelming and increasing demand for EVD diagnosis. From the beginning of the EML operation in Freetown, NICD teams undertook training of Sierra Leonean scientists and technical personnel in the operational logistics of the facility, biosafety and diagnostic procedures. This effort culminated in the successful handover of the EML to the Sierra Leonean Ministry of Health and Sanitation on 24 March 2015. The EML remains operational and now plays an important role in the WHO-recommended enhanced surveillance of Ebola cases post-Ebola outbreak. As of 17 April 2016 the NICD-established EML has tested 9837 clinical specimens (blood and buccal swabs) from suspected EVD cases.

Figure 5: Prof. Janusz Paweska training a Sierra Leonean scientist in extraction of Ebola virus RNA from clinical specimens using MagMax Express 96 automated system (AB Applied Biosystems) donated to NICD EML by CDC, Atlanta. Sierra Leone, Free Town, March 2015.

TEACHING AND TRAINING

In addition to extensive training of staff and national and international research fellows in laboratory techniques, and introducing them to working in BSL3 and BSL4 biocontainment facilities and special laboratory techniques such as electron microscopy, the CEZD was actively involved in supporting post-graduate studies in the fields of medical microbiology, medical virology and public health through collaborative projects with South African and international universities. The CEZD is also involved in the training of microbiology and clinical pathology registrars, intern scientists and technologists on an ongoing basis. The CEZD co-ordinates a number of formal training programmes and is often requested to co-ordinate specialist diagnostic workshops. The CEZD also provides routine training in support of its plague surveillance programme. This includes training of environmental health officers from the City of Johannesburg (Gauteng Province) on the dissection and storage of rodent organs for plague surveillance purposes.

In February 2016, CEZD hosted a workshop on packaging and shipping of class 6.2 dangerous goods in collaboration with the African Centre for Integrated Laboratory Training (ACILT).

PROFESSIONAL DEVELOPMENT

CEZD staff members are intricately involved in the postgraduate training of students in the field of virology and other related fields. During the reporting period, a total of 15 postgraduate students were supervised or co-supervised by CEZD staff members. These students were enrolled for programmes at the Universities of Pretoria and Witwatersrand and the Sokoine University in Tanzania. From this cohort, two students graduated with PhDs and another two students with MSc degrees. Two post-doctoral fellows are also supported in their research through the Southern African Centre for Infectious Disease Surveillance (SACIDS). In addition, CEZD staff members are also completing further qualifications. Dr Jacqueline Weyer enrolled for a Master's in Public Health at the Sefako Makgatho Health Sciences University in January 2016. Mrs Naazneen Moolla is registered at the University of Witwatersrand in the PhD programme.

HONOURS AND AWARDS

Dr Jacqueline Weyer was elected as the President of the South African Biorisk Association, whilst Dr Jennifer Rossouw was appointed as Board Member and Mrs Anastasia Trataris-Rebisz as Treasurer of the Association. Drs Jacqueline Weyer and Jennifer Rossouw were nominated as members of the newly established Institutional Biosafety and Biosecurity Committee of the NICD. Mrs Anastasia Trataris-Rebisz was elected as the regional, SADC representative council member for the African Biological Safety Association. In addition, Prof. Janusz Paweska was appointed as a member of WHO Global Outbreak Alert and Response Steering Committee and as a member of WHO Ebola Virus Persistence Study Independent Data Monitoring Committee.

RESEARCH OUTPUT

Journal articles

1. Beechler BR, Bengis R, Swanepoel R, **Paweska JT**, Kemp A, **Jansen van Vuren P**, Joubert J, Ezenva VO, Jolles AE. Rift Valley fever in Kruger National Park: Do buffalo play a role in the inter-epidemic circulation of virus? *Transboundary and Emerging Diseases* 2015; **62**(1): 24–32.

2. Dietz P, Jambai A, **Paweska JT**, Yoti Z, Ksiazek TG. Epidemiology and risk factors for Ebola virus infection in Sierra Leone, May 23, 2014 - January 31, 2015. *Clinical Infectious Diseases* 2015. **61**(11): 1648–54.

3. Dietrich M, Tjale MA, **Weyer J**, Kearney T, Seamark EC, Nel LH, Monadjem A, Markotter W. Diversity of Bartonella and Rickettsia spp. in Bats and Their Blood-Feeding Ectoparasites from South Africa and Swaziland. *PloS One* 2015; **11**(3): p.e0152077

4. **Jansen van Vuren P, Grobbelaar A, Storm N**, Conteh O, Konneh K, Kamara A, Sanne I, **Paweska JT**. Comparative evaluation of the prototype Cepheid GeneXpert® Ebola Assay diagnostic performance. *J. Clin. Microb.* 2016; **54**(2): 350–367.

5. **Jansen van Vuren P**, Shalekoff S, **Grobbelaar AA**, Archer BN, Thomas J, Tiemessen CT, **Paweska JT**. Serum levels of inflammatory cytokines in Rift Valley fever patients are indicative of severe disease. *Virology Journal*, 2015; **12**: 159.

6. **Jansen van Vuren P, Weyer J, Kemp A, Dermaux-Msimang V,** McCarthy K, Blumberg L, **Paweska J**. Is South Africa at risk for Zika virus disease? *SAMJ* 2016; **106**(3):232–233.

7. **Jansen van Vuren P**, Wiley MR, Palacios GF, **Storm N,** McCulloch SD, Markotter W, **Birkhead M**, Kemp A, **Paweska JT**. Isolation of a novel fusogenic orthoreovirus from Eucampsipoda africana bat flies in South Africa. *Viruses* 2016; **8**(3): 1–25.

8. Jivan R, Damelin LH, **Birkhead M**, Rousseau AL, Veale RB, Mavri-Damelin D. Disulfiram/copper-disulfiram damages multiple protein degradation and turnover pathways and cytotoxicity is enhanced by metformin in oesophageal squamous cell carcinoma cell lines. *J Cell Biochem*. 2015; **116**(10): 2334–43.

9. Jori F, Alexander K A, Mokopasetso M, Munstermann S, Moagabo K, **Paweska JT**. Serological evidence of Rift Valley fever virus circulation in domestic cattle and African buffalo in Northern Botswana (2010-2011). *Frontiers in Veterinary Science* 2015; **2**: 63

10. Massangaie M, Pinto G, Padama F, Chambe G, da Silva M, Mate I, Chirindza C, Ali S, Agostinho S, Chilaule D, **Weyer J**, **le Roux C**, Abilio AP, Baltazar C, Doyle T, Cliff J, **Paweska J**, Samo GE. Clinical and epidemiological characterisation of the first recognised outbreak of dengue virus-type 2 in Mozambique, 2014. *Am J Trop Med Hyg*. 2016; **94**(2): 413–416.

11. Mortlock M, IV Kuzmin, **Weyer J**, Gilbert AT, Agwanda B, Rupprecht CE, Nel LH, Kearney T, Malekani JM, Markotter W. Novel paramyxoviruses in bats from sub-Saharan Africa, 2007-2012. *EID* 2015; **21**(10): 1840–1843.

12. Munhenga G, Brooke BD, Gilles JRL, Slabbert K, **Kemp A**, Dandalo LC, Wood OR, Lobb LN, Govender D, Renke M, Koekemoer LL. Mating competitiveness of sterile genetic sexing strain males (GAMA) under laboratory and semi-field conditions: Steps towards the use of the Sterile Insect Technique to control the major malaria vector *Anopheles arabiensis* in South Africa. *Parasit. Vect.* 2016; **9**(2): 1–12.

13. **Paweska JT, Storm N, Grobbelaar AA**, Markotter W, **Kemp A**, **Jansen van Vuren P**. Experimental inoculation of Egyptian Fruit Bats (*Rousettus aegyptiacus*) with Ebola virus. *Virology* 2016; **8**(2): 1–11.

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15. Sindato C, Pfeiffer DU, Karimuribo ED, Mboera LEG, Rweyemamu MM, **Paweska JT**. A spatial analysis of Rift Valley fever virus seropositivity in domestic ruminants in Tanzania. *PLoS ONE* 2015; **10**(7): e0131873.

16. Urata S, **Weyer J**, **Storm N**, Miyazaki Y, **Jansen van Vuren P, Paweska JT**, Yasuda J. Analysis of assembly and budding of Lujo virus. *Journal of Virology* 2015; **90**(6): 3257¬61.

17. **Weyer J**. Rabies in South Africa: Where do we stand in 2015? *SAJID* 2015; **30**(2): 40-41.

18. **Weyer J**, **Msimang Dermaux V, Paweska JT,** le Roux JT, Govender P, Coertse J, Markotter W, Nel LH, Blumberg LH. A case of human survival of rabies, South Africa. *SAJID* 2015; **1**: 1-3.

Chapters in books

1. **Paweska JT, Jansen van Vuren P**. (2015). Crimean-Congo haemorrhagic fever. OIE Manual of Standards for Diagnostic Tests and Vaccines, 2015.

2. **Paweska JT**. Rift Valley fever. *In*: Zientara S, Verwoerd D, Pastoret P-P, (eds). *New developments in major vector-borne diseases – Part II: Important diseases for veterinarians.* Rev. Sci. Tech. Off. Int. Epiz., 2015; 34(2): 375-389.

Conference presentations

Type	Number
International	7
National	14

CENTRE FOR HIV AND SEXUALLY TRANSMITTED INFECTIONS

Centre Head: Prof. Adrian Puren

Centre Co-Head: Prof. Lynn Morris

Centre Co-Head: Prof. Caroline Tiemessen

Centre Co-Head: Dr Ranmini Kularatne

BACKGROUND

UNAIDS has set three treatment targets to be achieved by 2020 to eradicate HIV by 2030 as follows: (1) 90% of HIV infected individuals must know their status; (2) 90% of the HIV diagnosed individuals are on treatment; and (3) 90% of those on treatment must be virally suppressed. If these three targets, also known as the "90:90:90" targets, are achieved, 73% of those on treatment will be virally suppressed.

The National Department of Health (DoH) has adopted the three "90s" targets and, while the focus of the targets is on treatment, there are also benefits for prevention. In 2015 the centre supported the DoH through efforts ranging from surveillance to quality assurance.

It is critical to assess the effectiveness of the programme in terms of treatment cascade. To this end, the DoH, through the World Bank, commissioned a prospective impact evaluation of antiretroviral therapy (ART) adherence and retention programmes in South Africa. The centre collaborated with the World Bank, HE^{2}RO and the NHLS to evaluate the determinants of CD4 recovery. The exercise demonstrated the utility of "Big Data" to inform policy, using the NHLS Corporate Data Warehouse (CDW).

"At birth" HIV testing in infants was introduced and the centre focused on programmatic implementation and monitoring, using the CDW. The accuracy of testing is critical for the first "90" target and through a PEPFAR grant, the centre, in collaboration with the DoH, provincial DoH, Centres for Disease Control and Prevention (CDC) and USAID implementing partners, implemented an extensive rapid testing quality improvement initiative. More than 3 million patients are on the treatment programme and thus drug resistance monitoring is crucial.

The centre expanded its integrated HIV and TB drug resistance programme using the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) facility-based platform. Sexually transmitted infection (STI) surveillance focused more broadly on aetiological surveillance and antimicrobial drug resistance. The centre supported, through various collaborations or laboratory-based activities, various HIV and STI surveillance studies in high-risk populations including young women, sex workers and men who have sex with men (MSM). Human papillomavirus (HPV) surveillance highlights the high-risk HPV genotypes found in young women and the need for the vaccine.

The centre conducted wide-ranging research that focused on HIV pathogenesis in search of a cure, vaccine-related research, and microbicides. The studies on anti-HIV broadly neutralising antibodies yielded finer details that will inform vaccine development. The centre has invested in the development of appropriate technologies to better answer surveillance questions and diagnostic challenges. These activities have yielded an array of peer-reviewed publications and participation in various national and international conferences and meetings. Integral to activities are several national and international collaborations and funding that have been sustained and expanded. In preparing for the future, extensive training for various professional staff continued, as well as postgraduate mentoring and technical assistance at both a national and regional level.

SURVEILLANCE AND MONITORING

HIV surveillance

The major emphasis for the centre in terms of HIV surveillance was a collaborative project with the World Bank, HE^{2}RO and NHLS as part of an impact evaluation of antiretroviral therapy (ART) adherence and retention programmes in South Africa. The focus was secondary analyses of available viral load data and CD4 count data available in the Corporate Data Warehouse (CDW) of the NHLS. Routinely collected CD4 count and viral load laboratory data that is stored in the CDW were linked to unique individuals using a probabilistic matching algorithm. A cohort of individuals who met eligibility criteria was identified and included in the analysis. The proportions of individuals 15 years or older who initiated ART between 2010 and 2014 and achieved CD4 count recovery to 200, 350 and 500 cells/µl, their time to CD4 count recovery, and extent of recovery in the first 12 months of follow up was studied. The HIV Sero-Molecular Laboratory supported various surveillance activities including co-ordination with NHLS laboratories of the 35th Annual Antenatal HIV Prevalence and Incidence Survey. The first HIV incidence at a district/sub-district level using the HIV Incidence Provincial Surveillance System (HIPPS) was concluded and the second baseline survey is currently under way.

An additional focus for the centre is the collaboration with the Human Sciences Research Council (HSRC) and the University of California, San Francisco (UCSF) in partnership with Anova Health, centred on surveillance in "key populations" including sex workers, truck drivers (Truck Driver and Commercial Sex Worker (KPN3) Study in KZN); men who have sex with men survey (South African Men's Health in Gauteng, Western Cape, Free State, Limpopo and North West provinces and Mpumalanga Men's Study Planning); and the Key Population Implementation Science (KPIS) studies. The KPIS study has, as part of the interventions, a focus on evaluating the use of multiple interventions including the use of multiple Point of Care Testing (POCT) to improve access to care and retention amongst MSM.

Early infant diagnosis (EID) HIV polymerase chain reaction (PCR) reports at facility level were provided monthly to ±200 stakeholders for monitoring prevention of mother-to-child transmission (PMTCT). HIV PCR results for action reports, detailing real time HIV PCR results, were distributed weekly to ±80 stakeholders to track HIV PCR positive children into care. Birth testing study cohorts were established at Rahima Moosa Mother and Child and Kalafong Hospitals in collaboration with Empilweni Research and Service Unit, the Medical Research Council (MRC), Kalafong Paediatrics Department and the DoH. EID HIV POC testing on two different HIV PCR technologies was under evaluation. The use of cell phone technology was investigated to close PMTCT cascade gaps for the elimination of mother to child transmission, in collaboration with the United Nations Children's Emergency Fund (UNICEF).

HIV Drug Resistance

The HIV Drug Resistance (HIVDR) Laboratory is a designated laboratory for national surveillance activities and also serves as a World Health Organization regional HIVDR testing laboratory and quality control reference centre. The laboratory expanded activities to include sequencing of the *Integrase* region for detection of mutations associated with *Integrase* inhibitor resistance, and to develop a 96-well plate system to sequence the protease and reverse-transcriptase regions using next generation sequencing (NGS) technologies. The centre developed an integrated TB-HIV surveillance study building on the GERMS-SA clinic facility-based surveillance platform. Surveillance for TB drug-resistance among persons initiating TB treatment and/or HIV Drug Resistance (HIVDR) surveillance among persons initiating ART in the same clinic was implemented. In each province, a single primary health clinic was selected, based on high TB and HIV caseloads. Thus far 334 specimens have been collected for HIVDR testing; 70 (21%) from Eastern Cape, 64 (19%) from Mpumalanga and 200 (60%) from North West Province. The median age of all enrolled participants was 32 years (IQR 26–40 years), and 71% were female. The median recent CD4 count at time of ART initiation was 257 cells/µl (IQR 160–389 cells/µl). Of the 326 case report forms with available data, prior exposure to ART (as PMTCT and/or previous ART initiation) was reported in 80 (25%) participants. Fourteen of these (17.5%) reported receiving PMTCT and 47 (58.8%) had previously received

standardised combination therapy (cART) for clinical management, whilst 19 (23.7%) participants reported receiving both PMTCT and cART. HIVDR testing was successful in 311 (93.1%) of specimens. Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) class resistance was detected in 18.6% (58/311) of specimens, and dual Nucleoside Reverse Transcriptase Inhibitor (N(t)RTI)/NNRTI drug resistance in 2.6% (8/311). When analysed according to prior ART exposure, HIVDR was present in 37.5% (30/80) of participants with any prior ART vs. 14.2% (35/246) of those with no reported prior ART. The data show that rates of NNRTI resistance are ~15% in patients initiating ART and are higher in patients reinitiating cART. However, the data should be interpreted with caution as the study is at an early stage and analysis is currently based on small sample size.

STI clinical syndromes, aetiological and gonococcal antimicrobial resistance surveillance

Sentinel surveillance of sexually transmitted infection syndrome aetiologies and HPV genotypes among patients attending public health facilities in South Africa (2015–2016)

The Gauteng STI surveillance project, managed in collaboration with the Gauteng Department of Health, continued to collect STI syndrome data from public clinics throughout the review period. The national STI aetiological surveillance, supported through a CDC grant, continued until August 2015. Specimens were collected in the 36 clinics (four per province) across the country. These data have been analysed and a report is being compiled for submission to the DoH and CDC. This analysis, and the resultant trends that will be observed over time, will provide important intelligence data for monitoring interventions to reduce STI/HIV transmission, and will also provide crucial baseline HPV prevalence genotyping data following the introduction of the HPV vaccine into South Africa. The new STI guidelines were introduced in South Africa in 2015, following review of surveillance data, with recommended changes for the treatment of STI syndromes.

Aetiological surveillance of STI syndromes in patients attending public health facilities in South Africa (NICD GERMS-SA)

The syndromic approach to the management of STIs in primary healthcare centres (PHCs) is based on the identification of a group of symptoms and easily recognisable signs associated with a number of well-defined aetiologies. Periodic aetiological surveillance of STI syndromes is critical in validating the existing treatment algorithms. Aetiological surveillance at selected sentinel sites (at least one per province) was carried out under the umbrella of the NICD GERMS-SA facility-based surveillance programme in 2015.

The main objective of this surveillance was to determine the microbial aetiologies of the three major STI syndromes i.e. male urethritis syndrome (MUS), venereal disease – syphilis (VDS) and genital ulceration syndrome (GUS) among adult (>18 years) patients. Secondary objectives were to determine (a) the prevalence of HIV co-infection in patients presenting with STI syndromes; and (b) the antimicrobial susceptibility of Neisseria gonorrhoeae isolates from MUS patients to extended-spectrum cephalosporins (ESCs) (c) the sero-prevalence of HSV-2, infectious hepatitis B and syphilis.

Surveillance sites have been established in the following provinces to date: Eastern Cape (Zwide Clinic); KwaZulu-Natal (Phoenix (mobile) clinic in Durban and Eastboom Clinic in Pietermaritzburg); Mpumalanga (Kabokweni Clinic in Nelspruit and Hluvukani Clinic near Bushbuckridge); North West (Jouberton Clinic); and Gauteng (Alexandra Health Centre). Surveys will be conducted annually in Gauteng and Durban and every two years in South Africa's other eight provinces.

Analysis of data from Gauteng (Alexandra Health Centre) was completed. In 2015, a total of 379 STI patients presenting to the PHC were tested: 169 MUS, 107 VDS and 103 GUS. Among MUS cases, Neisseria gonorrhoeae remained the most common aetiological agent detected (152/169, 89.9%) followed by *Chlamydia trachomatis* (31/169, 18.3%). There was a statistically significant increase in the prevalence of *Neisseria gonorrhoeae* and a relative decrease in the prevalence of *Chlamydia trachomatis* compared to 2014. Most infections (134/169, 79.3%) had a single aetiology.

Among VDS patients, bacterial vaginosis (BV) was the commonest cause (68/107, 63.6%), followed by *Chlamydia trachomatis* (28/107, 26.2%) and *Trichomonas vaginalis* (27/107, 25.2%), respectively. Of the 68 patients with BV, 32 (47%) were co-infected with one or more STI pathogens. A relative increase in the prevalence of *Trichomonas vaginalis* in 2015 was not statistically significant ($p = 0.07$). There were no significant differences in aetiological findings between 2014 and 2015.

The prevalence of genital ulcer disease (GUD) pathogens was as follows: HSV (57/103, 55.3%) and *Treponema pallidum* (4/103, 3.9%). No cases of lymphogranuloma venereum (LGV), chancroid or donovanosis were detected in either 2014 or 2015. Only one patient had mixed ulcer aetiology (herpes simplex virus [HSV] and *Treponema pallidum*) detected by polymerase chain reaction (PCR). An ulcer-derived pathogen was not identified in 42/103 (41%) GUS cases.

HIV seroprevalence rates were as follows: approximately 30% in MUS, 40% in VDS and 55% among GUS cases. HIV co-infection rates in 2015 were not significantly different from those in 2014.

All gonococcal isolates from male urethral discharge specimens demonstrated low extended-spectrum-cephalosporin minimum inhibitory concentrations that were within the susceptible range.

Human papillomavirus surveillance

Sexually transmitted infections (STIs), including human immunodeficiency virus (HIV) infection, continue to be highly prevalent among individuals of reproductive age within South Africa. A total of 241 endo-cervical samples were collected from family planning clinic attendees aged 18–20 years. The samples were tested for human papillomavirus (HPV) to determine the prevalence and genotypes. HPV was detected in 73.9% (178/241) of the specimens. Single HPV infection was detected in 19.9% (48/241) while multiple (2-14) HPV infection was detected in 53.9% (130/241). High-risk (HR)-HPV infection was detected in 52.7% (127/241) women, probable HR-HPV infection in 27.0% (65/241) and LR-HPV infection in 53.5% (129/241). Prevalence of HPV-6 was found to be 7.5%, HPV-11 to be 4.6%, HPV-16 to be 10.8% and HPV-18 to be 7.9%. High prevalence of one or more HPV types found in the bivalent vaccine (HPV-16/18) and quadrivalent vaccine (HPV-6/11/16/18) was observed (18.3% and 25.7% respectively). HPV-16 and HPV-66 were the two most frequently detected HPV types (10.8% and 10.4% respectively). The range of HPV prevalences in all provinces was found to be between 55.6% and 83.3%. The highest HPV prevalence was observed in KwaZulu-Natal (83.3%) and the lowest in Western Cape (55.6%). The high HPV prevalence (73.9%) among young women attending family planning clinics is a concern; in particular HPV-16 prevalence (10.8%) which is associated with the majority of cervical cancer cases. The high prevalence of HPV types found in bivalent and quadrivalent vaccines in South African women demonstrate that this population will greatly benefit from current HPV vaccine that was introduced in South Africa in 2014.

HIV-1 rapid testing quality assurance and post-marketing surveillance of HIV rapid test devices

The Department of Health (DoH) has, since 2010, sustained high levels of HIV rapid testing in its effort to enrol infected individuals into treatment and care. The objective is to test 10 million South Africans per year. It is thus essential to have a national quality assurance programme in place to ensure accuracy of testing. The centre participated in a regional President's Emergency Plan for AIDS Relief (PEPFAR)-initiated training programme on Rapid Testing Quality Improvement Implementation. The training provided the opportunity to build institutional capacity to implement quality assurance and monitoring. Subsequent to the regional training, the centre, in co-ordination with the CDC-Pretoria, DoH, provincial departments of health and implementation partners, undertook a week-long training course for staff that currently implement HIV rapid testing, training and monitoring and evaluation. Ninety-seven staff members from a range of organisations were trained and it is expected that these staff will roll out training in the next financial year.

The centre staff has, in parallel, presented training in the various provinces on quality assurance and has trained 1 578 staff in 819 facilities that form part of the PEPFAR priority-focused facilities. Post marketing surveillance (PMS) continued and 70 batches of the HIV rapid tests were tested through the system established in the centre. In addition, and as part of the surveillance, the use of a serum-based internal quality control (IQC) was rolled out to various facilities in provinces for both government run and implementation partner-supported facilities. The purpose of the IQC is to provide testers with a key control step prior to testing and also an early warning indicator of test-kit performance. In 2015 the laboratory distributed 24 000 panels to the various testing facilities. The participation of HIV rapid testing facilities in proficiency testing (PT) is part of surveillance and currently over 800 facilities have been enrolled in the NLHS PT scheme.

The centre has also contributed to other related HIV rapid testing activities including the formation of a National Technical Working Group for HIV rapid testing under the auspices of the DoH, and the development of HIV Rapid Testing Guidelines that also form part of the national HIV Testing Service Policy. The expansion of this type of testing has become necessary because the HIV rapid tests currently in use do not detect early HIV infection. The centre evaluated various test devices that aim to shorten the serological window period including devices that incorporate the p24 antigen. One potential device that may be suited to self-testing is the Atomo HIV Rapid Test device (Atomo Diagnostics). A first test phase was done in collaboration with the Society for Family Health, using trained testers. A second phase for evaluating self-testing would be the next logical step, based on the results of testing under ideal conditions.

As part of the Technical Working Group nominated by the DoH, a comprehensive strategy for STI management in South Africa will be developed, which includes the use of rapid syphilis point-of-care tests.

Support for HIV vaccine trials

The centre performs validated end-point humoral antibody and molecular HIV assays for the HIV Vaccine Trials Network (HVTN). A major activity of the past year has been the finalisation of the HVTN 097 safety trial. This clinical trial tested the same vaccine regimen that was used in the moderately effective RV144 vaccine trial, conducted in Thailand in 2009. Data showed that the binding and neutralising antibody responses in HVTN 097 were similar to RV144, suggesting that these products were immunogenic in the South African population. This has paved the way for HVTN 100, in which vaccines have been redesigned to target clade C viruses that circulate in South Africa. The NICD commenced immunogenicity testing in HVTN 100 at the end of 2015 and this

will be a major focus for the year ahead. Data from HVTN 100 will be used to make the crucial go/no-go decision that will trigger the large efficacy trial of this vaccine regimen (the HVTN 702 trial). The centre also continued to provide results from validated end-point results for HIV infection. The current protocols for end-point testing include HVTN802, 404, 910, 097, 915 and 100 that used revised testing algorithms in line with current diagnostic approaches.

RESEARCH PROJECTS

The use of dried blood spot specimens for HIV-1 drug resistance genotyping in young children initiating antiretroviral therapy

Collaborators: *Dr A Coovadia (Wits), Dr EJ Abrams (CU), Dr K Technau (Wits), Dr L Kuhn*

Paired plasma and dried blood spots (DBS) from 232 South African HIV-infected children initiating antiretroviral therapy (ART), most of whom had prior exposure to ART for prevention-of-mother-to-child-transmission, were genotyped for drug resistant mutations. Non-nucleoside reverse transcriptase inhibitor mutations were most commonly detected in both specimen types, particularly Y181C/I and K103N/S. Resistance interpretation concordance was achieved in 97% of pairs, with seven children having mutations detected in DBS only. These results validate the preferential use of DBS specimens for HIVD drug resistance genotyping in this patient group.

Genetic changes in HIV-1 gag-protease associated with protease inhibitor-based therapy failure in paediatric patients

Collaborators: *Dr A Coovadia (Wits), Dr L Kuhn (CU), Dr EJ Abrams (CU), Dr R Strehlau (Wits)*

Studies have shown a low frequency of HIV-1 protease drug resistance mutations in patients failing Protease Inhibitor (PI)-based therapy. Recent studies have identified mutations in Gag as an alternate pathway for PI drug resistance in subtype B viruses. Genotyping was done on the Gag and protease genes from 20 HIV-1 subtype C infected paediatric patients failing a PI-based regimen. Major Protease resistance mutations were identified in eight (40%) patients, as well as Gag cleavage site (CS) mutations in nine (45%) patients. Four of these Gag CS mutations occurred in the absence of major Protease mutations at PI failure. Changes in Gag during PI failure therefore warrant further investigation of the Gag gene and its role in PI failure in HIV-1 subtype C infection.

Contribution of Gag and protease to HIV-1 phenotypic drug resistance in paediatric patients failing protease-inhibitor based therapy

Collaborators: *Dr K Sutherland (University College London), Dr CM Parry (Public Health England), Dr PA Cane (Public Health England), Dr A Coovadia (Wits), Dr L Kuhn (CU)*

This study investigated the phenotypic consequences of amino acid changes in Gag and Protease on lopinavir (LPV) and ritonavir (RTV) susceptibility among paediatric patients failing protease inhibitor (PI) therapy. The Gag-Protease from 20 HIV-1 subtype C infected paediatric patients, failing a LPV and/or RTV-based regimen, were phenotyped using a non-replicative *in vitro* assay. Phenotypic resistance or reduced susceptibility to RTV and/or LPV was observed in 10 (50%) patients all of whom had been treated with RTV. In most cases, this was associated with Protease resistance mutations but substitutions at Gag cleavage and non-cleavage sites were also detected. Three patients had reduced drug susceptibilities despite having wild-type Protease. All patients who received LPV exclusively were phenotypically susceptible, supporting the continued use of this drug in paediatric patients. Overall, these data suggest that including both Gag and Protease provides a more comprehensive assessment of the effect of PI-induced amino acid changes on PI phenotypic resistance.

Studies on implementation of HIV birth testing including point of care testing (2014 – 2017)

Collaborators: *Prof. A Coovadia (ESRU, RMMCH), Dr K Technau (ESRU, RMMCH), Prof. L. Kuhn (Columbia University, NY), Dr S Carmona (NHLS), Prof. S Velaphi (CH-Bara), Dr Firdose Nakwe (CH-Bara), Dr Nosisa Sipambo (CH-Bara), Prof. A Goga (MRC),Prof. T Avenant (Kalafong, University of Pretoria), Dr N du Plessis (Kalafong, University of Pretoria)*

Cohorts of HIV-exposed neonates at three academic hospitals, Rahima Moosa Mother and Child, Chris Hani Baragwanath and Kalafong Hospitals, are being studied to: 1. Determine performance of the Cepheid and Alere HIV EID instruments in a clinical setting in comparison to the laboratory-based Roche CAP/CTM Qualitatitve HIV PCR; 2. Assess the implementation of targeted versus universal birth testing of HIV-exposed neonates in terms of the yield of HIV-infected neonates; 3. Document the outcomes of early diagnosis and treatment in neonates; 4. Measure adherence to maternal and neonatal prevention of mother-to-child transmission of HIV (PMTCT) prophylaxis; and 5. Develop a strategy for indeterminate HIV PCR test results to elucidate the final HIV infection status.

Women Initiative in Sexual Health (2013-current): Human Papillomavirus (HPV) section of the Project

Collaborators: *A/Prof. Jo-Ann Passmore (UCT), Lynn Morris (NICD)*

This study investigated cervical HPV prevalence and its association with *Chlamydia trachomatis* (CT), Neisseria gonorrhoeae (NG), Trichomonas vaginalis (TV), Mycoplasma genitalium (MG), herpes simplex virus-2 (HSV-2), bacterial vaginosis (BV) and sexual behaviour in HIV-negative adolescents and young women of Soweto (n=143) and Cape Town (n=148). The overall HPV prevalence was found to be 66.7% (194/291) and did not differ between Cape Town and Soweto women (68.2% 101/148; 65.0%, 93/143 respectively, P=0.649). Overall, the prevalence of HPV-6 was 6.5%, 2.4% for HPV-11, 11.7% for HPV-16 and 7.6% for HPV-18. Women with BV (Nugent >7) were more likely to be HPV positive than women with no BV (OR: 2.8, 95% CI: 1.3-6.8, P=0.014). This was similarly the case in women with intermediate flora (Nugent 4-6; OR: 2.2, 95% CI: 1.3-3.7, P=0.004). HPV infection was not associated with HSV-2, CT, NG, TV or MG infection. An increased number of lifetime sex partners (4-10) was associated with HPV infection (OR: 3.0, 95% CI: 1.2-7.6, P=0.018). The high HPV prevalence in young South African women, particularly HPV types that are targeted by current HPV vaccines, suggests that South African women will greatly benefit from these vaccines.

Investigation of GeneXpert Human Papillomavirus Performance

Collaborators: *Prof. Cynthia Firnhaber (Right to Care), Carla Chibwesha (Right to Care), Timothy Wilkin (Weill Cornell Medical College, New York), Bridgette Goeieman (Right to Care)*

This study investigated the performance of the Cepheid Xpert human papillomavirus (HPV) assay in South African human immunodeficiency virus (HIV)-infected women and compared its performance with that of Hybrid Capture-2 (hc2). Stored cervical specimens from HIV-infected women that had previously been tested using hc2 were tested using Xpert. The overall HR-HPV prevalence was found to be 62.0% (720/1161) using Xpert and 61.2% (711/1161) using hc2. 13.6% (158/1161) were HPV16 positive; 18.8% (218/1161) were HPV18/45; 37.3% (434/1161) were HPV31/33/35/52/58; 12.7% (147/1161) were HPV51/59; and 23.3% (270/1161) were HPV39/68/56/66. Overall agreement with hc2 was 90%; Cohen's kappa was 0.78 (95% CI 0.74-0.82) indicating substantial agreement. Detection of HPV16, HPV18/45, and HPV31/33/35/52/58 were independently associated with cervical intraepithelial neoplasia (CIN)-2+ (P<0.0001 for each); while HPV51/59 and HPV39/68/56/66 were not. Xpert and hc2 were similarly sensitive (88.3% and 91.5%, respectively) and specific (48.4% and 51.0%) for CIN2+ and CIN3 (sensitivity: 95.8% and 97.9%; specificity: 41.4% and 42.8%). Conclusions: Xpert is a promising screening test in HIV-infected women that performs similarly to hc2.

The natural history of oral human papillomavirus (HPV) infection in South African heterosexually active couples

Collaborators: *David Coetzee*

HPV genotyping was performed in cervical, penile and buccal cells of 662 women and men using the Roche linear array HPV genotyping assay. Oral HPV infection was observed in 6.9% (46/662) of study participants. Men had a higher oral HPV prevalence than women (8.8% 29/331; 5.1% 17/331 respectively, P=0.07) however, this is not statistically significant. Among the observed infection 15.2% (7/46) were multiple HPV infection (2-4 HPV types). HPV-72 (2.7%), HPV-55 (1.4%) and HPV-62 (1.1%) were the most prevalent HPV types. Oral HPV prevalence was not found to significantly differ between HIV-positive and HIV-negative women (5.2%, 9/173; 5.1%, 8/158, P=0.956 respectively) and men (11.8%, 12/102; 7.4%, 17/229 P=0.198 respectively). Among those that were oral HPV positive, individual oral-genital HPV concordance was observed in 35.3% (6/17) women and 20.7% (6/29) men and it was not significantly higher between genders (P=0.288). Couples oral-genital HPV concordance was observed in 47.1% (8/17) women and 13.8% (4/29) men and it was significantly higher between genders (P=0.015). Couples oral-oral HPV concordance was observed only in two couples. The high oral-genital concordance in couples suggests that there was oral-genital contact. The increasing rate of oral sex, especially in adolescents, may increase the oral HPV prevalence and lead to increased oral cancer in our communities.

Molecular characterisation and detection of fluoroquinolone and macrolide resistance determinants in Mycoplasma genitalium in Gauteng, South Africa (2007-2014)

Mycoplasma genitalium is responsible for 20-35% of non-chlamydial non-gonococcal urethritis and cervicitis. Isolation of M. genitalium from clinical specimens is difficult and time consuming and diagnosis is mainly achieved by using nucleic acid amplification tests. Due to the difficulties in culturing the organism, the antibiotic susceptibility profiles of clinical strains of M. genitalium are limited, especially in South Africa. Known profiles have shown M. genitalium to be highly susceptible to macrolides but less susceptible to tetracyclines and quinolones. Current treatment guidelines recommend the use of either a 1-week oral course of doxycycline or a 1 g single dose of azithromycin as first-line therapy for uncomplicated M. genitalium infection. Macrolide resistance is caused by mutations in region V of the 23S rRNA gene, while fluoroquinolone resistance is associated with gyrA and parC gene mutations.

To further understand the epidemiological aspects of M. genitalium infection and to determine associations between different M. genitalium types and resistance profiles it is necessary to genetically characterise M. genitalium by generating DNA sequence data profiles. A single-locus typing system, based on single nucleotide polymorphisms (SNPs) in the mgpB gene (locus MG191) encoding the M. genitalium adhesion MgPa, provides adequate discriminatory power for epidemiological studies. The genetic characterisation of M. genitalium strains from South African patients is important for studies of antimicrobial susceptibilities which in turn will allow development of effective treatment regimens and better management of patients with non-gonococcal urethritis. For the current study all M. genitalium positive DNA from Gauteng STI National Microbiological Surveillance (NMS) samples, obtained between 2007 and 2014, will be tested, as well as M. genitalium positive DNA obtained from HIV positive patients previously recruited at an urban HIV outpatient clinic in Johannesburg, South Africa, in 2007. All stored M. genitalium positive DNA from these studies will be tested for specific drug resistance determinants to investigate the possible emergence of macrolide and fluoroquinolone resistance. Additionally, all M. genitalium tested in this study will be characterised using the M. genitalium MG191 typing system.

Prevalence of HPV infection in Men who have Sex with Men (MSM)

Co-investigators: *Dr Kevin Rebe, Dr Tobias Chirwa, Prof. Helen Struthers, Prof. James McIntyre, Prof. David Lewis*

The prevalence of human papillomavirus infection was investigated in an MSM population cohort in Cape Town, South Africa and the behavioural risk factors associated with HPV infection were assessed. MSM were enrolled at the Ivan Toms Centre for Men's Health in Cape Town. A psychosocial and sexual behavioural risk questionnaire was completed for each participant and urine, oro-pharyngeal and anal swabs were collected for HPV testing, using the Linear Array HPV Genotyping Test. Logistic regression analyses were performed to determine sexual risk factors associated with HPV infection at the three anatomical sites. The median age of participants was 32 years (IQR 26-39.8), of which 31.0% were black, 31.5% mixed race/coloured and 35.5% white. The majority of the participants (73.0%) had completed high school, 42% had a tertiary level qualification and 67.5% were employed. HPV genotypes were detected in 72.8%, 11.5% and 15.3% of anal, oro-pharyngeal and urine specimens, respectively. Prevalence of high-risk (HR)-HPV types was 57.6% in anal samples, 7.5% in oro-pharyngeal samples and 7.9% in urine, with HPV-16 being the most common HR-HPV type detected at all sites. HPV-6/11/16/18 were detected in 40.3%, 4.5% and 3.2% of anal, oro-pharyngeal and urine samples, respectively. Multiple HPV types were more common in the anal canal of MSM while single HPV types constituted the majority of HPV infections in the oropharynx and urine. Among the 44.0% of MSM that were HIV positive (88), 91.8% had an anal HPV infection, 81.2% had anal HR-HPV and 85.9% had multiple anal HPV types. Having sex with men only, engaging in group sex in lifetime, being HIV-positive and practising receptive anal intercourse were the only factors independently associated with having any anal HPV infection. Anal HPV infections were common among MSM in Cape Town with the highest HPV burden being among HIV co-infected MSM and those that practiced receptive anal intercourse. Behavioural intervention strategies and the possible rollout of HPV vaccines among all boys are urgently needed to address the high prevalence of HPV and HIV co-infections among MSM in South Africa.

Studies on HIV-1 functional cure – paediatric and adults (2014 – 2018)

Collaborators: *Prof. L Kuhn (Columbia University, NY), Dr A Coovadia (ESRU, RMMCH), Dr K Technau (ESRU), Dr N Martinson (PHRU), Dr D Spencer (Right to Care), Dr P Ive (CHRU), Prof. M Ramsay (SBIMB), Dr P Kiepiela (MRC), M Vermeulen (SANBS)*

Two large collaborative studies in the field of HIV cure have continued, one in adults who effectively control their infection without intervention, and the other a clinical trial in children (started in September 2015) to explore early antiretroviral therapy as a modality to reduce viral reservoir size in HIV-1 infected infants identified at birth, and to determine biomarkers that may be associated with functional cure. Most of the research efforts in the past year have gone into the development of appropriate assays for measuring the HIV-1 subtype C reservoir (several have been developed) and the evaluation and optimisation of multicolour flow cytometry panels that allow for the longitudinal monitoring of various reservoir CD4 T cell subsets as well as CD8 T cell and B cell subsets. Four panels have been finalised and to date these have been applied to 12 trial infants, recruited on treatment within 48 hours of birth, who are at varying stages of follow-up (flow assays are being done at birth, 1-month, 3-months and 18/24-months of age; assays are also done on mothers' samples at delivery). The study being conducting in adults explores natural control of HIV-1 that occurs in rare individuals called elite controllers, and includes other interesting groups of patients that may control their disease progression through different mechanisms. The overall purpose is to identify and recruit such HIV-1 controllers across South Africa (to date samples have been collected from approximately 100 elite controllers), with the aim of identifying viral and host targets that can be developed for functional cure strategies in our populations. The approach includes establishing biosignatures (combinations of host, viral, bacterial, environmental factors) that ultimately will distinguish different clinical phenotypes of HIV-1 control, and the incorporation of unbiased systems biology approaches such as whole genome DNA sequencing, and whole genome transcriptional profiling of mRNA and miRNA. This study will contribute to the discovery of novel factors that play a role in natural control of HIV-1 infection.

Microbicides for HIV and STIs: Alginate microbead-encapsulated silver complexes for selective delivery of broad-spectrum silver-based microbicides

Collaborators: *Dr M Fernandes (Molecular Sciences Institute, School of Chemistry, Wits University)*

This study describes the synthesis of various silver complexes, using ligands saccharin, benzimidazole and 8-hydroxyquinoline, and the assessment of their antimicrobial activity against HIV-1, HSV-2 and *N. gonorrhoea*. Findings showed the following: (i) A silver saccharinate benzimadazole complex (AgSB) exhibited activity against all three organisms and at effective concentrations exhibited low toxicity towards a vaginal cell line, (ii) Acid-stable alginate microbeads rapidly dissolve in seminal fluid stimulant, and (iii) Microbead-encapsulated AgSB, dissolved in seminal fluid stimulant, retained its antimicrobial activity. This ongoing research highlights, for the first time, the potential use of silver compounds encapsulated in alginate microbeads as a novel system for delivery and selective release of broad-spectrum silver-based microbicides within the vaginal milieu during sexual intercourse/after ejaculation.

Virological features associated with the development of broadly neutralising antibodies to HIV-1

Collaborators: *Prof. C Williamson (UCT)*

The development of a preventative HIV-1 vaccine remains a global public health priority. This will likely require the elicitation of broadly neutralising antibodies (bNAbs), able to block infection by diverse viral strains from across the world. Understanding the pathway to neutralisation breadth in HIV-1 infected humans will provide insights into how bNAb lineages arise, a process that probably involves a combination of host and viral factors. This review focuses on the role of viral characteristics and viral evolution in shaping bNAbs during HIV-1 infection, and describes how these findings may be translated into novel vaccine strategies.

HIV broadly neutralising antibody targets

The HIV-1 envelope glycoprotein spike mediates viral entry, and is the sole target of neutralising antibodies. Recent advances in B-cell technologies have dramatically expanded the number of antibodies isolated from HIV-infected donors with broadly neutralising plasma activity. These, together with the first high resolution crystal and cryo-EM structures of a cleaved, pre-fusion HIV-1 trimer, have defined new regions susceptible to neutralisation. Three epitopes in the gp120-gp41 interface were structurally characterised in 2015, highlighting the importance of pre-fusion gp41 as a target. Collectively, the epitopes for broadly neutralising antibodies now reveal a continuum of vulnerability, spanning the length of the HIV-1 envelope trimer. This review provides an update on neutralising antibody targets in the context of the recent HIV-1 envelope trimer structure, describes new antibody isolation technologies, and discusses the implications of these data for HIV-1 prevention and therapy.

New member of the V1V2-directed CAP256-VRC26 lineage that shows increased breadth and exceptional potency

Collaborators: *Dr NA Doria-Rose (VRC), Dr RS Roark (VRC), Dr CA Schramm (CU), Dr J Gorman (VRC), Dr G Chuang (VRC), Dr M Pancera (VRC), Dr EM Cale (VRC), Dr MJ Ernandes (VRC), Dr MK Louder (VRC), Dr M Asokan (VRC), Dr RT Bailer (VRC), Dr A Druz (VRC), Dr IR Franschilla (VRC), Dr NJ Garrett (CAPRISA), Dr M Jarosinski (VRC), Dr RM Lynch (VRC), Dr K McKee (VRC), Dr S O'Dell (VRC) Dr A Pegu (VRC), Dr SD Schmidt (VRC), Dr RP Staupe (VRC), Dr MS Sutton (VRC), Dr K Wang (VRC), Dr BF Haynes (Duke University), Prof. SS Abdool Karim (CAPRISA), Dr L Shapiro (CU), Dr PD Kwong (VRC), Dr JR Mascola (VRC)*

The CAP256-VRC26 lineage of monoclonal antibodies, isolated from an HIV-1 clade C-infected donor that targets a quaternary epitope in the V2 region of the HIV-1 envelope glycoprotein was previously described. This study reports on the isolation and characterisation of 21 new members of the family that displayed greater neutralisation potency than previously published antibodies. One of these, CAP256-VRC26.25, neutralised 57% of diverse clade viral isolates and 70% of clade C isolates with remarkable potency. Interestingly, while all known bNAbs targeting the V1V2 region interact with N160 glycan, the CAP256-VRC26 antibodies showed an inverse correlation of neutralisation potency with dependence on this glycan. Overall, the results describe more potent and broadly neutralising members with potential clinical utility, particularly in areas where clade C is prevalent.

Viral variants that initiate and drive maturation of V1V2-directed HIV-1 broadly neutralising antibodies

Collaborators: *Dr C Anthony (UCT), Dr NA Doria-Rose (VRC), Dr CA Schramm (CU), Dr G Botha (UCT), Dr J Gorman (VRC), Dr NJ Garrett (CAPRISA), Prof. SS Abdool Karim (CAPRISA), Dr L Shapiro (CU), Prof. C Williamson UCT), Dr PD Kwong (VRC), Dr JR Mascola (VRC)*

The elicitation of broadly neutralising antibodies (bNAbs) is likely to be essential for a preventative HIV-1 vaccine, but this has not yet been achieved by immunisation. In contrast, some HIV-1-infected individuals naturally mount bNAb responses during chronic

infection. Recent studies have shown that viral diversification precedes the emergence of bNAbs, but the significance of this observation is unknown. Here the key viral events that drove neutralisation breadth within the CAP256-VRC26 family of monoclonal antibodies were delineated. Firstly, minority viral variants that efficiently engaged the bNAb precursor were identified. Secondly, deep sequencing revealed a pool of diverse epitope variants (immunotypes) that were preferentially neutralised by broader members of the antibody lineage. Thus, early viral escape at key antibody-virus contact sites selects for antibody sublineages that can tolerate these changes, thereby providing a mechanism for the generation of neutralisation breadth within a developing antibody lineage.

Broadly neutralising antibody specificities detected in the genital tract of HIV-1 infected women

Collaborators: *Dr V Ashley (Duke University), Dr D Archary (CAPRISA), Dr NJ Garrett (CAPRISA), Prof. Q Abdool Karim (CAPRISA), Prof. SS Abdool Karim (CAPRISA), Dr N Yates (Duke University), Dr JS Passmore (UCT), Dr GD Tomaras (Duke University)*

This study investigated whether antibodies in the genital tract from HIV-1 infected women share similar epitope specificities and functional profiles to those in blood. Immunoglobin G (IgG) antibodies were isolated from cervicovaginal lavages or Softcups from 13 HIV-infected women in the CAPRISA 002 cohort. Using a binding antibody multiplex assay, these antibodies were shown to react with multiple envelope antigens, including V1V2, gp120, gp140 and gp41. Furthermore, IgG from the genital tract had neutralising activity against both Tier 1 and Tier 2 primary HIV-isolates. Epitope mapping revealed antibodies targeting glycan epitopes in the V2 and V3 of gp120, as well as the membrane proximal region of gp41 that matched specificities in plasma. This suggests that broadly, antibodies transudate from blood to the genital tract. These data provide evidence that induction of systemic HIV-specific bNAbs can lead to antiviral immunity at the portal of entry for HIV infection.

GRANT FUNDING

Funding to support the centre's work was obtained from the following organisations:

- Centers for Disease Control and Prevention (CDC, PEPFAR and Global Disease Detection funds)
- Canadian HIV Vaccine Initiative's CANSSA HIV/AIDS network pilot grant
- Department of Science and Technology/National Research Foundation Chair of HIV Vaccine Translational Research
- Medical Research Council
- National Institutes of Health (NIH)
- National Health Laboratory Service Research Trust
- National Research Foundation Incentive Funding for Rated Researchers
- National Research Foundation Professional Development Programme
- Poliomyelitis Research Foundation.

TEACHING AND TRAINING

The centre contributed to various teaching and training activities. This included specialist registrar training encompassing topics on surveillance of HIV and STIs, antimicrobial drug resistance, diagnostics for HIV and STIs and HIV vaccine developments. Lectures in Medical Microbiology were given to undergraduate medical and dental/pharmacy/nursing students, as well as to postgraduate students enrolled for the Diploma in Tropical Medicine and Hygiene (DTM&H) at the University of the Witwatersrand.

An affiliation exists with the Department of Clinical Microbiology & Infectious Diseases at the University of the Witwatersrand. This includes participation in the registrar training programme and departmental academic activities.

The centre successfully hosted a South African Field Epidemiology and Laboratory Training Programme resident, and provided technical training and support for SADC countries. The latter included implementation of quality assurance for HIV at the National Reference Laboratory in Lesotho and HIV drug resistance training for the National Reference laboratory of Zimbabwe. In addition, training was provided for the NRF (Zimbabwe) for national surveillance activities such as the investigation of STI aetiologies and gonococcal antimicrobial resistance testing. The centre also contributed to the SADC Health Project on the harmonisation of Minimum Standards for National Reference Laboratories.

PROFESSIONAL DEVELOPMENT

Students graduated:	5 PhD, 2 MSc and 2 BSc (Hons) students
Students registered:	7 PhD and 6 MSc students
Postdoctoral fellows:	7

HONOURS AND AWARDS

- Prof. CT Tiemessen: HIV Cure: A formidable challenge **10th James Gear Memorial lecture**, NICD Johannesburg, 16 Nov 2015 – Invited speaker
- Prof. Lynn Morris received the Medical Research Council Gold Scientific Achievement Award in recognition of the excellence of her research. Prof. Morris is listed on the Thompsons Reuters 2015 ISIS list of the 3 000 highest cited researchers in the world.
- Prof. Penny Moore was selected as a the NRF/DST South African Research Chair (SARChI) of Virus-Host Dynamics for Public Health
- Jinal Bhiman received a PhD Fellowship from the L'Oreal UNESCO for Women in Science Programme as well as the 2015 Wits Faculty Research Prize
- Dr A Haeri Mazanderani was awarded a Discovery Foundation PhD Scholarship.

RESEARCH OUTPUT

Journal articles

1. Archary D, Liebenberg LJ, Werner L, Tulsi S, Majola N, Naicker N, Dlamini S, Hope TJ, Samsunder N, Abdool Karim SS, **Morris L**, Passmore JS and Garrett NJ. Randomised cross-sectional study to compare HIV-1 specific antibody and cytokine concentrations in female genital secretions obtained by menstrual cup and cervicovaginal lavage. *PLoS One* 10 (7): e0131906. [Epub ahead of print]

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8. **Damelin LH**, Fernandes MA and **Tiemessen CT**. Alginate microbead-encapsulated silver complexes for the selective delivery of broad-spectrum, silver-based microbicides. *Intl J of Antimicrobial Agents* 2015; **46**: 394–400.

9. Fairlie L, **Sherman GG**, Madevu-Matson C, Black V. Nine-month infant HIV testing in South Africa: Time to act and Implement? *SAMJ S Afr Med J*. 2015; **105**(9): 765–768. DOI:10.7196

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41. **Wibmer CK, Moore PL, Morris L**. HIV Broadly Neutralising Antibody Targets. *Curr Opin HIV and AIDS*. 2015; **10**(3): 135–43

Chapters in books

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Conferences and congresses

Type	Number
International congresses	17
National congresses	41
Local congresses	11

CENTRE FOR OPPORTUNISTIC, TROPICAL AND HOSPITAL INFECTIONS

Centre Lead: A/Prof. John Frean

Centre Lead: A/Prof. Nelesh Govender

Centre Lead: A/Prof. Basil Brooke

Centre Lead: A/Prof. Olga Perovic

BACKGROUND

The surveillance, reference and research activities of the centre include opportunistic infections, particularly those that are related to HIV/AIDS; tropical infections, especially malaria and its vectors; and nosocomial infections, concentrating on antimicrobial resistance, molecular epidemiology and outbreak investigations in the hospital setting.

SURVEILLANCE, DIAGNOSTIC AND REFERENCE SERVICES

In the Antimicrobial Resistance Reference Laboratory (AMRRL), phenotypic and genotypic characterisation of mechanisms of bacterial resistance was aimed at ESKAPE organisms (*Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas* and extended-spectrum beta-lactamase (ESBL)-producing *Enterobacter* and *E. coli)* and especially focused on *Staphylococcus aureus, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* with the aim of completing situational analyses of resistance patterns to all organisms. Once baseline data have been collected, periodic surveillance for ESKAPE pathogens will be performed. A reference service was offered for all multidrug-resistant organisms, such as emerging colistin and linezolid resistance, and carbapenem-resistant *Enterobacteriaceae* (CRE). Other specialised diagnostic services offered by the Parasitology and Mycology Reference Laboratories are in the fields of opportunistic or unusual parasitic and fungal infections. Surveillance functions encompassed national and regional monitoring of cryptococcal meningitis, candidaemia, pneumocystosis (including emerging antifungal-

resistant pathogens), protozoal diarrhoea, and antibiotic-resistant hospital infections. The centre has played a leading role in the national implementation and monitoring of a screen-and-treat intervention for cryptococcal disease, included in South Africa's HIV programme.

The centre provides an identification service for medically-important arthropods for entomologists, medical practitioners and environmental health officers. Malaria vector mosquitoes were routinely identified and insecticide resistance studies carried out by the Vector Control Reference Laboratory for the Mpumalanga and KwaZulu-Natal Provincial Malaria Control Programmes. Malaria parasite infection surveillance has been expanded to support the Department of Health's plans to halt malaria transmission in selected districts in South Africa; going forward, these activities include antimalarial drug resistance studies, submicroscopic and gametocyte infection detection, malaria serology, rapid diagnostic test quality assessment, and the evaluation of novel point of care malaria case management tools. Advice and expertise were provided to the Department of Health (DoH) both at the national and provincial levels, with active participation on the SA Malaria Elimination Committee.

In the field of laboratory quality improvement, the centre has played an active role in reporting on laboratory capacity in the WHO African region for the past 14 years, and has supported African laboratories involved in crucial international malaria vaccine trials. The National Biological Sample Collection maintains characterised bacterial and fungal pathogens of national importance, as a resource for scientists and quality controls for routine laboratory tests.

During the year, the centre was involved in outbreak investigations and responses. These included cases of malaria affecting Gauteng residents without recent travel history, that is, odyssean malaria, which has increased substantially (including some deaths) over previous years. Entomological investigations revealed no evidence of local breeding of vector anophelines. The interrelated issues of antimicrobial resistance and nosocomial infections are growing public health problems. In this regard, the centre led investigation of outbreaks of neonatal candidaemia at a Gauteng hospital, and carbapenem-resistant *Enterobacteriaceae* in private health facilities.

RESEARCH AND SURVEILLANCE PROJECTS

Insecticide resistance in malaria vectors

Collaborators: *Dr Katey Glunt (University of Barcelona, Spain), Dr Andrew Jones (Oxford Brookes University, UK), Dr Michael Osae (University of Ghana & Ghana Atomic Energy Commission), Provincial Malaria Control Programmes of Mpumalanga and KwaZulu-Natal provinces.*

Anopheles arabiensis is a major malaria vector in much of sub-Saharan Africa, including South Africa. Resistance to insecticides in populations of this species is widespread, necessitating ongoing surveillance and research into the mechanisms conferring resistance. Recent investigations affirmed the presence of pyrethroid and DDT resistance previously detected in a population in Mamfene, northern KwaZulu-Natal, South Africa, and also indicated the comparatively recent emergence of resistance to the carbamate insecticide bendiocarb. These data show that special attention and commitment needs to be given to the principles of insecticide resistance management as well as to investigations into alternative control techniques designed to target outdoor-resting *An. arabiensis* in northern KwaZulu-Natal. *Anopheles funestus* is also a major malaria vector in the southern African region. It was demonstrated that long-lasting insecticidal nets do not effectively kill pyrethroid-resistant mosquitoes of this species, showing that alternative control methods are necessary where resistant populations of this species occur. Pyrethroid resistance in the major malaria vector *An. gambiae* increased in frequency at a gold mine in Sadiola District, Mali, during the period 2005–2012. The introduction of organophosphates for malaria vector control in 2013 resulted in a subsequent 70% decrease in malaria incidence there. Resistance to the insecticide dieldrin is widespread in *An. gambiae* and is primarily based on the A296G rdl mutation. Recent research shows that an additional mutation, T345M, is also associated with dieldrin resistance in this species by offsetting the structural impact of A296G. A malaria vector survey in southern Ghana revealed the presence of the three major vector species *An. gambiae, An. coluzzii* and *An. funestus*, all of which contribute significantly to malaria transmission in the region. A high prevalence of insecticide resistance in populations of all three species highlights the need for alternative vector control technologies to complement existing methods.

Malaria vector control and transmission dynamics

Collaborators: *Dr Marit Farenhorst (In2Care, the Netherlands), Prof. LEO Braack (University of Pretoria, South Africa)*

Understanding the biology of malaria vector mosquitoes is critical for disease epidemiology and vector control. Recently, the genomes of 16 *Anopheles* malaria vector species were sequenced. The samples used included material from the *Anopheles* mosquito cultures maintained in the Botha DeMeillon Insectary of the NICD in Johannesburg. In addition, it was demonstrated

that malaria vector mosquitoes prefer to obtain blood meals close to ground level and are therefore most likely to bite people on their lower legs, ankles and feet. Therefore, the development of repellent anklets has the potential to reduce vector-human contact. Given the burgeoning incidence of insecticide resistance in malaria vector mosquito populations, alternative technologies and insecticidal active ingredients for malaria vector control are urgently needed. New technologies and products assessed for their efficacy against insecticide resistant and susceptible malaria vector mosquitoes included a novel method of insecticide application via electrostatic coating and an organic fatty acids mixture (C8910). In these evaluations it was demonstrated that application of electrostatically adhered particles boosts insecticide efficacy even against resistant mosquitoes and that C8910 is equally effective as an adulticide against pyrethroid resistant and susceptible *An. funestus* mosquitoes.

Laboratory-based antimicrobial resistance surveillance for nosocomial bacteria (LARS)

NICD principal investigator: *Olga Perovic*

Collaborators: *Andrew Whitelaw, Adriano Duse, Anwar Hoosen, Catherine Samuel, Colleen Bamford, Mark Nicol, Jeannette Wadula, Preneshni Naicker, Ranmini Kularatne, Sharona Seetharam, Trusha Nana, Norma Bosman, Nontombi Mbelle, Ruth Lekalakala, Halima Dawood, Sumayya Haffejee, Koleka Mlisana, Yacoob Coovadia, K. SweSwe Han, P. Ramjathan, P Bohla*

Laboratory surveillance for antimicrobial resistance (AMR) provides a platform for generation of reliable AMR data from different geographical regions. A limited number of nosocomial bacterial pathogens such as *Staphylococcus aureus, Klebsiella pneumoniae* (2010–2012), and *Pseudomonas aeruginosa* (2014–2015) were chosen to monitor trends in resistance at NHLS sentinel sites over the period. Baseline antimicrobial resistance data on other ESKAPE organisms and tests for genes implicated in emerging resistance will also be conducted in future.

Molecular testing includes an array of assays (real time and conventional PCRs) for the detection of various mechanisms of resistance including antibiotic resistance genes, efflux pumps and porin loss. The presence of the methicillin resistance determinants mecA and mecC were investigated in *Staphylococcus aureus* along with the linezolid resistance gene (*cfr*) and the Panton-Valentine leukocidin toxin. In Gram-negative organisms, the following antibiotic resistance genes were investigated: extended spectrum beta-lactamases (ESBLs) bla_{TEM}, bla_{SHV} and bla_{CTX-M}; carbapenemase-producing genes: bla_{NDM}, bla_{VIM}, bla_{MP}, bla_{OXA-48} & Variants, bla_{KCP} and bla_{GES}; plasmid mediated *ampC* and colistin resistance (*mcr-1*) genes and *VEB-1*. Porin loss (*OprD*) and efflux pumps were also investigated. Molecular characterisation techniques also include SCCmec element and spa typing in *Staphylococcus aureus* and multilocus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE) in various organisms.

Antimicrobial resistance prevalence and transmission between animal feed and humans

NICD investigators: *Ashika Singh-Moodely, Olga Perovic*

Collaborators: *Professor Moritz Van Vuuren; Dr Deryn Petty*

Given that antimicrobial resistance is a major global health concern, and that South Africa has high-density industrial farming of food animals, including cattle, poultry and pigs, the routine use of antibiotics for therapeutic, prophylactic and growth promotion on these farms is worrying as antibiotics in food animals have been linked to increases in clinical resistance in human medicine. There has been little regulation of antibiotics administered to animals, with overlaps in the classes of antibiotics used for farming and human therapy. These animals, animal products, farm workers and the farming environment itself are potential reservoirs for resistance determinants. Antimicrobial resistance has been detected in farms; however, the extent of resistance and spill over in the country remain largely unknown. Hence, the transmission of resistance between animal feed and humans is important and requires investigation. We aim to describe the antibiotic resistance genes present in food animals and livestock workers which result in a reservoir from which spillover may occur into the community and/or hospital environments.

Carbapenemase characterisation in *Klebsiella pneumoniae*

NICD principal investigator: *Olga Perovic*

Collaborators: *Andrew Whitelaw, Adriano Duse, Anwar Hoosen, Catherine Samuel, Colleen Bamford, Mark Nicol, Jeannette Wadula, Preneshni Naicker, Ranmini Kularatne, Sharona Seetharam, Trusha Nana, Norma Bosman, Nontombi Mbelle, Ruth Lekalakala, Halima Dawood, Sumayya Haffejee, Koleka Mlisana, Yacoob Coovadia, K. SweSwe Han, P. Ramjathan, P Bohla*

Carbapenem non-susceptible isolates obtained during sentinel surveillance from four provinces were confirmed at the NICD and further characterisation for carbapenemase genes and plasmid mediated *ampC* were performed.

Enhanced surveillance for hospital versus community associated infections by methicillin-resistant *Staphylococcus aureus*

NICD principal investigator: *Olga Perovic*

Collaborators: *Ranmini Kularatne, Trusha Nana, Ruth Lekalakala, Catherine Samuel, Andrew Whitelaw, Preneshni R Naicker, Colleen Bamford*

The epidemiology of *S. aureus* is changing. *Staphylococcus aureus* is one of the most significant pathogens responsible for causing nosocomial and community-associated infections, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), which has a high prevalence worldwide, as well as a high morbidity and mortality rate. Previously MRSA was considered as a nosocomial pathogen, now it is recovered from patients at admission in hospitals. This community-associated MRSA (CA-MRSA) occurs either from patients that have never been exposed to healthcare settings or from patients that have been exposed to recent hospital admissions or any interventions in healthcare settings. Since the introduction of antimicrobial agents, bacterial antimicrobial resistance has become a global problem, and as the use of antimicrobial drugs increases the complexity of resistance mechanisms demonstrated by bacterial pathogens becomes increasingly important. Due to the increase in antibiotic resistance, the prevalence of hospital- and community-acquired infections is high with a significant number of deaths. As *S. aureus* is a causative agent for a wide variety of infections and diseases, varying from less serious episodes of food poisoning to more serious wound infections and life-threatening conditions such as bacteraemia, necrotizing pneumonia and endocarditis, there is a need for epidemiological characterisation of the organism. MRSA has spread increasingly to the community over the past three decades, becoming endemic in most countries. The aim of this project is to identify virulence factors and evolution patterns of *S. aureus*, in order to determine the prevalence and extent of these nosocomial and community-acquired infections that result in antimicrobial resistance in the South African setting.

Enhanced surveillance for carbapenem-resistant Enterobacteriaceae

NICD principal investigator: *Olga Perovic*

Collaborators: *Colleen Bamford, P Bohla, Norma Bosman, Vindana Chibabhai, Yacoob Coovadia Halima Dawood, Adrian Duse, Sumayya Haffejee, K Swe Swe Han, Anwar Hoosen, Nontombi Mbelle, Yesholata Mahabeer, Motlatji Maloba, Caroline Maluleka, Koleka Mlisana, Preshni Naicker, Maphoshane Nchabeleng, Sibongile Mahlangu, P Ramjathan, Sharona Seetharam, Teena Thomas Jeannette Wadula, Andrew Whitelaw*

The Enterobacteriaceae are part of the commensal human gut flora and are the cause of community and healthcare-associated infections. Over the last decade, Enterobacteriaceae have increasingly developed resistance to all beta-lactam antibiotics, fluoroquinolones and aminoglycosides. Of concern are carbapenem resistant Enterobacteriaceae (CREs) that have become a threat to healthcare and patient safety worldwide. The LARS project aims to gather information about the spread of CPEs in South Africa from sentinel sites at public healthcare facilities.

Pneumocystis jirovecii pneumonia (PCP) in hospitalised patients with severe acute respiratory infections (SARI) using an existing surveillance network in South Africa

Collaborators: *Severe Acute Respiratory Infections (SARI) partnership*

Surveillance is being done for PCP in adults and children at sentinel sites in North West and KwaZulu-Natal provinces. The relative contribution of PCP to the burden of severe acute respiratory infections is being determined together with the Centre for Respiratory Diseases and Meningitis, NICD. Because colonisation, rather than infection, can confound the interpretation of molecular detection of *P. jirovecii*, an attempt is being made to correlate gene copy number with serum β-D-glucan levels and clinical findings in enrolled patients.

Sentinel surveillance for parasitic causes of diarrhoea in hospitalised children

Five sentinel sites provide stool samples from under-five children with diarrhoea, as part of a rotavirus surveillance programme; residual samples are screened for bacterial and parasitic pathogens. Of 3 443 samples received, 12.4% contained pathogenic parasites. The vast majority (>95%) were *Cryptosporidium* species. Genotyping has previously shown that these are predominantly *C. hominis*. This human-specific species is therefore emerging as an important cause of childhood diarrhoea in South Africa. Molecular detection of microsporidial species in these samples has started. These organisms are probably underappreciated as diarrhoeal agents, because they are difficult to detect by conventional laboratory methods.

Malaria parasite infection surveillance

Collaborators: *Provincial Malaria Control Programmes of Limpopo, Mpumalanga, and KwaZulu-Natal provinces; Prof. I Kleinschmidt, London School of Hygiene and Tropical Medicine*

The NICD is re-establishing the country's only molecular antimalarial drug resistance surveillance programme, as well as initiating surveillance for sub-microscopic malaria infections, gametocyte carriage, and plans to undertake malaria sero-prevalence surveys. The rate of false results of malaria rapid diagnostic tests will also be investigated. These efforts are all in support of DoH plans to eliminate transmission of malaria in South Africa by 2018.

Assessing the safety and efficacy of single low-dose primaquine

Collaborators: *Provincial Malaria Control Programmes of Limpopo and Mpumalanga; Prof. KI Barnes, University of Cape Town; Prof. L Braack and Dr H Swanepoel, University of Pretoria*

In 2015 the WHO recommended that single low-dose primaquine be incorporated into standard antimalarial treatment in regions nearing malaria elimination. This recommendation is based on preliminary data which showed that the addition of single low-dose primaquine greatly reduced the number of circulating gametocyctes, the parasite stage associated with onward transmission. Unfortunately, high doses of primaquine are associated with acute heamolysis, particularly in glucose-6-phosphate dehydrogenase deficient individuals. To help inform a single low-dose primaquine policy in South Africa, a primaquine safety, efficacy and tolerability clinical trial will be conducted at selected high malaria burdened clinics in Mpumalanga and Limpopo provinces during the 2016/2017 malaria season.

Implementation of a public health intervention: Cryptococcal antigen screening and pre-emptive treatment

Programme partners: *Department of Health, USAID, CDC, PEPFAR partners, Boston University*

Cryptococcal meningitis, a common AIDS-defining fungal opportunistic infection, has a fatal outcome in >50% of cases in routine care in South Africa. In 2014, 5 772 new cases of laboratory-confirmed cryptococcal disease were detected by the NICD. In randomised clinical trials, cryptococcal antigen (CrAg) screening of HIV-infected persons with a baseline CD4 count <100 cells/µL and pre-emptive antifungal treatment, resulted in a ~30% relative reduction in 6–12 month mortality on ART. Two laboratory-based CrAg screening approaches have been evaluated in South Africa. Reflex laboratory CrAg testing was implemented at three NHLS CD4 laboratories serving 199 healthcare facilities in four districts of Gauteng and Free State; this was paired with intensive health worker training and prospective monitoring and evaluation. From September 2012 through to September 2015, 53 241 patients were screened, 1 971 (3.7%) of whom tested positive for cryptococcal antigenaemia. In parallel, provider-initiated laboratory CrAg screening was implemented at all ART facilities in five Western Cape districts with no specific health worker training. From 2012 through to 2013, of 4 395 eligible patients only 26.6% were screened, 2.1% of whom screened CrAg-positive. In this province, unscreened patients were nearly twice as likely to develop disseminated cryptococcal disease compared to screened patients. Detailed clinical guidance for the screen-and-treat intervention was included in the 2015 national consolidated guidelines for HIV and in the updated standard treatment guidelines/essential medicine lists for primary healthcare and hospital levels. Both screening strategies are included in national guidelines. To inform the decision-making processes within the DoH, a decision analytic model was developed to compare reflex and provider-initiated CrAg screening in terms of programmatic and health outcomes (number screened, number identified for pre-emptive treatment, lives saved, and discounted years of life saved) and screening and treatment costs (2015 USD). A base case was estimated with prevalence and other parameters based on data collected during CrAg screening pilot projects integrated into routine HIV care in Gauteng, Free State, and Western Cape Provinces. Sensitivity analyses were conducted to explore how results change with underlying parameter assumptions. Reflex screening compared to provider-initiated screening saves more lives and is likely to be cost saving or have low additional costs per additional year of life saved. In 2016, the DoH made a decision to implement reflex laboratory CrAg screening across the country and the National Cryptococcal Disease Technical Working Group (co-chaired by the centre) has been tasked to compile an implementation project plan. If properly implemented, this intervention has the potential to directly reduce deaths associated with cryptococcal disease.

Emergence of a cluster of novel dimorphic fungal pathogens in humans

Collaborators: *Prof. C Kenyon (University of Cape Town), Dr Ilan Schwartz (University of Antwerp), Prof. Sybren de Hoog (CBS) and others*

At least seven new species of *Emmonsia*-like fungi, with phylogenetic and clinical similarities to *Blastomyces dermatitidis* and *Histoplasma capsulatum*, have emerged over the last 50 years as causes of systemic human mycoses worldwide. They differ from classical *Emmonsia* species by producing a thermally-dependent yeast-like phase rather than adiaspores, causing disseminated infections, predominantly in immunocompromised patients, and often with high case-fatality rates. Such differences will be important for clinicians to consider in diagnosis and patient management, and for microbiologists who may encounter these fungi with increasing frequency. It remains unclear whether this cluster of *Emmonsia*-like species only emerged recently as human pathogens, or whether previous infections were merely under-appreciated. Support for the latter hypothesis comes from South Africa, where the introduction of molecular identification protocols resulted in a dramatic increase in the number of cases of disseminated *Emmonsia* disease, commensurate with a decline in cases of histoplasmosis. Preliminary phylogenetic analyses suggest that most of the new species of *Emmonsia*-like fungi form a single, derived clade in the Ajellomycetaceae, while agents of adiaspiromycosis (or classic emmonsiosis) appear to be polyphyletic.

Surveillance for candidaemia

Collaborators: *GERMS-SA network*

Patients with candidaemia were identified through active laboratory-based surveillance at sentinel hospitals in seven provinces (except Gauteng and Western Cape where surveillance was conducted in previous years) from January 2014 through to December 2015. In January 2016, surveillance was expanded to public- and private-sector laboratories in all provinces. The objective of this expanded surveillance project is to compare *Candida* species distribution and antifungal resistance with baseline data collected through national surveillance five years ago (2009–2010). An incident case of candidaemia was defined as the isolation of *Candida* species from the first submitted blood culture. Detailed clinical information will continue to be collected including underlying diseases and in-hospital mortality. Identification of isolates and antifungal susceptibility testing will continue to be performed at the centre. The centre has assisted with the investigation of a series of outbreaks of candidaemia at a sentinel hospital. During 2014, 80/118 (68%) cases of candidaemia (all *Candida* species), occurred in the neonatal intensive care unit (NICU) of this sentinel hospital; 48 neonatal cases due to *Candida krusei* were detected in July through October, among 589 admissions in the NICU (attack rate 8.1%). Overlapping collection dates for the first positive specimen suggested a propagated outbreak. Risk factors, which were significantly associated with *C. Krusei* candidaemia, included necrotising enterocolitis (NEC), birth weight <1 500 g and being admitted during the months of July and August. Neonates weighing 1 000 g to 1 500 g at birth were seven times more likely to develop candidaemia than those with BW >2 500 g. At the time of audit, the patient census was 12% above the ward's bed capacity and staff adherence to hand washing protocols was 76%. *C. Krusei* was not isolated from the environment.

RESEARCH FUNDING SOURCES

Carnegie-Wits Alumni Diaspora Programme

Centers for Disease Control and Prevention through NHLS/CDC Co-operative Agreement

Deutscher Akademischer Austausch Dienst

Gates Grand Challenges Explorations

Hillel Friedland Fellowship

Innovative Vector Control Consortium

International Atomic Energy Agency (IAEA)

London School of Hygiene and Tropical Medicine

International Centre of Excellence in Malaria Research - National Institutes of Health (ICEMR – Johns Hopkins Malaria Institute)

Medical Research Council of South Africa

National Health Laboratory Service Research Trust

National Institutes of Health

National Research Foundation (SARChI, NRF Incentive, DST-NRF Centre of Excellence for Invasion Biology, and DST-NRF Research Chair awards)

Pennsylvania Department of Health (Tobacco Settlement Funds)

Research and Policy for Infectious Disease Dynamics (RAPIDD) Programme

Sir Ratanji Dalal Research Scholarship

South African Nuclear Energy Corporation (Necsa)

Stellenbosch University Hope Project

UK-MRC/DFID/Wellcome Trust Joint Global Health Trials Scheme

TEACHING AND TRAINING

Teaching and training in various aspects of bacteriology, parasitology, mycology, medical entomology and communicable diseases was provided to students at postgraduate level (MSc, PhD), medical students, technicians, medical technologists, intern medical scientists, pathology registrars, South African Society of Travel Medicine (SASTM) travel medicine course participants as well as doctors enrolled in a postgraduate Diploma in Tropical Medicine and Hygiene (DTM&H). The centre assisted the DoH with development of laboratory and clinical training materials for the relevant disease programmes.

PROFESSIONAL DEVELOPMENT

Postgraduate students enrolled:	17 (10 MSc, 7 PhD)
Postgraduate students graduated:	6 (3 MSc, 1 MPH, 2 PhD)

HONOURS

Prof. Maureen Coetzee was the winner of the Distinguished Women Researchers (Life Sciences), DST/NRF Women in Science awards for 2015.

Dr Nelesh Govender was appointed as an Associate Professor in the Division of Medical Microbiology at the University of Cape Town.

RESEARCH OUTPUT

Top five publications

1. Vallabhaneni S, Longley N, Smith M, Smith R, Osler M, Kelly N, Cross A, Boulle A, Meintjes G, Govender NP. Evaluation of a public-sector, provider-initiated cryptococcal antigen screening and treatment programme, Western Cape, South Africa. *J cquir Immune Defic Syndr.* 2016; Feb 29 [Epub ahead of print]

   Synopsis: Just over a quarter of eligible patients were screened under the Western Cape provider-initiated cryptococcal antigen (CrAg) screening and treatment programme. Unscreened patients were nearly twice as likely to develop disseminated cryptococcal disease. CrAg screening can reduce the burden of disseminated cryptococcal disease but needs to be implemented well. Countries should consider laboratory reflex CrAg screening, where possible.

2. Govender NP, Maphanga TG, Zulu TG, Patel J, Walaza S, Jacobs C, Ebonwu JI , Ntuli S, Naicker SD, Thomas J. An outbreak of lymphocutaneous sporotrichosis among mine-workers in South Africa. *PLoS Negl Trop Dis.* 2015; **9**(9): e0004096.

   Synopsis: In an outbreak of lymphocutaneous sporotrichosis that was investigated in a South African gold mine in 2011, S. schenckii sensu stricto was identified as the causative pathogen. Although genetically distinct species were isolated from clinical and environmental sources, it is likely that the source was contaminated soil and untreated wood underground. No cases occurred following recommendations to close sections of the mine, treat timber and encourage consistent use of personal protective equipment. Sporotrichosis is a potentially re-emerging disease where traditional, rather than heavily mechanised, mining techniques are used. Surveillance should be instituted at sentinel locations.

3. Moodley K, Govind CN, Peer AKC, van der Westhuizen M, Parbhoo D, Ming Sun L, du Plessis DC, Frean JA. First detection of human dirofilariasis in South Africa. *Infect Dis Rep*. 2015; **7**: 5726 [doi: 10.4081/idr2015.5726]

   Synopsis: Dirofilariasis is a zoonotic condition caused by filarial nematodes that are transmitted by mosquitoes. Humans are accidental hosts, and while the condition is generally benign, its medical importance is related to its mimicking of tumours in the lungs and elsewhere. This is the first report of cases in humans in South Africa, involving masses in the eye and groin respectively of two patients, who acquired the infection locally.

4. Wragge S-E, Toure D, Gilbert A, Christian R, Segoea G, Hunt RH, Coetzee M. Malaria control at a gold mine in Sadiola District, Mali, and impact on transmission over 10 years. *Trans Roy Soc Trop Med Hyg*. 2015; **109**: 755-762.

   Synopsis: Pyrethroid resistance in the major malaria vector *Anopheles gambiae* increased in frequency at a gold mine in Sadiola District, Mali, during the period 2005–2012. Data presented in this manuscript show that the introduction of organophosphates for malaria vector control in 2013 resulted in a subsequent 70% decrease in malaria incidence there, highlighting the importance of evidence-based management of insecticide resistance.

5. Perovic O, Iyaloo S, Lowman W, Kularatne R, Bosman N, Wadula J, Seetharam S, Duse A, Mbelle N, Bamford C, Dawood H, Mahabeer Y, Bhola P, Abrams S and Singh-Moodley A. Prevalence and trends of *Staphylococcus aureus* bacteraemia in hospitalised patients in South Africa, 2010 to 2012: Laboratory-based surveillance mapping of antimicrobial resistance and molecular epidemiology. *PLoS One.* 2015; **10**(12): e0145429. doi:10.1371/journal.pone.0145429.

   Synopsis: An in-depth understanding of recent antimicrobial resistance and molecular epidemiology trends was obtained for *S. aureus* bacteraemia. These data were collected from thirteen academic centres in South Africa for the period June 2010 to July 2012. It was found that the Methicillin-Resistant *Staphylococcus aureus* rate was high in South Africa. The majority of isolates were SCCmec type III and IV with a dominance of spa types 037 and 1257. Monitoring these trends was recommended in order to detect changes in existing patterns.

Other publications

1. Wake R, Sriruttan C, Glencross DK, Harrison TS, Govender NP. Cryptococcalantigen screening in HIV-infected adults - Let's get straight to the point-of-care. *AIDS* 2016; **30**(3): 339–342.

2. Schwartz IS, Kenyon C, Feng P, Govender NP, Dukik K, Sigler L, Jiang Y, Stielow JB, Muñoz JF, Cuomo CA, Botha A, Stchigel AM, de Hoog S. 50 years of Emmonsia disease in humans: The dramatic emergence of a cluster of novel fungal pathogens. *PLoS Pathogens* 2015; **11**(11): e1005198.

3. Prakash A, Sharma C, Singh A, Singh PK, Kumar A, Hagen F, Govender NP, Colombo AL, Meis JF, Chowdhary A. Evidence of genotypic diversity among Candida auris isolates by multilocus sequence typing, matrix-assisted laser desorption ionisation-time of flight mass spectrometry and amplified fragment length polymorphism. *Clin Microbiol Infect.* 2015; pii: S1198–743X(15)00947-7.

4. Schwartz IS, Govender NP, Corcoran C, Dlamini S, Prozesky H, Burton R, Mendelson M, Taljaard J, Lehloenya R, Calligaro G, Colebunders R, Kenyon C. Clinical Characteristics, diagnosis, management, and outcomes of disseminated emmonsiosis: a retrospective case series. *Clin Infect Dis*. 2015; **61**(6):1004–12.

5. Espinel-Ingroff A, Alvarez-Fernandez M, Cantón E, Carver PL, ChenSC-A, Eschenauer G, Getsinger DL, Gonzalez GM, Govender NP, Grancini A, Hanson KE, Kidd SE, Klinker K, Kubin CJ, Kus JV, Lockhart SR, Meletiadis J, Morris AJ, Pelaez T, Quindós G, Rodriguez-Iglesias M, Sánchez-Reus F, Shoham S, Wengenack NL, BorrellSolé N, Echeverria J, Esperalba J, Gómez G, de la Pedrosa E, GarcíaGarcía I, Linares MJ, Marco F, Merino P, Pemán J, Pérez del Molino L, Roselló Mayans E, Rubio Calvo C, Ruiz Pérez de Pipaon M, Yagüe G, Garcia-Effron G, Guinea J, Perlin DS, Sanguinetti M, Shields R, Turnidge J. A multi-centre study of epidemiological cut-off values and detection of resistance in *Candida* spp. to anidulafungin, caspofungin and micafungin using the Sensititre Yeast One colorimetric method. *Antimicrob Agents Chemother.* 2015; **59**(11): 6725–32. doi: 10.1128/AAC.01250–15. Epub 2015 Aug 17.

6. Du Plessis D, Msimang V, Davidsson L, Cohen C, Govender N, Dawood H, Karstaedt A, Frean J. Laboratory-based surveillance for Pneumocystis pneumonia in South Africa, 2006 through 2010. *South Afr J Infect Dis*. 2016; **1**(1): 1–6.

7. Van Hougenhouck-Tulleken WG, Mathole G, Karstaedt A, Govind N, Moodley M, Seetharam S, Govender NP, Menezes CN. Disseminated fungal infection in an HIV-infected patient due to *Aureobasidium pullulans*. *South Afr J Infect Dis*. 2016; **1**(1): 1–3.

8. Mogoye BK, du Plessis D, Poonsamy B, Frean J. Characterisation of *Pneumocystis jirovecii* DHPS genotypes using a simple, inexpensive restriction fragment length polymorphism analysis. *South Afr J Infect Dis.* 2015; **30**: 46-50.

9. Singh-Moodley A, Ekermans P and Perovic O. Emerging carbapenem-resistant *Enterobacter* cloacae producing OXA-48-, VIM- and IMP-Type-β-lactamases in Eastern Cape hospitals in South Africa. *Open J MedMicrobiol.* 2015; **5**: 246-253. DOI: 10.4236/ojmm.2015.54030.

10. Singh-Moodley A, Marais E and Perovic O. Discrepancies between genotypic and phenotypic identification of methicillin-resistant *Staphylococcus aureus* and absence of *mecC* in surveillance isolates in South Africa. *South Afr J Infect Dis*. 2015; **1**(1):1-3. http://dx.doi.org/10.1080/23120053.2015.1107256

11. Neafsey DE, Waterhouse RM, Abai MR, Aganezov SS, Alekseyev MA, Allen JE, Amon J, Arca B, Arensburger P, Artemov G, Assour LA, Basseri H, Berlin A, Birren BW, Blandin SA, Brockman AI, Burkot TR, Burt A, Chan CS, Chauve C, Chiu JC, Christensen M, Costantini C, Davidson VLM, Deligianni E, Dottorini T, Dritsou V, Gabriel SB, Guelbeogo WM, Hall AB, Han MV, Hlaing T, Hughes DST, Jenkins AM, Jiang X, Jungreis I, Kakani EG, Kamali M, Kemppainen P, Kennedy RC, Kirmitzoglou IK, Koekemoer L, *et al*. Highly evolvable malaria vectors: the genomes of 16 *Anopheles* mosquitoes. *Science*. 2015; **347**(6217): 1258522, 1–8.

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13. Samuel M, Oliver SV, Wood OR, Coetzee M, Brooke BD. Evaluation of the toxicity and repellence of an organic fatty acids mixture (C8910) against insecticide susceptible and resistant strains of the major malaria vector *Anopheles funestus* Giles (Diptera: Culicidae). *Parasit Vectors*. 2015; **8**: 321.

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16. Taylor-Wells J, Brooke BD, Bermudez I, Jones AK. The neonicotinoidimidacloprid, and the pyrethroiddeltamethrin, are antagonists of the insect Rdl GABA receptor. *J Neurochem*. 2015; **135**: 705–713.

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18. Brooke BD, Robertson L, Kaise M, Raswiswi E, Munhenga G, Venter N, Wood O, Koekemoer LL. Insecticide resistance in the malaria vector *Anopheles arabiensis* (Diptera: Culicidae) in Mamfene, KwaZulu-Natal. *S Afr J Sci.* 2015; **111**(11/12): 2015–0261

CONFERENCES

Type	Number
International conferences and meetings	27 (mycology=4; PRL=1; AMRRL=2; VCRL=10)
National conferences	11 (mycology=3; PRL=2; VCRL=6)
Local conferences	9 (VCRL= 1;AMRRL=8)

CENTRE FOR RESPIRATORY DISEASES AND MENINGITIS

Centre Head: Associate Professor Cheryl Cohen

Centre Head: Dr Florette Treurnicht

BACKGROUND

The year under review continued to see the centre perform its core functions of syndromic surveillance for pneumonia and influenza-like illness (ILI) at sentinel sites within South Africa; and laboratory-based surveillance for important bacterial causes of invasive bacterial disease and meningitis throughout the country through the GERMS-SA platform. Using these surveillance programmes the centre monitored and reported on the key seasonal and long-term variations of viral and bacterial pathogens under surveillance. Specifically, this year, the centre published important data documenting the burden of influenza-associated mortality in pregnant women. The burden of pneumonia overall was also described, as well as specific bacterial and viral pathogens in HIV-exposed-uninfected infants, an important risk group, comprising approximately 30% of all infants born in South Africa. These data are important to guide recommendations for interventions to reduce the burden of severe disease and death due to pneumonia and meningitis.

Centre Head: Associate Professor Anne von Gottberg

In addition, during 2015 the centre set up and validated phenotypic and molecular assays for detecting *Corynebacterium diphtheriae* and supported regional laboratories with reference laboratory testing for diphtheria. Work continued to improve awareness about legionellosis and ensure that clinicians and other stakeholders were made aware of the reference diagnostic capacity available at the centre – from routine cultures to sequencing for linking of clinical and environmental isolates. In line with the World Health Organization (WHO) decision to focus on respiratory syncytial virus (RSV) surveillance activities to assess the impact of future vaccines, the centre established capability for RSV subtyping to distinguish infections caused by RSV-A and RSV-B strains by real-time reverse transcription-polymerase chain reaction (RT-PCR). The National Influenza Centre (NIC) within the CRDM has also been identified by WHO as a reference laboratory for RSV in the African Region and a pilot site for assessing WHO's RSV surveillance strategy.

SURVEILLANCE PROGRAMMES

Pneumonia surveillance

The National Pneumonia Surveillance Programme (NPSP) is fully operational in five provinces. The protocol includes surveillance for severe respiratory illness (SRI), irrespective of duration of symptoms, and testing for core pathogens of public health importance. In the year under review, at all surveillance sites, the NPSP programme described the contribution of influenza, other respiratory viruses and pneumococcus to the syndrome of severe acute respiratory illness (SARI). At two of the surveillance sites, surveillance was enhanced to describe the pathogens associated with more chronic respiratory illness and include collection and testing of

specimens for atypical bacterial causes of pneumonia: *Pneumocystis jirovecii, Mycobacterium tuberculosis, Streptococcus pneumoniae, Bordetella pertussis, Haemophilus influenzae, Legionella species, Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. Surveillance for influenza-like illness (ILI) is ongoing at outpatient clinics at two sites.

Ongoing influenza surveillance

In 2015, the influenza season started in week 20 and continued through week 25. It was predominated by influenza A(H1N1)pdm09, followed by influenza A(H3N2) and influenza B. Of the samples tested between January and September 2015 that were positive for influenza (516/1089), the majority of samples were identified as influenza A(H1N1)pdm09 (49%), while a large proportion were identified as influenza A(H3N2) (37%). To a lesser extent, influenza B was detected in samples collected during this time period (14%).

Cell culture derived influenza virus isolates were obtained with a 94% success rate. A four-fold or greater reduction in hemagglutination inhibition titre against relevant vaccine strain antisera was observed at frequencies of 7% for A(H1N1)pdm09; 38% for B/Yamagata; and 72% for A(H3N2) virus isolates. All influenza A(H1N1)pdm09 viruses are in the 6B genetic lineage and continued drift was observed whereas almost all influenza A(H3N2) viruses were in the 3C.2a genetic lineage. Influenza B viruses identified in 2015 were in clade 3 of the B/Yamagata lineage of which 31% of South African strains formed a subcluster with the B/Phuket/3073/2013 vaccine strain with 58% bootstrap support whereas the other 69% formed a subcluster with 92% bootstrap support. No genetic mutations associated with reduced susceptibility to oseltamivir were observed in the neuraminidase of influenza A and B viruses.

Laboratory-based surveillance

CRDM continues to contribute to the evaluation of the impact of both the pneumococcal conjugate vaccine (PCV) and the *Haemophilus influenzae* serotype b conjugate vaccine (Hib CV) through national, laboratory-based, population-based, active surveillance for invasive pneumococcal and Hib disease and case-control and other epidemiologic studies. CDRM also contributes data on numbers and serogroups of *Neisseria meningitidis* and supports diagnostic testing and outbreak response for suspected cases of meningococcal meningitis.

RESEARCH PROJECTS

Molecular epidemiology and circulation of influenza B Yamagata and Victoria lineages in South Africa, 2005–2014

The aim of this study was to describe the seasonal circulation of influenza B lineages and to determine the association of lineage type with severe acute respiratory illness (SARI). A total of 40 524 respiratory samples from patients with influenza-like-illnesses (ILI) and severe acute respiratory infection (SARI) were tested over 10 years. The influenza detection rate was 23% (9255/40 524), with 24% (2191/9255) influenza B. B/Victoria lineage accounted for 39% (857/2191) of infections. Compared with B/Victoria, B/Yamagata was more frequently associated with SARI in the ≥65 age group (aOR: 6.1; 95% CI: 1.5-24.8) and less likely to be associated with severe acute respiratory infection (SARI) in the 25-44 age group (aOR: 2.2; 95% CI: 0.9-5.0). HIV positive (48%, 194/405) SARI patients with influenza B infection were less likely to be infected with B/Yamagata (aOR: 0.5; 95% CI:0.2-0.9) than B/Victoria.

Enterovirus genotypes in patients with severe acute respiratory illness and influenza-like illness and among asymptomatic individuals in South Africa, 2012–2014

The objective of the study was to determine the prevalence, clinical and genetic characteristics of enteroviruses from South African patients who presented with SARI and influenza-like illness (ILI) during 2012–2014. The detection rate for enterovirus was 5.5% (87/1592), 3.2% (103/3211), and 3.1% (46/1499) among SARI, ILI and controls, respectively. Neighbour-joining phylogenetic analysis indicated that EV-B (25.9%; 61/236) and EV-A (9.3%; 22/236) are more commonly identified. The majority of EV-A (40.9%, 9/22) and EV-D (57.1%, 4/7) was detected among ILI and the majority of EV-B (55.7%, 34/61) and EV-C (54.6%, 6/11) was detected among SARI cases ($p<0.05$). A total of 33 genotypes were detected of which E30 was the most prevalent (9.9%, 10/101), followed by CVB5 (7.9%, 8/101), EV-D68 (6.9%, 7/101) and CVB4 (5.9%, 6/101).

Molecular epidemiology of RSV-A ON1 and RSV-B BA genotypes in South Africa, 2012–2015

In this study the molecular epidemiology of RSV-A ON1 and RSV-B BA9/BA10 genotypes in South African patients with ILI and SARI from 2012 to 2015 was investigated. Unravelling the molecular epidemiology of these genotypes may provide important data to understand the role of insertions and deletions in RSV G proteins for evading cytotoxic T cell and neutralising antibody responses. RSV positive samples were subtyped to identify RSV-A and RSV-B strains. The near-full length G protein genes were amplified and sequenced (2012: n=44; 2013: n=73; 2014: n= 25; 2015: n= 173) using strain specific primers and were analysed in the context of genetic data available from South Africa and in public databases. Continued circulation and dominance of the ON1 and BA10 genotypes was observed in 2015.

Effectiveness of the 13-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in South African children: A case-control study

A case-control study was conducted of pneumococcal conjugate vaccine (PCV13) effectiveness against invasive pneumococcal disease (IPD) in HIV-uninfected and infected children in a middle-income country. PCV13 was shown to be highly effective [vaccine effectiveness (VE) 85% (95% CI: 37, 96)] against PCV13 serotype IPD in HIV-uninfected children when implemented in a 2+1 schedule aligned with the WHO-recommended Expanded Programme of Immunisation (EPI) schedule. Although the vaccine effectiveness point estimate of PCV13 in HIV-infected children was high, this was not significant, possibly due to inadequate power to estimate effectiveness in this group. Combining data from this study with data from a previous study of PCV7 effectiveness showed that ≥2 doses of PCV7 or PCV13 are effective against PCV7-serotype IPD in HIV-exposed-uninfected children and in HIV-negative malnourished children.

Epidemiology of serotype 1 invasive pneumococcal disease in all ages in South Africa, 2003–2013

Using national, laboratory-based invasive pneumococcal disease (IPD) surveillance, the pneumococcal serotype 1 epidemiology was described in the pre- and post-pneumococcal conjugate vaccine (PCV) era. Of 46 483 IPD cases, 10% (4 544) were serotype 1. Serotype 1 incidence decreased after 2011 ($p<0.001$) and two clusters were detected in 2003–2004 and 2008–2012. Compared with IPD caused by other serotypes in children <5 years of age, cases with serotype 1 disease had shorter hospital stays ≤3 days (4–14 days odds ratio [OR]=0.58, 95% confidence interval [CI] 0.33-1.02; ≥15 days OR 0.44, 95% CI 0.23–0.85), less penicillin non-susceptibility (OR 0.02, 95% CI 0.01–0.05), lower HIV prevalence (OR 0.19, 95% CI 0.12–0.31) and lower in-hospital mortality (OR 0.38, 95% CI 0.19–0.76).

HIV infection and the epidemiology of invasive pneumococcal disease (IPD) in South African adults and older children prior to the introduction of a pneumococcal conjugate vaccine (PCV)

The baseline clinical characteristics of adult IPD pre-PCV were described in order to interpret potential indirect effects following vaccine use. National, active, laboratory-based surveillance for IPD was conducted from January 2003 through December 2008. The clinical characteristics of IPD individuals in those HIV-infected and -uninfected were compared using multivariable analysis. 17 604 IPD cases occurred amongst persons ≥5 years of age, with an average incidence of seven cases per 100 000 person-years. IPD incidence in HIV-infected individuals was 43 times higher than in HIV-uninfected persons (52 per 100 000 vs. 1.2 per 100 000). Most HIV-infected individuals presented with bacteraemia (74%, 3 091/4 190). HIV-uninfected individuals were older; and had more chronic conditions (excluding HIV) than HIV-infected persons (39% (210/544) vs. 19% (790/4 190), $p<0.001$).

Burden of potentially vaccine-preventable pneumococcal disease in children <5 years of age in South Africa, 2005–2008 and 2013

In the pre-pneumococcal conjugate vaccine (PCV) era (2005–2008) a total of 109 711 cases of pneumococcal disease was estimated to have occurred in children <5 years of age; an incidence of 2 125/100 000 child-years and a mortality rate of 68/100 000 child-years. The incidence and mortality in HIV-infected children was at least 20-fold greater than in uninfected children. Post-PCV introduction (2013) there was a 66% reduction in all serotype disease in children <5 years of age and a 79% reduction in mortality. The overall reduction was greater in HIV-infected children due to some replacement disease in the HIV-uninfected group.

An economic model for introducing a quadrivalent conjugate meningococcal vaccine among adolescents in South Africa

Invasive meningococcal disease is rare but can have devastating long-term complications. A quadrivalent conjugate vaccine has the potential to reduce costs related to this disease. Based on national surveillance data as well as published literature, the cost-effectiveness of introducing such a vaccine in South Africa routinely, was compared in a longitudinal population of annual 11 year-old cohorts, in a school-based programme over a 10-year period from governmental departments' perspective, to compare the current strategy of no routine vaccination. Routine vaccination of 11-year-olds would result in an estimated 49% reduction of vaccine-preventable disease and savings of R62 million in direct medical costs. However, the vaccine campaign would have a net cost of R2.4 billion over the ten-year period; with a cost per disability-adjusted life year (DALY) saved of R850 000. The finding is that routine vaccination of 11-year-olds in a school-based programme would not be cost-effective from a governmental perspective, when compared with the current situation of no routine vaccination.

Epidemiology of invasive meningococcal serogroup C (MenC) and W (MenW) disease in South Africa, 2003–2014

Cases of invasive meningococcal disease (IMD) were reported through national laboratory-based surveillance. Seventy-four percent (3 675/4 962) of cases had viable isolates. MenW caused 51% (1 869/3 675) of disease followed by B (22%, 816/3 675), Y (11%, 439/3 675), C (9%, 341/3 675) and A (5%, 187/3 675). The average annual incidence of IMD was 0.9/100 000 population. MenW and MenC accounted for 0.4 and 0.07 cases/100 000 persons, respectively. Compared to MenW, MenC disease occurred more frequently in older age groups (MenC: Median age 13 years, interquartile range (IQR) 3-24 years vs. MenW: 5 years, IQR 19 months–21 years; p<0.001); amongst males [(MenC: 59% (199/338) vs. MenW: 52% (955/1 822); p=0.03)]; and was cultured more frequently from cerebrospinal fluid (MenC: 80% (273/341) vs. MenW: 72% (1 347/1 869); p=0.004).

Assessing the impact of pneumococcal conjugate vaccines on invasive pneumococcal disease (IPD) using polymerase chain reaction-based surveillance: An experience from South Africa

Two independent IPD surveillance programmes were implemented among individuals hospitalised at Chris Hani Baragwanath Hospital, Soweto during 2009–2012: PCR-based syndromic pneumonia surveillance and culture-based laboratory surveillance. From 2009–2012, there were 607 *lytA*-positive and 1 197 culture-positive cases that were serotyped. Rates of PCV-7 serotypes/serogroups decreased -63.8% (95% CI: -79.3% to -39.1%) among lytA-positive cases and -91.7% (95% CI: -98.8% to -73.6%) among culture-positive cases. Rates of *lytA*-positive non-vaccine serotypes/serogroups also significantly decreased (-71.7%; 95% CI: -81.1% to -58.5%) over the same period. Such decline was not observed among culture-positive non-vaccine serotypes (1.2%; 95% CI: -96.7% to 58.4%). Until PCR methods improve further, culture methods should continue to be used to monitor the effects of PCV vaccination programmes on IPD incidence.

Comparison of in-house and commercial real-time PCR assays for the detection of bacterial pneumonia

Using a commercial real-time PCR panel and in-house real-time PCR assays for detection of common pneumonia-causing bacteria, 194 nasopharyngeal (NP) and sputum specimens were tested. Overall, inter-assay agreement was 76% (K=0.52, 95% CI: 0.403-0.637, p<0.001). Agreement was 99% each for *M. pneumoniae, C. pneumoniae, Legionella* spp. and *B. pertussis*. The detection rate for *S. pneumoniae* was 30% (58/194) using the commercial assay compared to 47% (90/193) for the in-house assay with an 83% assay agreement (K=0.66, 95% CI: 0.56-0.76, p<0.001). For *H. influenzae*, the detection rate was 36% (69/194) and 46% (89/193) using commercial and in-house PCR, respectively, with 76% assay agreement (K=0.51, 95% CI: 0.39-0.63, p=0.005).The commercial assay was significantly less sensitive for the detection of *S. pneumoniae* and *H. influenzae*.

Two cases of serotypeable and non-serotypeable variants of Streptococcus pneumoniae detected simultaneously during invasive disease

A serotype 1 and 18C isolate were each co-detected with a non-serotypeable isolate in two cases of invasive pneumococcal disease in South Africa in 2009 (case A) and 2010 (case B). The genomes of three available isolates were sequenced. Comparison of the case A non-serotypeable isolate with a serotype 1 genome revealed deletion of genes in the capsular locus. The isolate had a multilocus sequence type (ST) associated with serotype 1 (ST217 and ribosomal ST3462) and its core genome clustered with other ST217 isolates. The case B non-serotypeable isolate had all serotype 18C capsular genes except for variation in *wchA* and *wze*, compared to the 18C isolate. Both case B isolates were ST9817 and their core genomes were identical.

ACKNOWLEDGEMENTS AND COLLABORATORS

1. The SARI Surveillance Programme investigators
2. The Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) investigators
3. The IPD Case Control Study investigators
4. Drs AL Cohen, S Tempia, J Duque, C Whitney, J Winchell, M McMarrow, L McGee, X Wang, L Mayer, L Tondella, Centres for Disease Control and Prevention, Atlanta, USA
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7. Prof. G Milne: University of Western Australia, Perth
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9. Drs SD Bentley, J Parkhill, R Gladstone: Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus
10. Drs R Heyderman, D Everett, G Mapurisa, Malawi Liverpool-Wellcome Trust, Blantyre Malawi
11. Dr R Breiman: Emory University

TEACHING AND TRAINING

CRDM staff lecture at the Universities of the Witwatersrand and Pretoria and are involved in registrar training and ongoing postgraduate supervision of MSc and PhD students. Prof. Cohen co-ordinated the Infectious Diseases Epidemiology 1-week module at the University of Witwatersrand, School of Public Health for the MSc Epidemiology course. Additionally, the centre continues to train medical scientist interns to facilitate their registration with the Health Professions Council of South Africa.

PROFESSIONAL DEVELOPMENT

Postgraduate graduations

- Dr Susan Nzenze was awarded a PhD degree (School of Public Health) at the graduation ceremony of the University of the Witwatersrand graduation ceremony, held on 10 December 2015, for her project *Effect of the introduction of pneumococcal conjugate vaccine on the epidemiology of pneumococcal nasopharyngeal carriage in South African children and adults.*
- Ms Maimuna Carrim was awarded an MSc (Med) degree (School of Pathology) at the University of the Witwatersrand graduation ceremony, held on 10 December 2015 for her dissertation entitled *Identification and prevalence of bacteria causing atypical pneumonia in patients with severe respiratory illness and influenza-like illness in South Africa, 2012–2013*.
- Ms Fahima Moosa was awarded an MSc (Med) degree (School of Pathology) with distinction at the University of the Witwatersrand graduation ceremony, held on 10 December 2015, for her dissertation entitled *Detection of* Bordetella *species in individuals presenting with severe respiratory illness and influenza-like illness in South Africa, June 2012–October 2014.*

Additional students supervised and registered during 2015/16

Five students registered for PhD degrees
Six students registered for MSc degrees
One student registered for B Tech

HONOURS

1. Prof. Cheryl Cohen was promoted to Associate Professor at the University of the Witwatersrand, School of Public Health in May 2015
2. Ms Noluthando Duma was selected for the 6-week Obama Mandela Fellowship in the United States of America, June–July 2015
3. Prof. Anne von Gottberg received the South African Medical Research Council Scientific Merit/Achievement Award, Silver Medal
4. Ms Malefu Moleleki was awarded the Federation of Infectious Diseases Societies South Africa GSK Fellowship for her project entitled *The prevalence and characterisation of Staphylococcus aureus causing severe respiratory infections in South Africa*

RESEARCH OUTPUT

Top publications

1. Tempia S, Walaza S, Cohen AL, von Mollendorf C, Moyes J, McAnerney JM, *et al.* Mortality Associated with Seasonal and Pandemic Influenza Among Pregnant and Nonpregnant Women of Childbearing Age in a High-HIV-Prevalence Setting – South Africa, 1999–2009. *Clin Infect Dis*. United States; 2015 Oct; **61**(7): 1063–70.

   From 1999–2009 the estimated mean annual seasonal influenza-associated mortality rates were 12.6 (123 deaths) and 7.3 (914 deaths) among pregnant and non-pregnant women, respectively. Among pregnant women the estimated mean annual seasonal influenza-associated mortality rates were 74.9 (109 deaths) among HIV-infected and 1.5 (14 deaths) among HIV-uninfected individuals. Among non-pregnant women the estimated mean annual seasonal influenza-associated mortality rate was 41.2 (824 deaths) among HIV-infected and 0.9 (90 deaths) among HIV-uninfected individuals. Pregnant women experienced an increased risk of seasonal influenza-associated mortality compared to non-pregnant women (relative risk [RR]: 2.8; 95% confidence intervals [CI]: 2.1–3.7). In 2009 the estimated influenza A(H1N1)pdm09-associated mortality rates were 19.3 (181 deaths) and 9.4 (1 189 deaths) among pregnant and non-pregnant women, respectively (RR: 3.2; 95% CI: 2.3–4.1).

2. Von Mollendorf C, von Gottberg A, Tempia S, Meiring S, de Gouveia L, Quan V, *et al*. Increased risk for and mortality from invasive pneumococcal disease in HIV-exposed but uninfected infants aged <1 year in South Africa, 2009–2013. *Clin Infect Dis*. United States; 2015 May; **60**(9): 1346–56.

   This project identified 2 099 IPD cases in infants from 2009 to 2013 from all sites. In infants from enhanced sites (n = 1 015), 92% had known HIV exposure status and 86% had known outcomes. IPD incidence was highest in HIV-infected infants, ranging from 272 to 654 per 100 000 population between time points (2013 and 2009), followed by HIV-exposed uninfected (HEU) (33–88 per 100 000) and HIV-unexposed and uninfected (HUU) infants (18–28 per 100 000). The case-fatality rate in HEU infants (29% [74/253]) was intermediate between HUU (25% [94/377]) and HIV-infected infants (34% [81/242]). When restricted to infants <6 months of age, HEU infants (37% [59/175]) were at significantly higher risk of dying than HUU infants (32% [51/228]; adjusted relative risk ratio, 1.76 [95% confidence interval, 1.09–2.85]).

3. Cohen C, Moyes J, Tempia S, Groome M, Walaza S, Pretorius M, *et al*. Epidemiology of Acute Lower Respiratory Tract Infection in HIV-Exposed Uninfected Infants. *Paediatrics*. 2016 Mar 29; pii: e20153272. [Epub ahead of print]

   A total of 3 537 children aged <6 months were enrolled in the study. HIV infection and exposure status were determined for 2 507 (71%) infants, of whom 211 (8%) were HIV infected, 850 (34%) were HIV-exposed uninfected (HEU), and 1446 (58%) were HIV-unexposed uninfected (HUU). The annual incidence of lower respiratory tract infection (LRTI) was elevated in HEU (incidence rate ratio [IRR] 1.4; 95% confidence interval [CI] 1.3–1.5) and HIV infected (IRR 3.8; 95% CI 3.3–4.5), compared with HUU infants. Relative incidence estimates were greater in HEU than HUU, for respiratory syncytial virus (RSV; IRR 1.4; 95% CI 1.3–1.6) and human metapneumovirus-associated (IRR 1.4; 95% CI 1.1–2.0) LRTI, with a similar trend observed for influenza (IRR 1.2; 95% CI 0.8-1.8). HEU infants overall, and those with RSV-associated LRTI had greater odds (odds ratio 2.1, 95% CI 1.1–3.8, and 12.2, 95% CI 1.7–infinity, respectively) of death than HUU.

4. Wolter N, Carrim M, Cohen C, Tempia S, Walaza S, Sahr P, *et al*. Legionnaires' Disease in South Africa, 2012-2014. *Emerg Infect Dis*. United States; 2016 Jan; **22**(1): 131–3.

   The prevalence of *Legionellae* as a cause of community-acquired pneumonia in South Africa is unknown. Syndromic surveillance for patients hospitalised with severe respiratory illness (SRI) was conducted at two hospitals from June 2012 through September 2014. Sputum specimens were tested for *Legionella* spp. by real-time PCR . Demographic and clinical information was collected, and a retrospective epidemiological case investigation conducted. Sputum was collected from 1 805/4 525 (40%) SRI cases, and *Legionella* spp. were detected in 21 (1.2%) of the1 805 cases. All 21 cases occurred in adults aged 19–59 years, of whom 75.0% (15/20) had HIV infection and 42.8% (9/21) were positive for tuberculosis. A previous history of tuberculosis was reported for 82.4% (14/17) of cases. Only one of the 21 cases received targeted *Legionella* treatment. It was found that most patients with Legionnaires' disease had concomitant HIV and/or tuberculosis infection, and the majority of cases were not diagnosed and appropriately treated.

5. Walaza S, Cohen C, Nanoo A, Cohen AL, McAnerney J, von Mollendorf C, *et al*. Excess Mortality Associated with Influenza among Tuberculosis Deaths in South Africa, 1999–2009. *PLoS One*. United States; 2015; **10**(6):e0129173.

   Pulmonary tuberculosis (PTB) deaths increased each winter, coinciding with influenza virus circulation. Among individuals of any age, mean annual influenza-associated PTB mortality rate was 164/100 000 person-years (n = 439). The rate of non-tuberculosis respiratory deaths was 27/100 000 (n = 1 125) for HIV-infected and 5/100 000 (n = 2367) for HIV-uninfected individuals of all ages. Among individuals aged <65 years, influenza-associated PTB mortality risk was elevated compared to influenza-associated non-tuberculosis respiratory deaths in HIV-infected (relative risk (RR): 5.2; 95% CI: 4.6–5.9) and HIV-uninfected individuals (RR: 61.0; CI: 41.4–91.0). Among individuals aged ≥65 years, influenza-associated PTB mortality risk was elevated compared to influenza-associated non-tuberculosis respiratory deaths in HIV-uninfected individuals (RR: 13.0; 95% CI: 12.0-14.0).

List of publications

1. Caini S, Andrade W, Badur S, Balmaseda A, Barakat A, Bella A, *et al*. Temporal Patterns of Influenza A and B in Tropical and Temperate Countries: What Are the Lessons for Influenza Vaccination? *PLoS One*. United States; 2016; **11**(3): e0152310.
2. Cohen AL, McMorrow M, Walaza S, Cohen C, Tempia S, Alexander-Scott M, *et al*. Potential Impact of Co-Infections and Co-Morbidities Prevalent in Africa on Influenza Severity and Frequency: A Systematic Review. *PLoS One*. United States; 2015; **10**(6): e0128580.
3. Cohen AL, Sahr PK, Treurnicht F, Walaza S, Groome MJ, Kahn K, *et al*. Parainfluenza Virus Infection Among Human Immunodeficiency Virus (HIV)-Infected and HIV-Uninfected Children and Adults Hospitalised for Severe Acute Respiratory Illness in South Africa, 2009–2014. *Open forum Infect Dis*. United States; 2015 Dec; **2**(4):ofv139.

4. Cohen C, Moyes J, Tempia S, Groome M, Walaza S, Pretorius M, *et al*. Epidemiology of Acute Lower Respiratory Tract Infection in HIV-Exposed Uninfected Infants. *Paediatrics*. 2016 Mar 29; pii: e20153272. [Epub ahead of print]

5. Cohen C, Naidoo N, Meiring S, de Gouveia L, von Mollendorf C, Walaza S, *et al*. Streptococcus Pneumoniae Serotypes and Mortality in Adults and Adolescents in South Africa: Analysis of National Surveillance Data, 2003–2008. *PLoS One*. United States; 2015; **10**(10): e0140185.

6. Du Plessis M, Allam M, Tempia S, Wolter N, de Gouveia L, Mollendorf C von, *et al*. Phylogenetic Analysis of Invasive Serotype 1 Pneumococcus in South Africa, 1989–2013. *J Clin Microbiol*. 2016 May; **54**(5):1326-34.

7. Groome MJ, Moyes J, Cohen C, Walaza S, Tempia S, Pretorius M, *et al*. Human metapneumovirus-associated severe acute respiratory illness hospitalisation in HIV-infected and HIV-uninfected South African children and adults. *J Clin Virol*. Netherlands; 2015 Aug; **69**:125–32.

8. Iyengar P, von Mollendorf C, Tempia S, Moerdyk A, Valley-Omar Z, Hellferscee O, *et al*. Case-ascertained study of household transmission of seasonal influenza – South Africa, 2013. *J Infect*. England; 2015 Nov; **71**(5): 578–86.

9. Madhi SA, Izu A, Nunes MC, Violari A, Cotton MF, Jean-Philippe P, *et al*. Longitudinal study on Streptococcus pneumoniae, Haemophilus influenzae and *Staphylococcus* aureus nasopharyngeal colonisation in HIV-infected and uninfected infants vaccinated with pneumococcal conjugate vaccine. *Vaccine*. Netherlands; 2015 May; **33**(23): 2662–9.

10. McAnerney JM, Treurnicht F, Walaza S, Cohen AL, Tempia S, Mtshali S, *et al*. Evaluation of influenza vaccine effectiveness and description of circulating strains in outpatient settings in South Africa, 2014. *Influenza Other Respi Viruses*. England; 2015 Jul; **9**(4): 209–15.

11. McAnerney JM, Walaza S, Cohen AL, Tempia S, Buys A, Venter M, *et al*. Effectiveness and knowledge, attitudes and practices of seasonal influenza vaccine in primary healthcare settings in South Africa, 2010–2013. *Influenza Other Respi Viruses*. England; 2015 May; **9**(3): 143–50.

12. McMorrow ML, Wemakoy EO, Tshilobo JK, Emukule GO, Mott JA, Njuguna H, *et al*. Severe Acute Respiratory Illness Deaths in sub-Saharan Africa and the Role of Influenza: A Case Series From Eight Countries. *J Infect* Dis. United States; 2015 Sep; **212**(6): 853–60.

13. Meiring S, Cohen C, Quan V, de Gouveia L, Feldman C, Karstaedt A, *et al*. HIV Infection and the Epidemiology of Invasive Pneumococcal Disease (IPD) in South African Adults and Older Children Prior to the Introduction of a Pneumococcal Conjugate Vaccine (PCV). *PLoS One*. United States; 2016; **11**(2): e0149104.

14. Milne GJ, Halder N, Kelso JK, Barr IG, Moyes J, Kahn K, *et al*. Trivalent and quadrivalent influenza vaccination effectiveness in Australia and South Africa: Results from a modelling study. *Influenza Other Respi Viruses*. 2015 Dec 12; doi: 10.1111/irv.12367. [Epub ahead of print].

15. Murray J, Cohen A, Walaza S, Groome M, Madhi S, Variava E, ***et al***. Determining the Provincial and National Burden of Influenza-Associated Severe Acute Respiratory Illness in South Africa Using a Rapid Assessment Methodology. *PLoS One*. United States; 2015; **10**(7): e0132078.

16. Page N, Groome MJ, Murray T, Nadan S, Netshikweta R, Keddy KH, *et al*. Sapovirus prevalence in children less than five years of age hospitalised for diarrhoeal disease in South Africa, 2009–2013. *J Clin Virol*. 2016 Mar; **78**:82–8.

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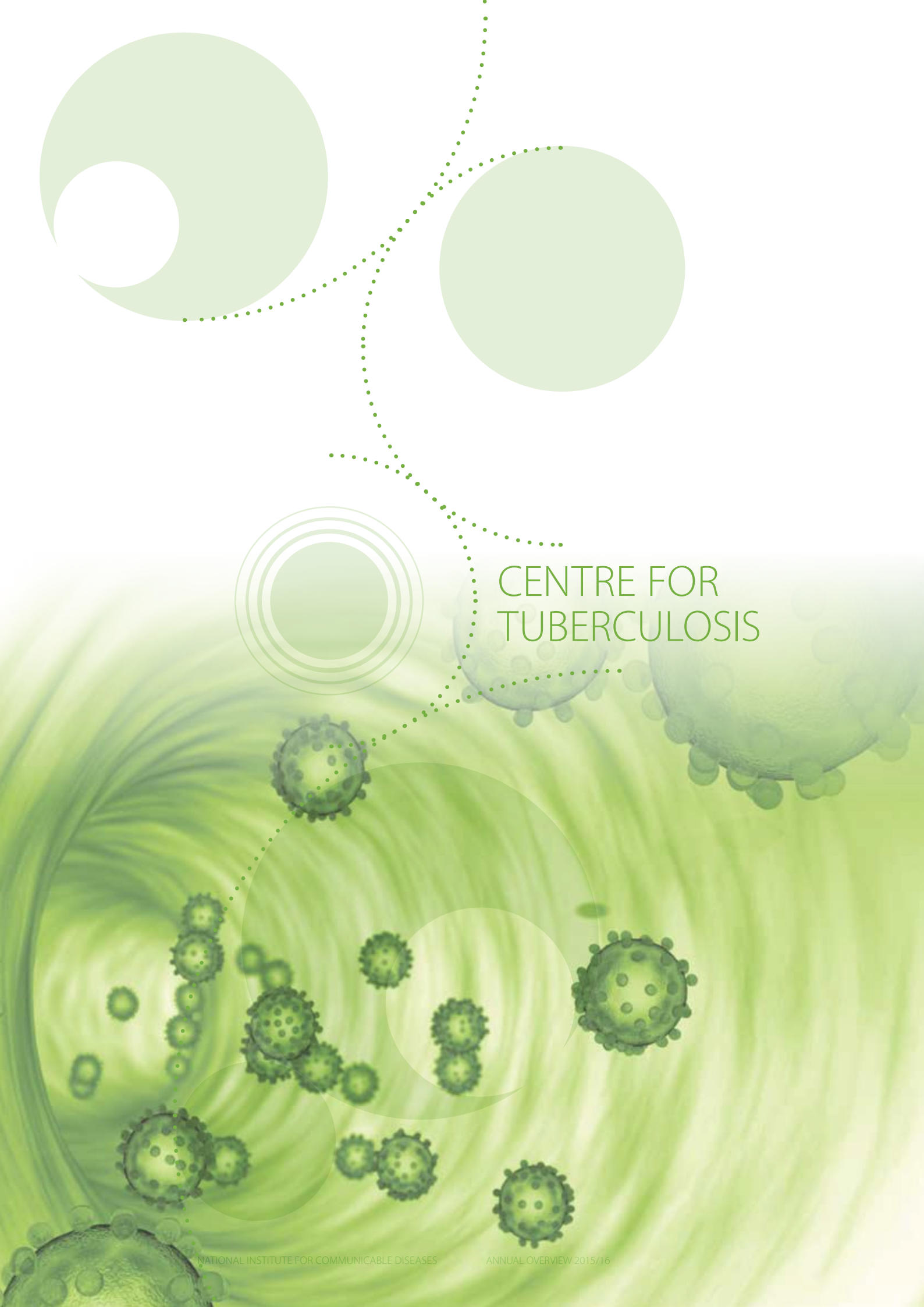
Conferences

Type	Number
International conference presentations	20
National conference presentations	3

CENTRE FOR TUBERCULOSIS



BACKGROUND

In line with the mandate of the National Institute for Communicable Diseases (NICD), the Centre for Tuberculosis (CTB) conducts laboratory-based public health surveillance of TB in South Africa, serves as a national TB reference laboratory (NTBRL) and participates in microbiology and epidemiology-oriented training programmes. The CTB also initiates applied public health research aimed at providing enhanced intelligence on the drivers and protective factors that underlie the TB epidemic in South Africa. It furthermore advises and works closely with the Department of Health (DoH) on strategic planning and assists with policy and guideline formulation concerning the diagnosis and treatment of TB in South Africa. Global policies and guidelines are initiated through the World Health Organization (WHO) and their formulation has included representation from the CTB which assisted in developing these strategic documents.

Centre Head: Dr Nazir Ismail

SURVEILLANCE AND DIAGNOSTIC SERVICES

The CTB uses an integrated approach, combining public health surveillance and reference laboratory functions to provide enhanced and strategic information to guide TB control activities for South Africa. National surveillance covers new cases of laboratory-confirmed TB as well as new drug-resistant TB, including rifampicin-resistant (RR), multidrug-resistant (MDR) and extensively drug-resistant (XDR) cases identified by NHLS laboratories which serve over 80% of the population. Surveillance findings are regularly analysed and reported to the National TB Programme (NTP) and have assisted in monitoring the 90:90:90 strategic targets of the DoH. Information on drug resistance prevalence determined through a national survey has been analysed and will guide the NTP in pursuit of the Sustainable Development Goals, providing direction in terms of human and financial resources for areas of need.

New specialised molecular techniques for *Mycobacterium tuberculosis* detection have now been integrated into the surveillance system to better define drug resistance mutation profiles and clonal strains, using whole genome sequencing (WGS), restriction fragment length polymorphism (RFLP) typing, spoligotyping and mycobacterial interspersed repetitive unit (MIRU) typing, all of which have become well established and are producing important findings. In addition, the centre is playing a leading role globally in the development and application of methods for drug resistance determination for new anti-mycobacterial agents, particularly bedaquiline.

Survey of drug resistance in TB, South Africa

The drug resistance survey (DRS) of TB cases in South Africa has been completed and results have been shared with the primary stakeholders. Further consultations are under way. This DRS has been the largest globally, with 200 358 presumptive TB cases screened into the survey from over 442 facilities, grouped into 343 clusters. For the first time results for the Northern Cape will become available, an objective that was not achieved in the previous survey. The majority of patients screened across the nine provinces were female (55.2%) and the median age was 39 years (IQR: 30–51 years). The unique design of this survey incorporates the recruitment of presumptive cases rather than smear-positive cases only, allowing assessment of the drug resistance burden inclusive of both smear-positive and smear-negative patients – an important issue for high HIV/AIDS burdened settings like South Africa. This was successfully achieved and the overall smear positivity rate among culture-confirmed cases was 55% nationally with a range of 45%–68% provincially. Among culture-positive cases, the percentage with a history of prior treatment was 22% nationally with the highest being in the Western Cape, at 35%. The HIV co-infection rate was 63.2% with the highest observed in Mpumalanga (76.8%). An additional difference, in comparison with the previous survey, was the testing of all culture-confirmed cases for a full range of drugs, including second-line agents. This data will be important in informing future drug regimens, both for novel regimens being tested among rifampicin susceptible cases, and for RR TB. The latter has emerged as an important concern with poor outcomes, especially in pre-XDR and XDR TB cases, but there is renewed optimism with the introduction of new drugs that could be combined with drugs to which lower levels of resistance, based on the survey findings, are present. The final report is expected to be released later in 2016, once consultations have been completed, and will be used to guide appropriate interventions.

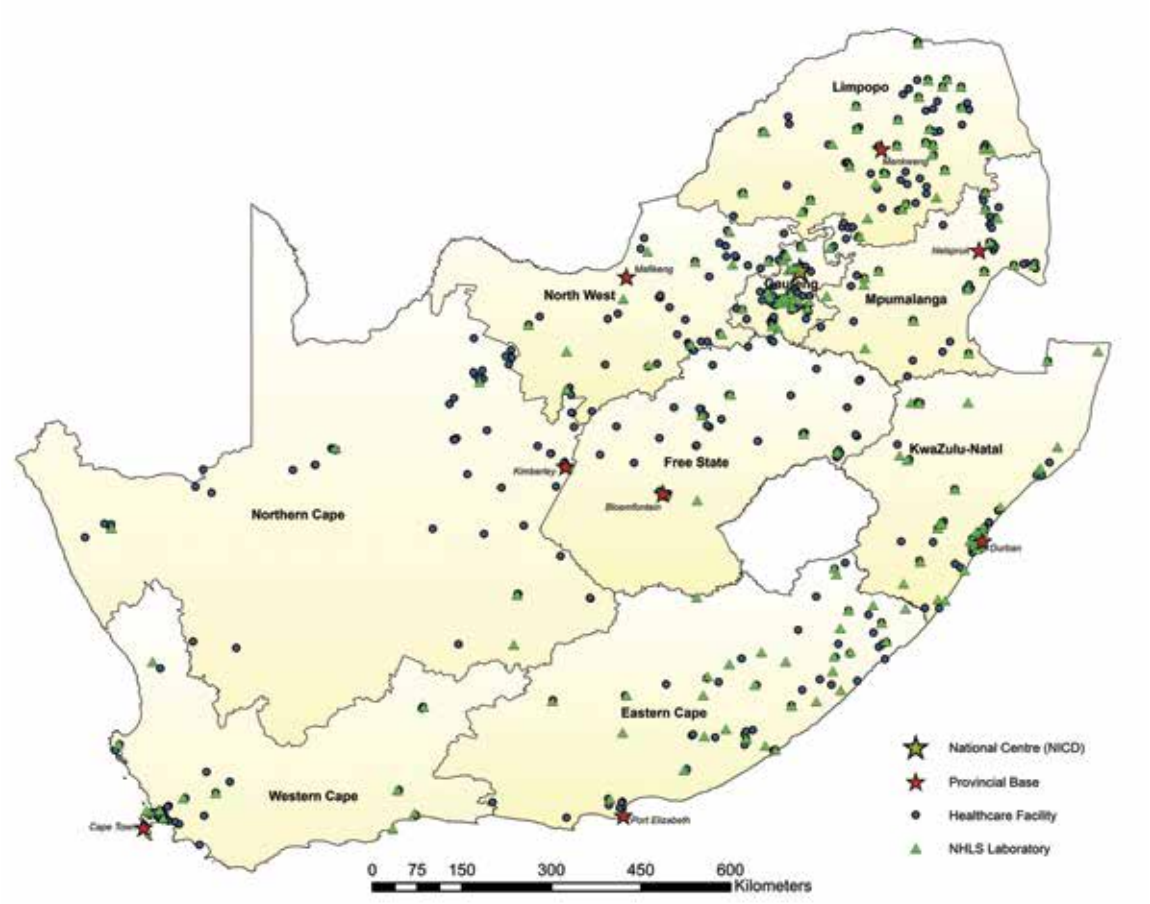


Figure 6: Map of randomly selected facilities included in the SA TB DRS 2012–14

Surveillance of microbiologically confirmed TB in South Africa

Electronic TB surveillance has been developed to provide trend analysis and relevant epidemiological data on TB in South Africa. The utility of the surveillance was demonstrated in the article titled *Nationwide and regional incidence of microbiologically confirmed pulmonary tuberculosis in South Africa, 2004–12: A time series analysis*, published in the *Lancet Infectious Disease Journal* in 2015. The activities described in this publication have formed the basis for surveillance reporting to the national and provincial Departments of Health on a quarterly basis, and provided information to WHO for the annual Global TB Report. Additional tasks have been undertaken, including trend analysis of drug resistant TB, and early assessment has shown an upward trend in RR TB. However, this is paralleled by an increased rate of testing for drug resistance. The initial introduction of mycobacteria growth indicator tube (MGIT)-based drug susceptibility testing (DST), followed by the line probe assay (LPA) and most recently the Xpert MTB/RIF system have been important contributors to improved detection of drug resistant TB. Further programme planning, adjusting for testing rates and methodologies, is in progress and will assist in contextualising the results of the DRS. In addition, it will present a potential avenue for monitoring trends in drug resistant TB into the future. The electronic TB surveillance system has proven to be a powerful tool in guiding TB control efforts; however, because the epidemic is heterogeneous, a more comprehensive understanding of the changes over time has facilitated efforts to geospatially map hotspots, and will in future allow targeted interventions to be applied.

Provincial and district-wide rifampicin resistance alerts for public health action

In the process of maximising benefits of the electronic reporting system for the NHLS, weekly alerts providing a line listing of cases diagnosed with RR TB by the Xpert MTB/RIF assay are emailed to the nine provincial and 52 district managers in each province for public health action. This was initiated in collaboration with the TB Cluster of the DoH, with the aim of reducing the gap between diagnosis and treatment. The reporting has been expanded to include cases diagnosed by LPA and DST, ensuring that all cases of drug-resistant TB (DR-TB) can be easily identified. This has provided an important tool for case management in the health system and to support continued efforts with patient tracing initiatives by the respective teams in the field. The electronic surveillance system has also been improved to provide a web-based interface, including quarterly data on both TB and DR-TB cases and allowing the information to be viewed down to sub-district level. The dashboard is still being refined and further automation of the data processing underlying the dashboard is being developed.

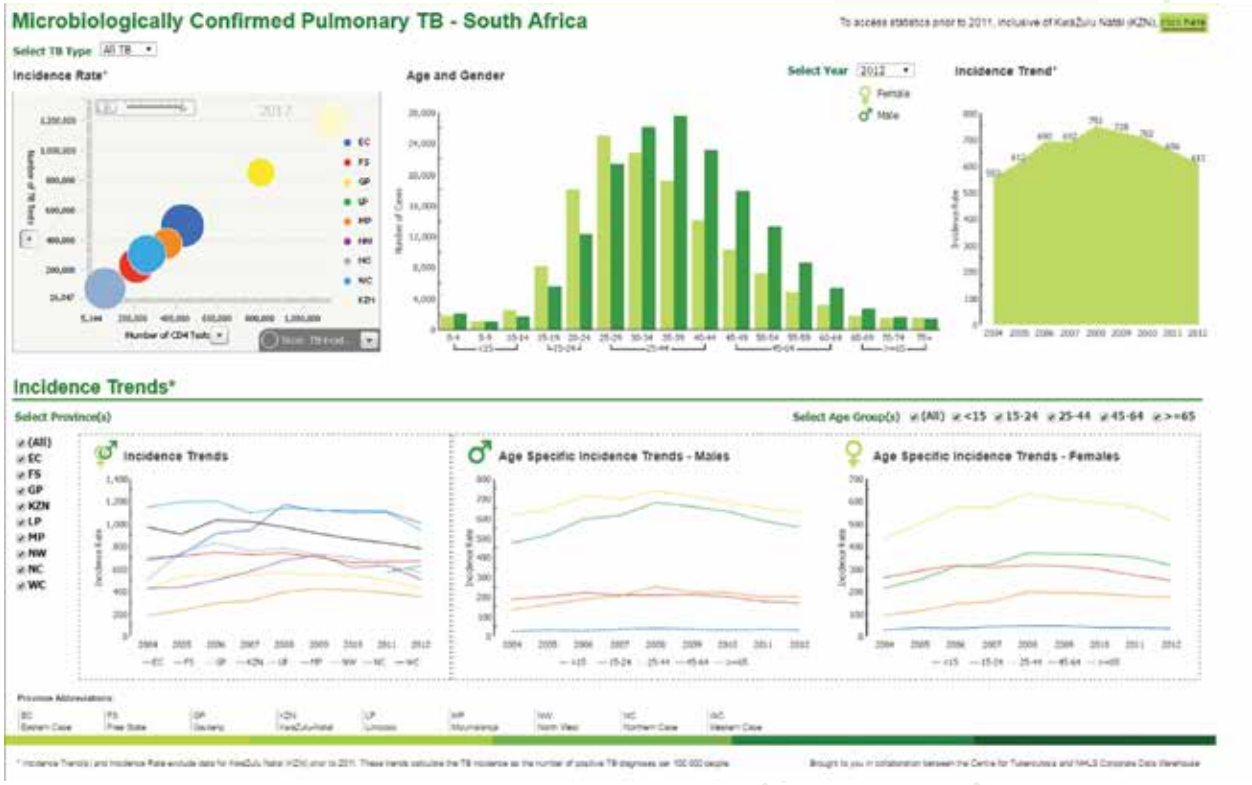


Figure 7: Beta-version of TB surveillance dashboard

Surveillance for bedaquiline resistance

Bedaquiline (BDQ) is a diarylquinoline antimycobacterial drug which specifically inhibits mycobacterial adenosine triphosphate synthase. It is the first new drug class with a novel mechanism of action to become available in forty years. Since October 2014, Sirturo (bedaquiline, BDQ) from Janssen Pharmaceutica, has been registered in South Africa for use in HIV-negative or HIV-infected ART-naïve patients, 18 years or older, who have laboratory-confirmed MDR-TB. Additionally, WHO has issued guidance on the treatment of MDR-TB with BDQ. Surveillance for early detection of BDQ resistance is advised by WHO and is incorporated into the South African policy framework according to which all patients starting BDQ treatment will have samples tested at baseline – week 8 and week 24 using BDQ minimal inhibitory concentration (MIC) determination. Results from the first 61 patients with isolates on which culture and MIC testing had been performed have shown that 47/61 had MIC values below 0.25ug/ml, 7/61 at 0.25ug/ml and 6/61 above 0.25ug/ml. The latter figures are above the European Committee on antibiotic susceptibility testing tentative Epidemiological cut off value, "breakpoint" concentration; however, of these only one had received prior treatment with clofazimine, a drug shown to have the potential to confer cross resistance to BDQ. Of these, none have shown mutations in atpE, the gene shown to be directly associated with resistance, and two have shown mutations in *Rv0678*, a putative efflux pump encoding gene thought to be associated with BDQ resistance. Monitoring is ongoing for the emergence of resistance and mechanisms associated with resistance in the South African context.

Population-based whole genome sequencing to inform new drug regimens for South Africa

In addition to the use of conventional drug resistance determination, the survey has included whole genome sequencing (WGS) of all *M. tuberculosis* isolates from two provinces: Gauteng and KwaZulu-Natal. This WGS project was initiated as part of a multi-country, WHO co-ordinated effort to determine baseline resistance to fluoroquinolones and pyrazinamide, as these drugs are planned for inclusion in new regimens currently under investigation. This study has been concluded and figures for pyrazinamide and fluoroquinolone resistance are approximately 3% and 1% respectively, irrespective of prior treatment history. These are positive findings for South Africa, which has recorded one of the lowest prevalence figures of resistance compared to other countries for which surveillance information is available. Furthermore, it suggests that first-line regimens, including these drugs in combination with new agents, are likely to show success and South Africa would be likely to be one of the countries to adopt such an approach expeditiously. The CTB has successfully established and optimised WGS as a method for use in surveillance and is recognised internationally for its contribution in this field. Based on research conducted by CTB in which closely comparable findings between the two approaches have been demonstrated, WGS has been shown to be a suitable alternative to phenotypic DST in surveys. It is likely to change the landscape of laboratory-based TB management in the future. Additional development of the technology is under way to improve the CTB's ability to maximise the use of WGS in investigating TB transmission as well.

Supporting NTBRLs and surveys in Africa – Supra-National TB Reference Laboratory

The CTB has recently been successfully evaluated for its reference laboratory potential, resulting in it being awarded the responsibility to function as the 3rd Supra-National Reference Laboratory of the WHO on the African continent. As part of this function, it provides technical support to two national reference laboratories in Africa, namely those in Malawi and Namibia. Progressive missions to improve quality management systems for laboratory testing, as well as programme initiatives towards TB surveillance activities, have been performed in these countries. In addition, the Centre provided reference laboratory support to the Malawi Prevalence Survey and the Namibia Drug Resistance Survey for both quality assurance and second-line DST. An expanded approach, aiming to assess the proficiency of nine of the 22 highest burden TB national reference laboratories in Africa on culture identification and DST, has been recently initiated through WHO-AFRO. Although the performance of countries in the first round was sub-optimal for several countries, feedback and further provision of quality-related support has resulted in an overall improvement in the quality of results generated by these laboratories. These developments have been important and have shown the great value of the support functions conducted by CTB.

SRLN

Prospective sentinel surveillance of RR TB, TB/HIV integration and hospitalised TB in South Africa

Complementing surveillance based on reporting of routine data, an enhanced surveillance programme has been introduced to provide additional quality assured data on demographic and clinical features, as well as risk factors. In addition, a surveillance specimen is collected and is used to generate complementary microbiological data. The first TB-related surveillance established was for RR TB and this has now been implemented in seven of the nine provinces. Important findings across the sites have shown that between 38% and 50% of patients with RR TB have never had TB treatment before, while between 9% and 33% have had household contact with TB, both indicating that transmission plays an important role. Striking differences have been observed in the prevalence of rifampicin mono-resistance between sites, with higher rates seen in selected districts/sites in Mpumalanga and the Northern Cape, and a mixed pattern in the other provinces. The percentage of RR TB patients with a final baseline resistance profile of XDR TB was highest in Eastern Cape at 18% and 8% across all sites.

The most recent surveillance introduced has been clinic-based rather than laboratory-based and will monitor TB and HIV programmatically, assessing trends and relative risk factors. In addition to clinic-based surveillance, monitoring of drug resistance has also been incorporated. Since both TB and HIV use empiric regimens for treatment without primary resistance testing, except for rifampicin resistance in TB, this surveillance is critical and will provide baseline data for the monitoring of resistance to isoniazid and other first-line drugs as well as baseline data on HIV resistance in those starting treatment. Both these are important and are missed in the routine system; notably for TB where isoniazid mono-resistance (IMR) is expected at >5% but is not detected by the widely implemented Xpert MTB/RIF assay. This has been observed with IMR at 11.1% at one site and 4.6% at a second site, though numbers are relatively small at this stage.

Molecular epidemiological surveillance for early detection of RR clusters in selected districts

Outbreaks of drug-resistant TB have been reported in South Africa, but are usually identified late and often with an accompanied high mortality. An early warning surveillance system has been introduced where strain typing of all RR TB cases is aimed at identifying areas of high risk transmission. This is being implemented at district level, with one district targeted per province. Spoligotyping has been applied as an initial measure to understand the population structure of TB strains and an example of its use is depicted in the Figure below. The challenge in endemic settings is the occurrence of endemic strains, the identification of which requires high resolution methodologies which are costly. An alternative strategy using molecular techniques has been developed and is discussed later. Using MIRU-VNTR 24 loci as the primary high resolution tool, clusters have been identified, primarily in Eastern Cape and Mpumalanga. These provide the first insights into transmission and will be followed up with further epidemiological investigations. These early developments are expected to define the threshold for investigations to further develop and utilise methodologies for intervention in endemic settings.

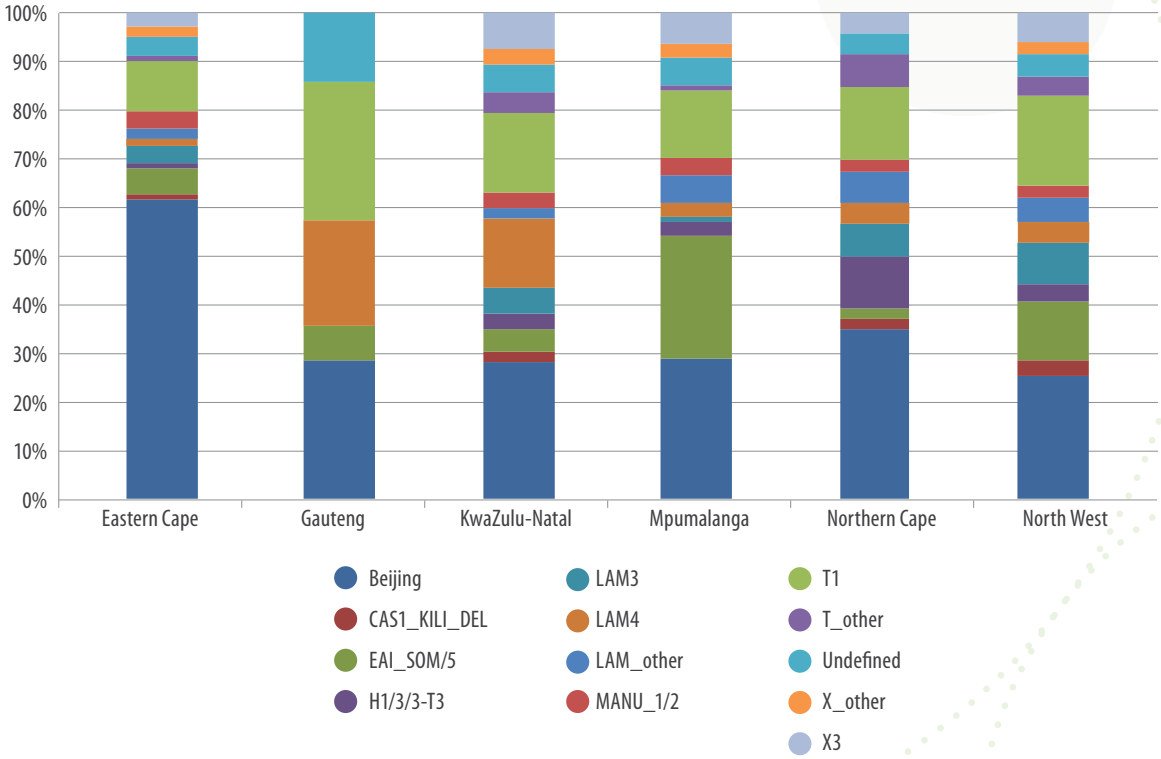


Figure 8: Rifampicin resistant TB Spoligotype distribution by province (N=624)

RESEARCH PROJECTS

The CTB was involved in the following research projects during the year under review:

Transmission of HIV-associated XDR-TB in households and hospitals in South Africa

Collaborator: *Neel R Gandhi; Departments of Epidemiology and Global Health, Emory University Rollins School of Public Health, Atlanta, GA, United States*

Recent data emphasises the importance of person-to-person transmission of drug-resistant TB worldwide. In KwaZulu-Natal up to 83% of XDR-TB cases are genotypically-clustered. In the present study, patients diagnosed with XDR-TB in KwaZulu-Natal from 2010–2014 were interviewed about their social networks and hospitalisations. An epidemiological link was defined as two participants having either a social network connection or overlapping admission at the same hospital. Among 404 XDR-TB patients, 311 (77%) were HIV-infected. Epidemiological links were identified for 287 (71%) patients: 83 (21%) were linked as social network contacts; 92% lived in the same home; and 267 (66%) overlapped with other XDR-TB patients in hospital. There were 63 (16%) patients with both hospital and social network links to other XDR-TB patients. The XDR-TB epidemic in this high HIV prevalence setting in South Africa is being driven by direct transmission of drug-resistant TB strains in both hospitals and households. Social network analysis has provided valuable insights into the multitude of interactions associated with transmission.

Optimising mycobacterial culture in smear-negative, HIV-Infected tuberculosis cases

Collaborators: *P Naidoo and Z Pininini; Desmond Tutu TB Centre, University of Stellenbosch, South Africa & TB/HIV Cluster, Gauteng Department of Health, South Africa*

Mycobacterial culture remains an important complementary tool and optimising its performance has important benefits. In this study, the effect of an increase in the number of specimens evaluated, addition of nutritional supplementation to the culture medium, sputum appearance and volume on diagnostic yield and time to detection of pulmonary TB among smear-negative, HIV-infected adults was investigated. The study was conducted at Tshwane District Hospital and Academic TB Laboratory, Pretoria, South Africa. Three sputum specimens from each of 236 patients were analysed. A single specimen identified 79% of pulmonary TB cases using standard media; the second and third specimens diagnosed identified an additional 12.5% and 8.3% cases respectively. Mean time to detection was reduced from 19.8 days in standard cultures to 11.8 days in nutrient supplemented cultures ($p = 0.002$). For every 1 ml increase in sputum volume, time to detection was decreased by a factor of 0.797 ($p = 0.011$).The use of an inexpensive culture supplement substantially reduced time to detection and could contribute to reducing treatment delay among HIV-infected cases.

Epidemiology of drug-resistant tuberculosis among children and adolescents in South Africa, 2005–2010

Collaborator: *B Moore; Centre for Disease Control, Atlanta, United Stated of America*

Demographic and clinical characteristics of children and adolescents diagnosed with drug-resistant tuberculosis (DR-TB) in South Africa were analysed using a retrospective review of medical records of children and adolescents with DR-TB at specialty hospitals in four South African provinces from 2005 to 2010. During this period, 774 children and adolescents were diagnosed with DR-TB and of these 45.2% had a history of previous TB treatment; 54.7% had HIV infection; 76.4% had contact with a TB case; while 60.8% were smear positive; and 38.7% had cavitary disease. Eighty-two per cent of patients with HIV infection received ART. Of the 626 patients diagnosed with multidrug-resistant TB, 561 (89.6%) received a regimen consistent with national guidelines and the median length of treatment was 22 months (IQR 16–25). Among 400 patients with any DR-TB and a known outcome, 20.3% died during treatment. History of previous treatment and contact with a TB patient provide opportunities for earlier diagnosis and treatment to improve outcomes.

Time to culture conversion and outcomes in HIV-positive multidrug-resistant-TB patients treated with regimens containing moxifloxacin or ofloxacin

Collaborator: *C Reddy, Field Epidemiology Training Programme, NICD, Johannesburg, South Africa*

Multidrug-resistant TB (MDR-TB) is associated with poor treatment outcomes among HIV-infected persons. A record review was conducted of HIV-positive, MDR-TB patients on ART at Sizwe Tropical Diseases Hospital, Johannesburg between 2007 and 2012, to compare culture conversion and outcomes in patients on regimens containing either ofloxacin or moxifloxacin. The review involved a descriptive analysis for socio-demographic factors; log-rank test for comparing time to sputum conversion; multivariable Cox regression for factors associated with time to culture conversion; and Chi-square test for association between regimens and treatment outcomes. Of 758 eligible patients, 405 received moxifloxacin and 353 received ofloxacin. Time to sputum culture conversion was not significantly different between treatment groups (HR=0.97; p=0.734) while the cure rate of 30% (n=122) was significantly better in the moxifloxacin group compared to 19% (n=67) in the ofloxacin group (p=0.001). South Africa should continue the use of moxifloxacin-containing regimens, paying special attention to individualised weight-based dosing. Additionally, interventions aimed at early diagnosis and treatment of HIV-positive, MDR-TB cases are essential to improve outcomes.

Multidrug-resistant tuberculosis and treatment in KwaZulu-Natal, South Africa: A long distance relationship

Collaborator: *B Moore; Centre for Disease Control, Atlanta, United States of America*

TB accounted for 14.3% of all deaths in South Africa in 2010. Mortality in MDR-TB patients who are HIV-positive is especially high and almost half of all MDR-TB cases in this country occur in KwaZulu-Natal (KZN) province. A retrospective cohort study involving a random selection of 6 600 patients was conducted to assess factors associated with treatment success. Final clinical outcome was determined two years after treatment initiation. Data were abstracted from the electronic drug resistance TB registry of the DoH for this cohort, as well as from medical files of the patients. The study found that proximity to an MDR-TB treatment facility was a significant factor in MDR-TB treatment success. High quality MDR-TB services should therefore be made available within geographically accessible distances in KZN.

A multi-laboratory, multi-country study to determine bedaquiline minimal inhibitory concentration quality control ranges for phenotypic drug susceptibility testing

Collaborator: *D Cirillo; San Rafelle Institute, Milan, Italy*

Bedaquiline is a new diarylquinoline antimycobacterial drug, used in the treatment of MDR-TB. A series of laboratory investigations was initiated to establish standardised drug susceptibility testing (DST) methodologies and reference minimal inhibitory concentration (MIC) QC ranges for this new anti-TB drug. Two tier-2 QC reproducibility studies of bedaquiline DST were conducted in eight geographically diverse laboratories, using Clinical Laboratory and Standards Institute (CLSI) guidelines. Agar dilution and broth microdilution DST methodologies were evaluated, using 7H10 and 7H11 agar from different manufacturers, and lots of frozen 7H9 broth polystyrene microtitre plates, respectively. CLSI criteria stipulate the QC range must encompass ≥95% of observed MICs centred round the mode or geometric mean over 3 or 4 dilutions. For outlying data, the entire laboratory dataset was excluded. Microbiological equivalence was demonstrated between Middlebrook 7H10 and 7H11 agar in the agar dilution method but not between agar dilution and broth microdilution methods. Bedaquiline DST methodologies and MIC QC ranges against H37Rv *M tuberculosis* reference strain were established at 0.015–0.12 mg/L for the 7H10 and 7H11 agar dilution method, and 0.015–0.06 mg/L for 7H9 broth microdilution method.

TEACHING AND TRAINING

Training accommodating experiential as well as didactic learning was provided on site in Malawi at their second and newly established TB Reference Laboratory. Additionally, CTB provided instruction in a train-the-trainer course for NTP/National Reference Laboratory managers with participants from SADC countries. Training was also provided for both reference mycobacteriology testing and public health aspects of TB, rotating registrars from university-based medical microbiology departments in South Africa, as well as for intern scientists in the country. In addition, CTB mentored a Field Epidemiology and Laboratory Training Programme (FELTP) student, further expanding capacity in epidemiology in South Africa. Lastly, training on Xpert MTB/RIF, LPA and WGS was conducted at an international skills building workshop, with participants from Africa, Europe and Asia.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates

Numbers of candidates enrolled: 3 PhD, 1 MMed, 4 MSc, 1 MPH
Numbers of candidates graduated: 1 PhD, 1 MMed, 1 MPH

HONOURS

The MRC UK/SA Newton Grant for Implementation Science was awarded to S Madhi and N Ismail.

RESEARCH OUTPUT

Scientific Publications

Walker TM, Kohl TA*, **Omar SV***, Hedge J, Elias CDO, Bradley P, Iqbal Z, Feuerriegel S, Niehaus KE, Wilson DJ. Whole-genome sequencing for prediction of *Mycobacterium tuberculosis* drug susceptibility and resistance: A retrospective cohort study. *The Lancet Infectious Diseases*. 2015; **15**: 1193–1202.

*indicates joint first authorship

Diagnosing drug resistance in patients with TB remains a priority for the elimination of TB and the use of whole genome sequencing for prediction of resistance and susceptibility was investigated. A training set of 2 099 *M. tuberculosis* genomes was sequenced and the 23 candidate genes identifying mutations were algorithmically characterised. The ability of these characterisations to predict phenotypic drug susceptibility was then assessed for an independent validation set of 1 552 genomes. A total of 120 training-set mutations were characterised as resistance determining, and 772 as benign. With these mutations, 89·2% of the validation-set phenotypes could be predicted with a mean sensitivity of 92·3% (95% CI: 90·7–93·7) and a 98·4% specificity (95% CI: 98·1–98·7). Prediction of 10.8% of the validation-set phenotypes was not possible due to uncharacterised mutations present. No additional resistance determinants were identified among mutations under selection pressure in non-candidate genes. The study showed that a broad catalogue of genetic mutations enable data from whole genome sequencing to be used clinically to predict drug resistance, drug susceptibility, or to identify drug phenotypes that cannot yet be genetically predicted. This approach could be integrated into routine diagnostic workflows, phasing out phenotypic drug susceptibility testing while reporting drug resistance early.

Bradley P, Gordon NC, Walker TM, Dunn L, Heys S, Huang B, Earle S, Pankhurst LJ, Anson L, de Cesare M, Piazza P, Antonina A, Golubchik VT, Wilson DJ, Wyllie DH, Diel R, Niemann S, Feuerriegel S, Kohl T, **Ismail N, Omar SV**, Smith EG, Buck D, McVean G, Walker AS, Peto TEA, Crook DW, Iqbal Z. Rapid antibiotic-resistance predictions from genome sequence data for *Staphylococcus aureus* and *Mycobacterium tuberculosis*. *Nature Communications.* 2015; 6.

The rise of antibiotic-resistant bacteria has led to an urgent need for rapid detection of drug resistance in clinical samples, and improvements in global surveillance. The present study shows how the de Bruijn graph representation of bacterial diversity can be used to identify species and resistance profiles of clinical isolates. This method is implemented here for *Staphylococcus aureus* and *Mycobacterium tuberculosis* in a software package ('Mykrobe predictor') that takes raw sequence data as input, and generates a clinician-friendly report within three minutes on a laptop. For S. aureus, the error rates of the method are comparable to gold-standard phenotypic methods, with sensitivity/specificity of 99.1%/99.6% respectively across 12 antibiotics (using an independent validation set, n¼470). For *M. tuberculosis*, the method predicts resistance with sensitivity/specificity of 82.6%/98.5% respectively (independent validation set, n¼1609); the lower sensitivity is probably linked to a limited understanding of the underlying genetic mechanisms. Evidence is provided here that minor alleles improve detection of extremely drug-resistant strains, and demonstrate feasibility of the use of emerging single-molecule nanopore sequencing techniques for these purposes.

Said HM, Kushner N, **Omar SV, Dreyer AW, Koornhof H, Erasmus L, Gardee Y, Rukasha I**, Shashkina E, Beylis N, Kaplan G, Fallows D, **Ismail NA**. A Novel Molecular Strategy for Surveillance of Multidrug-Resistant Tuberculosis in High Burden Settings. *PLoS One* 2016; 11: e0146106.

Transmission of tuberculosis (TB) is a significant contributor to rising rates of multidrug-resistant TB (MDR-TB) requiring an early detection system for transmission clusters suitable for high burden settings. The discriminatory power and clustering concordance of a novel and simple genotyping approach were evaluated, combining spoligotyping with pncA sequencing (SpoNC), against two well-established methods: IS6110-RFLP and 24-loci MIRU-VNTR typing methods. A total of 216 MDR-TB isolates was used for the study and clustering rates. Hunter-Gaston Discriminatory Indexes (HGI) and Wallace coefficients were compared between the methods. Overall, clustering rates were high by both IS6110-RFLP (52.8%) and MIRU-VNTR (45.8%), indicative of ongoing transmission. Both 24-loci MIRU-VNTR and IS6110-RFLP had similar HGI (0.972 and 0.973, respectively), while spoligotyping alone was the least discriminatory (80.1% clustering, HGI 0.903). The discriminatory power of spoligotyping when combined with pncA sequencing using the SpoNC approach was closely comparable to the established methods (61.8% clustering, HGI 0.958). A high proportion of MDR-TB isolates had mutations in pncA (68%, n = 145), and pncA mutations were significantly associated with clustering (p = 0.007 and p = 0.0013). This study shows that the SpoNC approach provides good discrimination for MDR-TB surveillance and early identification of outbreaks in South Africa, with 24-loci MIRU-VNTR typing applied for pncA wildtype strains as needed.

Lim JR, Gandhi NR, Mthiyane T, Mlisana K, Moodley J, Jaglal P, Ramdin N, Brust JC, **Ismail N**, Rustomjee R. Incidence and Geographic Distribution of Extensively Drug-Resistant Tuberculosis in KwaZulu-Natal Province, South Africa. *PloS one* 2015; 10: e0132076.

The Tugella Ferry XDR-TB outbreak was initially thought to be localised; however subsequent epidemiologic data confirmed that XDR-TB was widespread in KwaZulu-Natal province. The changes in XDR-TB incidence and geographic distribution between October 2010 and December 2012 were investigated. A total of 776 XDR-TB cases was identified in KwaZulu-Natal province, equating to a province-wide incidence of 3.5 XDR-TB cases per 100 000 population. Women represented over half (59%, 329 of 555 with available data) of the cases. The median age was 33 years (range: 0–76 years; data available for n = 616). Although the incidence varied by district, nearly all districts had an incidence of greater than 1.0 XDR-TB cases per 100 000 population, with the highest incidences in Umzinyathi (12.1 cases/100 000), Ethekwini (4.3 cases/100 000), and uMgungundlovu (3.4 cases/100 000). The average number of XDR-TB cases in the province each year increased from 270 to 358 and the overall incidence increased by 13%, compared to 2007. XDR-TB incidence increased in 8 of 11 districts. In Umzinyathi and uMgungundlovu, although the incidence declined over this time period, it remained among the highest in the province at 12.1 and 3.4 XDR-TB cases/100 000, respectively. The burden of XDR-TB cases remains concentrated in Umzinyathi eThekwini, and uMgungundlovu; eThekwini; and uMgungundlovu are home to Durban and Pietermaritzburg, the two largest cities in KwaZulu-Natal that account for nearly half of the provincial population.

Matabane MM, Ismail F, Strydom KA, Onwuegbuna O, **Omar SV, Ismail N**. Performance evaluation of three commercial molecular assays for the detection of *Mycobacterium tuberculosis* from clinical specimens in a high TB-HIV-burden setting. *BMC Infect Dis* 2015; 15: 508.

A major challenge for countries with a high burden of tuberculosis (TB) is early detection, especially in individuals with paucibacillary disease which is common in HIV endemic settings. In this laboratory-based retrospective study, the diagnostic performance of three commercial molecular assays was evaluated for the detection of *Mycobacterium tuberculosis* complex from clinical specimens in a high TB-HIV-burden setting. Samples selected for inclusion were stratified by smear-positive TB, smear-negative TB and TB culture-negative cases. Samples were also processed for liquid TB culture and time-to-culture positivity was recorded. The overall sensitivity was highest for Xpert® MTB/RIF (89.1 %) followed by GenoType MTBDRplus (70.9 %) and Anyplex™ plus (65.5 %). There was a significant difference in sensitivity between Xpert® MTB/RIF and the other two assays for smear-negative cases (P < 0.05). The performance in cases where the time-to-culture positivity was ≥20 days was also significantly poorer for both Anyplex™ plus and GenoType MTBDRplus compared to Xpert® MTB/RIF (P < 0.05). Xpert® MTB/RIF achieved 100 % specificity, while Anyplex™ plus and GenoType MTBDR plus achieved 96.2% and 92.3 % respectively. In this study, the Xpert® MTB/RIF was superior to the other two assays for the detection of TB in smear-negative specimens, notably when bacterial loads are very low in sputum. It is important that studies reporting on test performance stratify their results by time-to-culture positivity to accurately assess clinical performance, especially in high HIV settings.

Conference presentations

Type	Number
International	9 Oral; 9 Poster
National	1 Oral

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CENTRE FOR VACCINES AND IMMUNOLOGY

Head: Dr M Suchard

SURVEILLANCE/DIAGNOSTIC SERVICES

National Poliomyelitis surveillance

The Poliovirus Isolation Laboratory, within the Centre for Vaccines and Immunology, serves as a national reference laboratory for polio as part of the Global Polio Eradication Initiative (GPEI). In this capacity the laboratory routinely serves seven countries within Africa; Angola, Botswana, Lesotho, Mozambique, Namibia, Swaziland and South Africa, with processing and testing of more than 2 600 stool samples during the reporting period. As per World Health Organization (WHO) recommendations, samples are inoculated into cell cultures and samples with suggestive cytopathic effects undergo molecular characterisation, including sequencing where applicable. The last case of endemic polio in South Africa was in 1989; however constant high quality surveillance is critical to promptly identify importations and halt transmission. The non-polio acute flaccid paralysis (non-polio AFP) rate in South Africa doubled from two to four cases per 100 000 population in the year. In order to differentiate circulating strains of Sabin vaccine virus from wild polio virus, suspected poliovirus isolates are subjected to molecular typing and characterisation, as the virus isolation process does not cater for this. Laboratory data is shared on a weekly basis with WHO and the Department of Health (DoH). When necessary, cases are classified by the National Polio Expert Committee based on history, clinical notes and laboratory findings. Additionally the centre provides expertise to the National Task Force and National Certification Committees (NCC) for polio containment in all laboratories nationally.

Regional Polio Surveillance

The centre serves as a Regional Reference Laboratory for poliovirus characterisation for the WHO African region. No wild poliovirus type 1 (WPV1) or wild poliovirus type 3 (WPV3) cases were reported for this period, although incidents of vaccine derived poliovirus (VDPV) have been periodically detected. Madagascar continues to be affected by a VDPV-1 outbreak. Seventeen cases were reported in the period, with 22 August 2015 as the onset date for the last reported case. A total of nineteen VDPV-2 cases were reported from Ethiopia, Democratic Republic of Congo, Republic of South Sudan, Guinea and an environmental sample from Niger. The majority of VDPV-2 cases were from Guinea (n=11) with the most recent having the date of onset as 14 December 2015. The 2015 cases are genetically linked to the initial case reported in 2014 with an onset date in August 2014.

As wild poliovirus type 2 has been globally eradicated, there will be a globally co-ordinated switch from the trivalent oral polio vaccine (tOPV) to bivalent oral polio vaccine (bOPV) in April 2016 (bOPV excluding the type 2 component). In the run up to the switch, monitors are being trained to ensure that no single health facility or vaccine storage centre continues to store or use stocks of trivalent OPV following the switch. As a member of the NTF for Polio Containment, Mr Wayne Howard is involved in the verification of the destruction of poliovirus containing/potentially containing materials in various laboratories in South Africa. He will also be assisting with the validation and verification of the success of the vaccine switch carried out following the switch.

The NICD has indicated to the DoH and the NTF that it intends to become a Polio Essential Facility (PEF) for the handling of wild polioviruses. This is in line with the requirements of the Global Action Plan (3rd Edition) for Poliovirus eradication. The Poliovirus Reference Laboratory is in the process of reviewing operating procedures of a BSL3 laboratory which will suit the requirements for testing to continue through the final stages of poliovirus eradication. Staff will also undergo intensive training to work under the higher containment conditions.

The NICD Regional Reference Laboratory, together with the Pakistan Regional Reference Laboratory, European Research Council (ERC) and National Institute for Biological Standards and Controls (NIBSC) participated in a validation study of an updated Real Time PCR assay for the intratypic differentiation of polioviruses. The assay has been modified to improve the detection rate of WPV1 and WPV3. The validation report from the NICD laboratory was submitted to CDC in March 2016. Once all data submitted from the participating laboratories have been evaluated and verified by the CDC, the protocol will be circulated to the Polio Network laboratories for use as part of the testing algorithm.

National measles surveillance

As the WHO African Region 2020 measles elimination goal approaches, the focus of surveillance indicators will need to change from outbreak identification indicators to case detection, tracking of chains of virus transmission and ultimately the verification

of measles elimination. Serology, specifically the detection of measles-specific immunoglobulin M (IgM) antibodies, was the diagnostic mainstay to detect acute measles infection in the outbreak detection phase of the measles control programme. However, serology alone is not sufficient to define an acute measles case in the elimination phase as the positive predictive value of the test is low when the disease prevalence is low. Thus epidemiological case investigations, in conjunction with other laboratory tests, need to be performed.

The Centre for Vaccines and Immunology is the national referral laboratory for measles surveillance. The laboratory tested 3 412 specimens during the financial year in support of the National Measles Surveillance Programme and identified 38 measles IgM-positive specimens. Following on from case investigations, one case of autoimmune diseases (SLE) and 20 vaccine-associated cases were discarded, leaving 17 cases which could be divided into 14 sporadic cases (genotype B3 was detected in specimens that were PCR-positive) and a cluster of three cases of nosocomial transmission (genotype D8) which resulted in the death of two oncology patients.

Rubella-specific IgM antibody testing on all specimens submitted for measles surveillance was discontinued in 2013 and resumed in May 2015. There were 620 rubella cases identified during the remainder of the review period. Gender was not captured for 14 cases and 49 cases were females of reproductive age.

The centre reports case-based data to the DoH and WHO, and aggregated data to the Multisectoral National Outbreak Response Team (MNORT). The centre works closely with the National Expanded Programme on Immunisation Task Group to classify cases and monitor susceptibility.

Regional Measles and Rubella Surveillance

Collaborators: *WHO*

A minority of countries in Africa offer a second measles vaccine dose in their routine immunisation schedules, thus most countries still rely on supplementary campaigns to provide the second dose. The Reaching Every District (RED) strategic approach has been very useful to improve immunisation coverage in the African Region. Nevertheless intensified efforts are required to increase coverage with two doses of measles vaccine through routine immunisation services, sustaining the implementation of the RED approach, use of supplementary immunisation activities and introduction of a second dose in the routine immunisation schedule where it is not yet offered.

The centre retested 377 serum specimens from nine southern African countries (Botswana, Lesotho, Madagascar, Malawi, Mozambique, Namibia, Swaziland, Zambia and Zimbabwe) for measles and rubella as part of the WHO Regional Quality Assurance Programme. Of these only eight were measles IgM-positive (reflecting the low prevalence of measles in southern Africa) while 85 were rubella IgM-positive (reflecting the high prevalence of rubella in the region as very few African countries offer rubella vaccine).

Environmental Polio Surveillance

In July 2014, the centre established an environmental surveillance laboratory for polio after a brief period of training with immediate implementation of virological testing in support of the GPEI. This service is currently restricted to four selected sites in Angola. From 01 April 2015 to 31 March 2016 a total of 54 samples were received. Results were available for 50 samples; 40 non-polio enteroviruses and nine Sabin vaccine polio viruses were isolated. This suggests good quality sample processing and laboratory techniques in use. In South Africa, approvals for environmental surveillance have been obtained for KwaZulu-Natal and Western Cape provinces. The surveillance sites are sewage treatment plants that were selected based on criteria set by the WHO and there will be one site per province.

Congenital Rubella Syndrome surveillance

The surveillance programme for congenital rubella syndrome (CRS) in South Africa was set up by the centre in the first quarter of 2015 and became fully functional in all nine provinces in February 2016. In preparation for rubella vaccine roll-out in the national Expanded Programme on Immunisation, baseline data on the burden of CRS is being collected. Between 2010 and 2014, retrospective data collection from nine sites in five provinces revealed 36 confirmed CRS cases. Prospective case reporting by 13 sites from six provinces resulted in 35 confirmed CRS cases being recorded from January to December 2015. This baseline data on disease burden will be compared to CRS incidence after the vaccine is introduced, since rubella vaccination has been known to lead to an increase in CRS cases when adequate coverage is not achieved. Using a sentinel site approach, 30 study sites in all nine provinces have been identified and established, where case detection and laboratory testing are being performed on a continuous basis.

RESEARCH PROJECTS

Use of dried blood spots (DBS) for measles serology

Many parents do not consent to the collection of venous blood from their infants. Since finger/heel prick for collection of DBS is widely used for HIV diagnosis and is more acceptable to parents, paired samples of serum and DBS were tested for both measles IgM and IgG serology to validate the use of DBS for routine serology.

Validation of serological assays for the detection of antibodies to Bordetella pertussis

There has been a marked increase in the prevalence of pertussis cases since the global introduction of the acellular pertussis vaccine. Diagnosis in the first six weeks of disease is mainly by culture or PCR, but there is a role for serology in the later course of disease. Various assay formats are being evaluated and validated for use.

Hepatitis C genotype distribution in patient and blood donor samples in South Africa for the years 2008–2012

Collaborators: *T Chirwa, H Smuts, M Vermeulen, AJ Puren*

This retrospective study describes hepatitis C genotypes in residual samples from a patient group (N=941) and from anonymous blood donor volunteers (N=294) between the years 2008–2012. Genotype 1 was predominant in blood donors. Genotype 5a was prevalent in clinical patients. Genotype 1 was predominant in the younger age groups. Genotype 5a was found at higher proportions in the older age groups for both the patient and blood donor groups. Genotypes 1 and 5a were in the highest proportions across all provinces. In blood donors, genotype 1 was predominant among Caucasians and genotype 5a among the Black population. Such information is required for planning the impact on the health sector with regard to newly emerging therapies for hepatitis C and burden of disease.

Naturally occurring resistance mutations within the core and NS5B regions in Hepatitis C genotypes, particularly genotype 5a, in South Africa

Collaborators: *JT Blackard, A Mahomed, W Abuelhassan, J Mahlangu, M Vermeulen, SM Bowyer*

HCV genotypes and/or mutations in the core/non-structural regions have been associated with response to therapy and/or disease progression. This study examines mutations in the core and NS5B regions on pre-treatment isolates from patients or asymptomatic blood donors. Two mutations, associated with interferon resistance – R70Q and T110N – were present in 29 genotype 5a core sequences. No resistance mutation to NS5B nucleotide inhibitor, sofosbuvir, was found. Six putative CD8+ and one CD4+ T-cell epitope sequence in the core region showed binding scores of <300 IC50nM to HLA alleles frequently observed in the South African population. This study provides new insight into one of the lesser studied HCV genotypes and compares the diversity seen in a large pre-treatment cohort with other subtypes.

The assessment of HIV infection or exposure on immune responses to routine oral polio vaccination in infants

Collaborators:*Respiratory and Meningeal Pathogens Unit, Soweto*

Deficiencies in poliovirus immunity in HIV infected and/or exposed infants following the routine immunisation schedule could impact adversely on the overall population immunity and may pose a risk for the development of clusters or even localised outbreaks of circulating vaccine derived polioviruses. Where the deficiency is substantial, it would motivate for supplementation to the routine polio immunisation schedule for HIV infected and/or exposed infants. Infant sera, archived at Chris Hani Baragwanath Hospital, were tested for polio neutralising antibodies at six weeks, to assess serotype-specific response to birth OPV dose, at 10 weeks following the second dose of OPV and at six weeks and 18 weeks following primary vaccine administration. Neutralising antibody responses were compared between non-exposed, exposed and HIV infected infants with or without antiretroviral therapy.

The assessment of the combined oral polio vaccine – inactivated polio vaccine immunisation (OPV – IPV) schedule in an African setting

Collaborators: *Respiratory and Meningeal Pathogens Unit, Soweto*

South Africa is the first country on the African continent to have introduced IPV as part of the routine immunisation schedule. Assessing the effect of the combined schedule versus that of the OPV only schedule may impact directly on determining future IPV strategy for Africa as the global programme for eradication of polio progresses to its final stages. Infant sera archived at Chris Hani Baragwanath Hospital were tested for neutralising antibodies to polio at 6 and 18 weeks; the latter following the complete primary vaccine administration. Neutralising antibody responses were compared between sera collected prior to and subsequent to the implementation of the combined OPV – IPV schedule in HIV non-exposed infants.

TEACHING AND TRAINING

- Mr J Manamela and Mr T Motsamai participated in the WHO IST data management training from 29 February to 4 March 2016 at the DoH, Pretoria.
- The centre hosted a workshop on environmental polio surveillance from 22–26 February 2016 in collaboration with the WHO and the Nigerian Polio Reference Laboratory. A database for environmental surveillance was developed and will be used by countries in the WHO AFRO region.
- The Polio Molecular Unit hosted a colleague from the Uganda Viral Research Institute during December 2015 as part of research collaboration. Training was given in sequencing of the VP1 gene and phylogenetic analysis.
- Dr N Prabdial-Sing and Mr J Manamela attended a WHO consultancy meeting in Pretoria to aid countries in Africa with Phase 1 poliovirus eradication activities. They provided technical support and expertise to poliovirus containment activities as follows:
- Dr Prabdial-Sing: Eritrea, 07–16 November 2015 and Swaziland, 15–18 December 2015
- Mr Manamela: Mozambique, 23–31 Dec 2015.
- Mrs R Williams attended a WHO training course in Uganda (Molecular training workshop in measles, Entebbe 2–12 November 2015) and inter-country Training on Molecular Techniques for Measles and Rubella for Rwanda, Tanzania, Ethiopia, South Africa Uganda and Zimbabwe.
- Dr M Suchard, Mrs M Mashele and Mr W Howard attended the Vaccine Preventable Disease Surveillance Training and Switch workshop for IST ESA countries, from 29 July–1 Aug 2015 in Harare, Zimbabwe.
- Mrs M Mashele conducted on-site training from 17–21 August 2015 when the WHO established a National Measles Serology Laboratory in Seychelles. The training covered measles and rubella testing as well as implementation of routine quality control procedures.
- Mr J Manamela and Dr NV Motaze attended the Congenital Rubella Sentinel Surveillance Training course, organised by WHO in Harare from 17–19 June 2015.
- During the reporting period the centre was involved in the training of three intern medical scientists, a Field Epidemiology Training Programme (FETP) resident, microbiology/virology registrars, two new medical technologists and a data administrator.

PROFESSIONAL DEVELOPMENT

- Mrs S Moonsamy submitted her dissertation for her MTech in Biomedical Technology, University of Johannesburg in December 2015.
- Mr W Howard completed his MSc dissertation and is in the final stages of submission at the University of the Witwatersrand.
- There are currently 10 students registered for higher degrees in the centre: 1 BSc (Hons), 5 MSc, 2 MMED and 2 PhD students.

RESEARCH OUTPUT

Scientific publications

Moonsamy S and Suchard M. Seroprevalence of polio antibodies in adult laboratory staff in South Africa, 2009 to 2013, *Southern African Journal of Infectious Diseases*, 2016; DOI: 10.1080/23120053.2016.1128149

Prabdial-Sing N, Blackard JT, Puren AJ, Mahomed A, Abuelhassan W, Mahlangu J, Vermeulen M and Bowyer SM. Naturally occurring resistance mutations within the core and NS5B regions in hepatitis C genotypes, particularly genotype 5a, in South Africa. *Antiviral Research*. 2016; **127**: 1-9

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Conference presentations

Type	Number
International	7 Oral; 1 Poster
National	2 Oral; 5 Poster

DIVISION OF PUBLIC HEALTH SURVEILLANCE AND RESPONSE

Centre Head: Prof. Lucille Blumberg

BACKGROUND

The Public Health Surveillance and Response Division includes the Outbreak Response Unit, the Epidemiology Support Unit, the GERMS-SA surveillance programme, Travel Health, and the Communications Unit. It provides evidence-based epidemiological and public health expertise and guidance to both the national and provincial health departments through support of surveillance and epidemiological activities as well as outbreak response. Additionally, the division supports other NICD units through collaborative surveillance projects. It facilitates communication and data sharing between the national and provincial health departments, the various centres within the NICD and the public.

During the past year, the division continued to expand significantly and now includes a Data Management Unit and an Emergency Operations Centre (EOC) to respond to public health emergencies. The provincial epidemiology team aimed at increasing the footprint of the NICD within the provinces to provide timely and relevant epidemiology and public health support at local levels has increased representation; now with an epidemiologist in seven of the nine provinces. The division, via the Epidemiology Support Unit, under a directive from the National DoH, is leading the development of an integrated Notifiable Medical Conditions (NMC) national surveillance system that builds on existing resources to provide a co-ordinated approach to the collection, collation, analysis, interpretation and dissemination of public and private sector NMCs in South Africa. Significant progress has been made, including the development, debate and approval of a national surveillance strategy with implementation well under way.

In the first half of 2015 the division continued its focus on intense Ebola response activities, but the need for these decreased, following the successes of a co-ordinated international and national response to the outbreak in West Africa. There were no imported Ebola cases in South Africa during the outbreak but a large number of 'suspected cases' were managed through a risk assessment process, instituted by the Outbreak Unit. Ebola's status as a 'Public Health Outbreak of International Concern (PHEIC)' was rescinded in April 2016, although small clusters of cases are likely to continue in Liberia, Guinea and Sierra Leone due to reintroduction from viable virus populations in sanctuary sites within survivors of the infection. The Outbreak Unit responded to the declaration of the Zika virus as a PHEIC, specifically the likely association with microcephaly, in February 2016, and established guidelines and managed communications for health professionals and the public, together with the NICD Centre for Emerging and Zoonotic Diseases.

The expansion of the GERMS-SA programme for surveillance for a number of priority conditions in rural and urban clinics in the provinces has continued, supported by a network of NICD-appointed epidemiologists. The Mass Gatherings Centre, as part of the WHO Mass Gathering Global Network, has supported research and operational activities on communicable disease monitoring and risk assessments for mass gatherings in the region and is currently in the process of being appointed as a WHO collaborating centre. The South African National Travel Health Network (SaNTHNet), established together with the National DoH and South African Travel Medicine Society in August 2013, has continued to provide reliable and current information and guidelines for travellers to the southern African region. The NICD Communications Unit has played a key role in conveying important public health messages and outbreak alerts to both the medical and allied professionals and to the public, through extensive interaction with the media around a number of outbreaks, including leptospirosis, malaria, Zika, Ebola, diphtheria and rabies to mention a few.

SURVEILLANCE/DIAGNOSTIC SERVICES

GERMS-SA

Surveillance and Diagnostic Services

The GERMS-SA laboratory-based surveillance programme for diseases of public health importance is co-ordinated by a core team within the division and spans many of the centres at the NICD. The laboratory surveillance pathogens include: *Candida* spp, *Salmonella* spp, *Shigella* spp, *Vibrio cholerae, Campylobacter* spp, Enterohaemorrhagic *E.coli*, Diarrheoagenic *E.coli, Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, Staphylococcus aureus*, Carbapenem Resistant *Enterobacteriaceae* (CRE), and *Cryptococcus* spp. GERMS-SA is an active surveillance programme and relies not only on participating laboratories to submit isolates, but also makes use of the NHLS Corporate Data Warehouse to ensure that all cases that meet the case definition are included in the database. Annually, approximately 50 laboratories that do cultures on Cerebrospinal fluid and blood send us

specimens (from both private and public sector), however the drainage for our surveillance specimens includes all ~150 NHLS microbiology laboratories as there is a set referral system for the flow of microbiology cultures. These laboratories report roughly 13 500 cases meeting the GERMS-SA case definitions. The enhanced surveillance arm is operational at 25 sentinel public sector sites across the country, where nurse surveillance officers collect clinical information on patients relating to specific pathogens, namely invasive *S. pneumoniae, H. influenzae, N. meningitidis, Salmonella* Typhi; *S. aureus*, CREs and *Candida* spp bacteraemia; *Cryptococcus* spp, and rifampicin-resistant TB.

The aim of GERMS-SA is to use the data to inform and guide public health policy makers in their decisions. The objectives include estimating the burden of both community- and hospital-acquired infectious diseases under surveillance; monitoring antimicrobial susceptibility trends; monitoring the impact of the HIV/AIDS Comprehensive Care, Management and Treatment Programme in SA on HIV-associated opportunistic infections; and evaluating the impact of vaccines included in the Expanded Programme on Immunisation (EPI). The Laboratory Antimicrobial Resistance Project has been set up to establish a functional, integrated, antimicrobial resistance surveillance system for common, nosocomial, bacterial pathogens. The work carried out by the GERMS-SA team has significantly contributed to the development of clinical guidelines for pneumonia, meningococcal disease, cholera, cryptococcosis and typhoid fever; contributed to the situational analysis of antibiotic resistance in South Africa; and the introduction of pneumococcal conjugate vaccine as well as a booster dose for *Haemophilus influenzae* type b into the EPI. Data emanating from GERMS-SA activities have also contributed to the DoH rollout of the Cryptococcal Antigen (CrAg) Screening Programme to facilitate the early diagnosis, and hence treatment, of cryptococcal meningitis. With these interventions in place it is imperative that surveillance continues in order to monitor the burden of *S. pneumoniae* and *H. influenzae*. *Cryptococcus* spp surveillance will help monitor the CrAg screen and treat programme. GERMS-SA work is funded through the NICD/DoH.

Expansion of the GERMs platform: Xpert MTB/RIF

Xpert MTB/RIF, a rapid diagnostic test that detects both *Mycobacterium tuberculosis* and resistance to rifampicin, has been implemented in all NHLS laboratories nationally and has become the initial diagnostic test for all suspected TB cases in South Africa. In response to this implementation, enhanced surveillance for Xpert rifampicin resistant TB was initiated at Chris Hani Baragwanath Hospital and selected surrounding clinics in late October 2012, and has subsequently been introduced into seven other provinces. This surveillance will monitor trends over time, estimate the proportion of Multi-Drug Resistant TB among rifampicin resistant TB cases and the burden of resistance to other TB drugs, and provide information on risk factors including close contact, previous TB treatment and HIV status. Surveillance will be initiated in the Free State and Western Cape provinces during 2016.

GERMS-SA clinic-based surveillance (STI, HIV and TB)

GERMS-SA recently expanded to include clinic-based surveillance. Three sites have been initiated to date in the North West, Mpumalanga and Eastern Cape provinces. Clinic-based surveillance includes integrated TB/HIV surveillance which aims to characterise the burden of TB-HIV co-infection, describe the proportion of patients actively managed through care, and describe the epidemiology of drug resistance among HIV-infected persons initiating ART and/or TB treatment at the selected sites, as well as undertake STI surveillance. The STI component includes surveillance of STI syndrome aetiologies, gonococcal antimicrobial resistance and HPV genotypes among patients attending the clinic. Additional sites were established in Gauteng, KwaZulu-Natal and Mpumalanga in late 2015 and early 2016.

Building on the zoonotic diseases study which was funded by the Swedish Civil Contingencies Agency (MSB) and Swedish International Development Cooperation Agency (SIDA) and by the Global Disease Detection Program in 2012–14, the Acute Febrile Illness Surveillance Project has been incorporated into clinic-based syndromic surveillance at one clinic site in rural Mpumalanga. The Mnisi area is bordered by the Kruger National Park and contact between wildlife, livestock and humans is frequent. This surveillance is a One-Health project and done in collaboration with veterinary practitioners and researchers from the University of Pretoria Veterinary Faculty.

The aim of the surveillance is to describe the prevalence of zoonotic infections in adult patients presenting with acute febrile illness and for whom the clinic sisters would do a malaria test. Laboratory testing includes PCR and serology for brucellosis, bartonella infections, leptospirosis, Q-fever, tick bite fever (TBF), West Nile virus, Sindbis, Rift Valley fever and chikungunya virus infections. Study data published show a high seroprevalence of TBF, Q-fever and leptospirosis in parallel with significant exposures at the human/animal interface.

Surveillance of Notifiable Medical Conditions (NMCs)

Following the resurgence of fatal epidemics in recent years, the Global Health Security Agenda (GHSA) was launched to enhance global capacities to prevent, detect, and rapidly respond to infectious diseases in line with International Health Regulations (IHR)

requirements. To help achieve the set national public health surveillance goals in South Africa, the DoH directed the NICD in early 2015 to develop an integrated Notifiable Medical Conditions (NMC) national surveillance system that builds on existing resources to provide a co-ordinated approach to the collection, collation, analysis, interpretation and dissemination of public and private sector NMCs in South Africa. Additionally the NICD is expected to build the required field epidemiology and analytical capacity in the public health service required to maximise the utility of NMC surveillance. A team is being established within the NICD to take forward this body of work under the guidance of the senior epidemiologist. In-depth consultations with provincial and local level DoH and relevant key stakeholders are in progress to inform the requirements of an efficient and robust national public health surveillance system. The first phase of the implementation strategy which involves facility and district level assessments to determine human, IT, support, infrastructural, regulatory and guidance resource levels, has been completed in six of the nine provinces. Assessment findings will guide the development and implementation of a re-engineered system, targeted to start in October 2016.

Provincial Epidemiology Team (PET)

Epidemiology is a rare skill in South Africa, but one that is critically needed at provincial and local levels to ensure efficient and real-time data analyses, the results of which are used to direct public health activities, resource allocation and policy decisions at regional and local levels. To address this deficiency, in the last few years, the NICD has deployed seven provincial epidemiologists to Gauteng, Limpopo, Western Cape, Mpumalanga, North West, Free State and Eastern Cape DoH provincial offices, under the guidance of a senior epidemiologist based at the NICD Sandringham campus. Efforts are still under way to provide support to Northern Cape and KwaZulu-Natal. The aim of this service, integral to the NICD's functions, is to ensure that the NICD's core services of surveillance, outbreak response, specialist microbiology and public health research are available to the Provincial Departments of Health in a timely, flexible and rapid manner.

Key achievements thus far include, the provincial epidemiologists' support and guide outbreak response at local levels and the Gauteng provincial epidemiologist supported contact tracing and management during the typhoid outbreak in January 2016. In the last quarter of 2015, the North West provincial epidemiologist supported the malaria outbreak response and was a key liaison between the province and the NICD in facilitating epidemiological and entomological investigations. During the diphtheria outbreak in KwaZulu-Natal which endured over three months in 2015, the then KwaZulu-Natal epidemiologist led and co-ordinated the epidemiology components of the outbreak, including contact tracing and monitoring, case mapping and the establishment of epidemiologic links and use of these data to contain transmission and direct vaccination efforts.

Significant milestones have been achieved in supporting the TB directorate to achieve the national 90-90-90 strategy. The PET is central to enhancing patient management following TB diagnosis via GeneXpert. Laboratory based alerts of all diagnosed rifampicin resistant TB patients are sent weekly to the provincial teams, including the respective provincial epidemiologist who ensures that the alerts are distributed to local levels to facilitate rapid patient tracing and timely treatment initiation. In the North West, Eastern Cape and KwaZulu-Natal provinces, these alerts have significantly benefited the TB programme by ensuring that diagnostic results are timeously channelled to the local levels for patient tracing. Additionally for KwaZulu-Natal, the TB programme provincial data from patient records is consolidated with laboratory based diagnosis data to identify gaps between patient diagnosis

Figure 9: NMC Surveillance National Meeting on 26 January 2016

and treatment initiation. Furthermore, results from epidemiological analyses of these data are utilised in identifying TB hotspots and high risk groups, and direct resource allocation and interventions to curb TB transmission. In the Eastern Cape, the provincial epidemiologist has developed a quarterly TB bulletin that highlights TB burden and TB programme performance per quarter.

Currently the PET, in collaboration with national and provincial DoH, is conducting a nationwide assessment of the standing NMC surveillance. The objective is to improve the existing system as mentioned earlier. As a way of building and strengthening the epidemiology capacity within the provinces, the provincial epidemiologists identify training needs and provide required training and mentoring either directly or via available structures of the NICD such as the SAFETP and centre-specific training programmes.

Through the PET, the NICD is better able to understand the diverse needs of each province, hence enabling relevant and timely communication between the NICD and local departments of health and deployment of required information, guidance and expertise. Additionally the provincial epidemiologists have been pivotal in the implementation of the NICD clinic-based surveillance programmes.

NICD Data/Information Centre

Work continues within the NICD Data/Information Centre which aims to centralise management of data generated within the NICD, provide technical support/expertise for GIS and provide a Data Repository for the NICD including the dashboards. Efforts still continue to build the required team and align the data centre goals with those of the DoH Information Management Directorate.

Outbreak Response Unit

The Outbreak Response Unit (ORU) provides technical support for all aspects of communicable disease outbreaks and control in South Africa. Through close collaboration with provincial and national health departments and other stakeholders, and together with systems for early detection and improved reporting of epidemic-prone communicable diseases, the ORU functions as a source of intelligence for outbreak detection and facilitates comprehensive outbreak response activities. In addition, close partnerships with NHLS diagnostic laboratories and NICD Centres provide appropriate laboratory diagnostic services during outbreaks and specialised diagnostic testing as required. The Unit is also kept abreast of international developments in outbreaks and outbreak preparedness through representation on key WHO advisory committees and international interest groups.

Public Health Services

The ORU's role in outbreaks includes outbreak detection and verification, field investigation, development of clinical and laboratory guidelines, management of laboratory data and interpretation of results, reporting, and recommendations for prevention and control. Over 450 outbreak verification calls were attended to by ORU directly or through the NICD Hotline over the financial year 2015–6. Figure 10 represents the number of calls per category, and Figure 11 represents the source of the verification event.

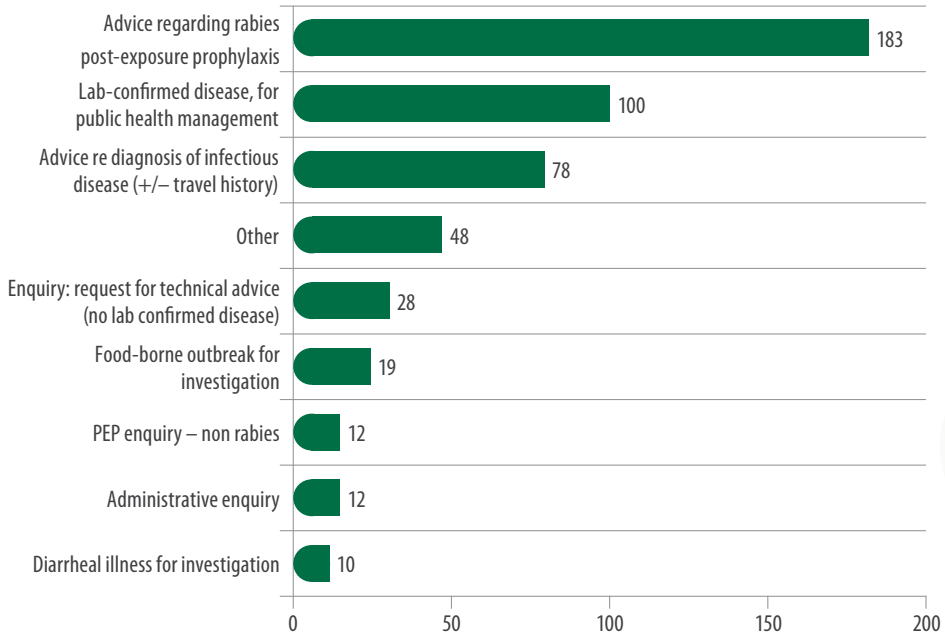

Figure 10: Number of outbreak verification events attended to over the financial year 2015/16 by category of event

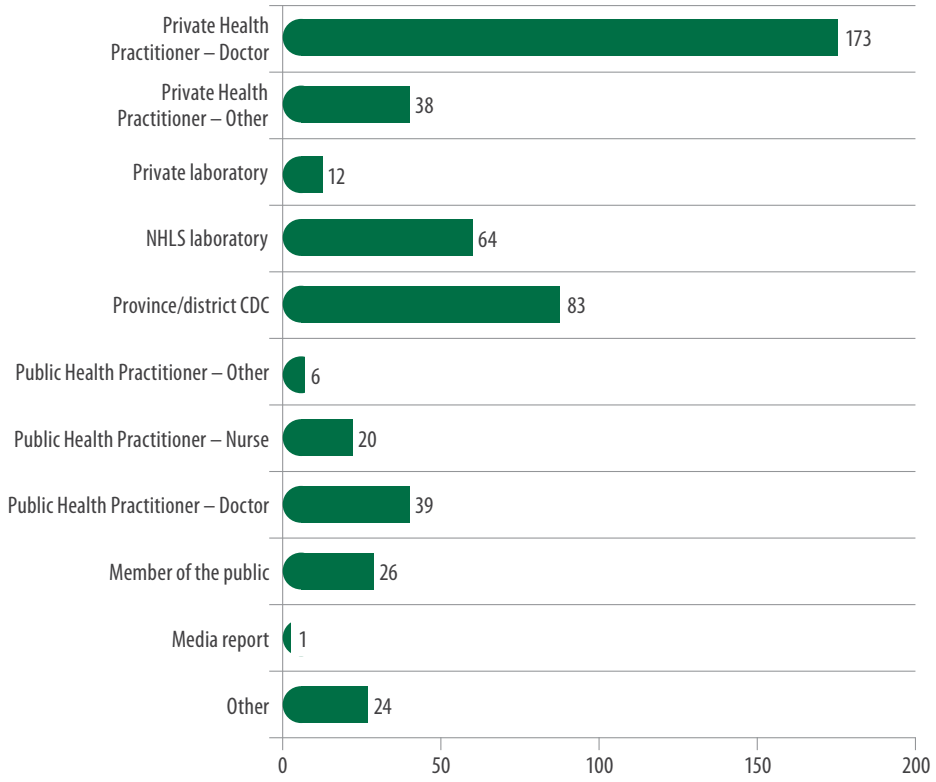

Figure 11: Source of outbreak verification events attended to during the financial year 2015/16

Among 486 events, 223 (45%) originated from the private sector, 212 (44%) from the public sector and 51 (10%) from the media or members of the public. The majority of calls originated from Gauteng (173) followed by Western Cape (98) and KwaZulu-Natal (90). The ORU, together with the NICD Centres, attended to a number of potentially major public health events over the course of the year as follows:

- **Diphtheria in KwaZulu-Natal** – Fifteen cases of diphtheria were identified (11 confirmed, 3 probable, 1 possible) with four deaths from March until June 2015. The ORU assisted the KwaZulu-Natal DoH through provision of guidelines; securing a donation of diphtheria anti-toxin; assigning of case definitions; maintenance of the outbreak line list; daily publication of a situational report; supervision of Field Epidemiology Training Programme (FETP) residents and provision of staff who supported field teams in contact tracing; vaccination campaigns; and monitoring and evaluation of outbreak activities. The Centre for Respiratory Disease and Meningitis assisted with specialist diagnostic testing and molecular epidemiological investigations.
- **Leptospirosis in Pollsmoor prison, Western Cape** – Three cases of leptospirosis were identified in offenders in the awaiting trial section of Pollsmoor prison in September 2015. ORU conducted a field visit to assess conditions in the facility and facilitated rodent capture and investigation for leptospirosis (done by the Centre for Emerging and Zoonotic Diseases (CEZD)). Subsequently, ORU, together with the provincial DoH, drew up a pre-emptive treatment and diagnosis plan for the institution, wrote guidelines on leptospirosis diagnosis and management, and facilitated diagnostic testing of offenders (conducted by CEZD). As a result of the NICDs intervention, the awaiting trial facility at Pollsmoor was temporarily closed and offenders relocated to facilitate rodent control and refurbishment.
- **Typhoid in Gauteng and South Africa** – In January 2016, a seemingly high number of typhoid cases were identified and reported by the media. The ORU together with the Centre for Enteric Diseases (CED) and provincial departments of health facilitated appropriate investigation of cases. ORU facilitated this through revision and dissemination of guidelines, FAQ and case investigation forms, collation of a national line list of typhoid cases and analysis of epidemiological and molecular data to interpret findings. As of 19 April 2016, over 50 typhoid cases have been identified nationally. ORU was able to reassure government departments and the public that the number of cases was not increasing year-on-year, and that the majority of cases were imported from other countries, including Zimbabwe, India and Bangladesh.
- **Listeriosis in Western Cape** – A cluster of cases of listeriosis was identified in infants and immunocompromised persons at hospitals in the Western Cape. Together with the NHLS Laboratory at Groote Schuur, the NICD's CED and ORU supported an epidemiological and laboratory investigation into the outbreak. A FETP resident contacted patients, their parents or relatives and obtained a history pertaining to food ingestion and exposures. CED identified that several cases of infant listeriosis were attributed to an identical clone. Further investigations are ongoing.

- **Zika virus** – The NICD, together with the Vector Control Unit and CEZD, facilitated a public health response to the emerging threat of Zika virus disease. In response to an alert from a private laboratory, ORU facilitated case investigation and management of the first case diagnosed in South Africa. ORU attended to many calls related to Zika diagnosis and management in returned travellers, particularly amongst pregnant women or persons wanting to conceive. ORU produced a travel advisory, diagnostic guide for clinicians and FAQ document. Guidelines are in development.
- **Support for the City of Johannesburg relating to striking refuse collectors** – ORU, together with the Centre for Opportunistic, Tropical and Hospital infections (COTHI), GERMS-SA and CDW provided data and information including plague and leptospirosis awareness FAQs to support the public health response to the accumulation of waste in the city following strikes by refuse collectors, and the finding of a plague seropositive rodent through the Rodent Surveillance Programme within the metro.
- **Legionellosis outbreak in a Cape Town Hotel** – Together with the Centre for Respiratory Disease and Meningitis, and the NHLS Infection Control Laboratory at Charlotte Maxeke Hospital, ORU facilitated an investigation into three Legionnaire's disease cases that had been identified by the European Legionnaire's Diseases Surveillance Network (ELDS-net) and reported to ORU. An environmental assessment of the hotel was done, revealing deficiencies in water system maintenance and contamination of the system with *Legionella pneumophila*. Remedial actions were taken by the hotel group.
- **Malaria in North West Province** – An outbreak of four cases of malaria at Madikwe in North West Province was investigated by ORU together with COTHI. A site visit and investigations revealed no malaria vectors in the area, nor gametocyte carriers amongst the affected community, leading to the conclusion that the cases were caused by importation of an infected mosquito from a malaria endemic area. Several staff and visitors to the area had recently returned from a malaria endemic area, lending support to the conclusion of the investigation.
- **Drug-resistant tuberculosis in Mpumalanga province** – A report by a NGO in Mpumalanga of an increased number of drug-resistant TB cases (DR-TB) prompted an investigation supported by ORU and the Centre for TB. An assessment of hospital records, interview of patients and healthcare workers in the NGO and local clinics concluded that the increased number of cases was an 'apparent' increase, resulting from improved adherence to the guidelines for TB diagnosis. Recommendations were made to the Mpumalanga DoH.

The ORU continues to publish a monthly Communicable Diseases Communiqué, which reports recent outbreaks and communicable disease cases/issues of relevance. This is distributed to a wide audience including general practitioners, specialists, infectious diseases and travel medicine societies, and national and provincial public health personnel. In addition, the unit published special urgent advisories and communiqués in response to acute events requiring immediate dissemination of information.

Research and Special Projects

Pertussis surveillance project

Investigators: *Dr Juno Thomas, Genevie Ntshoe and Prof. Lucille Blumberg (ORU, NICD); Dr Gary Reubenson (Rahima Moosa Mother and Child Hospital and University of the Witwatersrand); Dr Ranmini Kularatne (National Health Laboratory Service), Prof. Theuns Avenant and Prof. Nicolette du Plessis (Kalafong Hospital and University of Pretoria)*

Research funding: *Sanofi Pasteur*

Case enrolment ended in October 2015. The project was a prospective, hospital-based, sentinel surveillance for pertussis and was undertaken at two paediatric departments in Gauteng Province (Johannesburg and Pretoria). The project aimed to describe the prevalence and characteristics of pertussis disease amongst children ≤10 years of age who were hospitalised with suggestive respiratory illness, or in the case of infants and young children, presented with apnoea for investigation. Where informed consent was granted, demographic, clinical and epidemiological data were recorded and nasopharyngeal specimens were collected for *B. pertussis* and *B. parapertussis* PCR testing as well as culture for *Bordetella* spp. From August 2013 to October 2015, 1 040 patients were enrolled in the study, 9% of whom were positive for *Bordetella* spp.

Representation on WHO committees and advisory groups

Prof. Blumberg served on the WHO Scientific Advisory Group for the Blueprint on Research and Development Preparedness for emerging pathogens, which conducted the following activities: i) Prioritisation of emerging diseases for preparedness planning, ii) Research and development pertaining to vaccines and therapy, and iii) Developing of funding opportunities to support preparedness activities. Prof. Blumberg also served on the WHO International Health Regulations Emergency Committee pertaining to EVD, which was responsible for declaring and rescinding the status of 'Public Health Emergency of International Concern' in respect of the Ebola virus outbreak in West Africa. Prof. Blumberg was appointed on the Ministerial Ebola Committee in South

Africa, which advised the Minister of Health on prevention and response to the introduction of Ebola into South Africa. She also continued to serve on a number of national advisory committees, namely the South African Malaria Elimination Committee (Chairperson), National Advisory Group for Immunisation and the Rabies Advisory Group.

Travel Health

This unit provides a consultative service for health practitioners regarding pre-travel advice for travellers and clinical consultations for returning travellers with suspected infectious diseases; develops guidelines for a number of travel-related diseases and neglected diseases; serves as a point of contact and liaison internationally for infectious diseases acquired in southern Africa, and assists with training of travel health practitioners and those studying tropical diseases. There is a focus on zoonotic diseases and emerging pathogens through the One Health approach brought about by the interactions between animal and human health and the environment. The unit was recently accepted as a Geosentinal Programme member. This programme includes 64 global sites that monitor imported infectious diseases in business and leisure travellers, as well as migrants and displaced persons.

South African National Travel Health Network (SaNTHNet)

http://www.santhnet.co.za

SaNTHNet is a travel health network run by the DoH, the NICD and the South African Society of Travel Medicine (SASTM), and was launched in August 2013. SaNTHNet provides up-to-date information on health risks for travel in the southern African region, with a primary South African focus by developing and providing guidelines on communicable diseases and up-to date information on disease outbreaks. An informative website has been developed which has attracted over 5 000 visits a month, a significant number of which are of international origin. The network will focus on developing further guidelines around travel related health matters and will serve as a surveillance platform to gather information around imported communicable diseases e.g. dengue, trypanosomiasis and leishmaniasis as well as expert advice on diagnosis and management of tropical and travel related diseases. The unit also manages a supply of essential drugs for a selection of tropical and neglected diseases e.g. leishmaniasis, trypanosomiasis and severe malaria.

WHO Collaborating Centres on Health at Mass Gatherings

http://www.who.int/ihr/publications/mass_gatherings/en/

The Mass Gatherings Centre was established for communicable disease surveillance and risk assessment for the 2010 FIFA World Cup and has now become part of the WHO Mass Gatherings Collaborating Centre Network, which includes the Disaster Research Centre, Flinders University, Australia; Public Health England, United Kingdom; National Institute of Communicable Diseases (NICD), South Africa; Institute of Public Health of Vojvodina, Serbia; School of Public Health, University of Washington, United States of America; Ministry of Health, Saudi Arabia.

WHO Collaborating Centres held a workshop on 19–20 April 2015 in Cape Town, as part of the World Congress for Disasters and Emergency Medicine. Prof. Blumberg conducted a consultancy with WHO for the All Africa Games in Brazzaville Republic of Congo (September 2015) and advised on the establishment of a surveillance and response system for communicable diseases for the games. Since the games took place during the Ebola outbreak in West Africa, there was concern around the introduction of Ebola in the Congo by participants or visitors to the games. The games were very successful and no major communicable diseases were reported.

NICD Communications Unit

The National Institute for Communicable Diseases (NICD) Communication Unit continues to expand its role in conveying important public health messages and outbreak alerts to both the medical and allied professionals through guidelines and information sheets, as well as to the general public through media releases, interviews on TV, radio and the print media and through the introduction of social media. The NICD has, over the past year, set up a Twitter account, which is an effective tool for public health messaging.

The NICD website is proving to be an invaluable resource for information regarding outbreak response, diseases surveillance and infectious diseases information. A case in point would be the setting up of a portal on the NICD website dedicated to updating information on a daily basis during the Typhoid incidents and the Zika virus outbreak . Information on the NICD Centres is updated monthly; the Alerts and News sections are updated daily, or as required. During the reporting period, the NICD website continued to grow in attracting the general public, the media and health professionals. To meet increasing demands, media training for senior staff was undertaken.

A new electronic format has been successfully developed for disseminating NICD publications to stakeholders in the public and private sector and to national and provincial Departments of Health. These include the monthly Communicable Disease Communiqué, which highlights current communicable disease events and outbreaks; the monthly NICD Surveillance Bulletin, which collates data from the NICD surveillance programmes; and the Quarterly Bulletin, which more formally documents outbreaks and surveillance programmes.

The unit continues to collate internal publications; the NICD Science Focus is a quarterly compilation of abstracts of scientific publications by NICD staff members, published in peer- reviewed journals; and the NICD Newsletter which has a more informal focus on events and happenings at the NICD, and includes staff awards and profiles of individual staff members and their work.

Over the past year a good partnership was forged with the DoH Communications team and there is now collaboration in media relations during high profile events such as outbreaks with international and local significance.

TEACHING AND TRAINING

Staff delivered lectures for training activities related to communicable diseases for the Gauteng DoH, the DoH, under- and post-graduates of the University of the Witwatersrand (School of Public Health, Departments of Medicine, Obstetrics and Gynaecology, Community and Family Medicine, Diploma in Tropical Medicine and Hygiene), University of Pretoria, Onderstepoort Veterinary Institute, University of the North West (School of Pharmacology) and Stellenbosch University (Department of Medicine).

The unit collectively supervised 15 FETP residents and four public health registrars on rotation through the unit.

Prof. Lucille Blumberg supervised one MSc (Environmental Health) (awarded April 2016); and one PhD (Public Health) (ongoing).

PROFESSIONAL DEVELOPMENT

- Dr Kerrigan McCarthy is registered for a PhD at the University of the Witwatersrand, School of Public Health
- 1 MSc
- 1 MPH

HONOURS

Prof. Blumberg received the WSAVA One Health Award (this award is presented to an individual/organisation that promotes the Global One Health concept) which included a small monetary reward which had to be donated to a worthy human/animal One Health cause. Prof. Blumberg donated the funds to the East London SPCA, a perfect One Health cause given that rabies is of special interest. Animal welfare workers are a very vulnerable group, as they deal with 'problem' animals that may have rabies, while pre-exposure vaccination is not always given because of cost. SPCA East London is situated in a rabies risk area but did not have funds to vaccinate staff.

RESEARCH OUTPUTS

Publications

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NICD Publications

1. Monthly NICD Communique
2. Quarterly NICD Communicable Diseases Surveillance Bulletin
3. Surveillance Officer monthly communiqué
4. Monthly NICD Surveillance report
5. Monthly MNORT reports
6. Quarterly GERMS-SA ESSORs, Provincial Statistics and PEPFAR report
7. Quarterly pertussis surveillance project progress report to Sanofi Pasteur.

Conference Presentations

Type	Number
International	11
National	19

SOUTH AFRICAN REGIONAL GLOBAL DISEASE DETECTION CENTRE

Centre Head: Dr Natalie Mayet

BACKGROUND

The South African Regional Global Disease Detection Centre (SARGDDC), established in 2010 in South Africa as the eighth of ten Global Diseases Detection Programmes, continues to integrate and embed its core activities with collaborating partners in the common goal of preventing, detecting and effectively building capacity for responding to infectious disease threats.

The SARGDDC has 14 collaborative projects with the DoH and NICD, and employs 34 staff members through Research and Non-research Co-operative Agreements. Five of the staff have been permanently employed in positions at the NICD. The Non-Research Co-operative Agreement, with principal investigators located at both the NICD and DoH, has entered into its fifth year since inception. The seven projects in this agreement include: Public Health Emergency Preparedness and Response Capacity Building in South Africa; Strengthening Malaria Surveillance in South Africa; PulseNet Africa; Emergency funds for VHF outbreaks in Africa – Mobile laboratory; Analysing the South African Notifiable Disease Surveillance System; Supporting Capacity for Field Epidemiology in South Africa; and the Permit Process for the Import and Export of Biological Agents.

The Research Co-operative Agreement is in its forth year and includes five flu-related projects: i) Investigation of influenza burden, interaction with other pathogens and nosocomial transmission at sentinel surveillance sites; ii) Effectiveness of trivalent inactivated influenza maternal vaccination and evaluation of the vaccination programme among pregnant women and their new-borns in South Africa; iii) Household transmission of influenza amongst HIV-infected and uninfected individuals in South Africa; iv) Healthcare utilisation survey, and v) Investigating the contribution of swine and or avian influenza A viruses to influenza-like illness and pneumonia in South Africa. Two projects have moved from the Non-Research Co-operative Agreement to the Research Co-operative agreement: Additional methods for controlling malaria in South Africa; and Harbouring of viral zoonotic agents by the Southern Africa bat population.

SARGDDC continues to contribute to activities including the development of field epidemiologists through the South African Field Epidemiology Training Programme; the development of the Emergency Operations Centre at the NICD; participation in the South African Malaria Elimination Committee; collaborating in various influenza research activities; formulation of the One Health Strategy for South Africa; technical support to the DoH Communicable Disease Cluster; assisting with re-engineering of the Notifiable Medical Condition Surveillance System and support for the formation of the National Public Health Institute of South Africa.

SPECIAL PROJECTS

Dr Mayet and Prof. Anne Von Gottberg represented the DoH at the Global Health Security Agenda (GHSA) meeting held in Seoul in September 2015. A number of follow up activities relating to the GHSA work packages are planned pending Parliamentary approval for participation in the GHSA.

The SARGDDC team commenced with the development of an e-learning basic epidemiology course, together with ITECH and the DoH, aimed at building in-service capacity for DoH staff. This tool will be also be used as a selection tool to access the eligibility to participate in the FETP advanced course.

The team hosted a number of international delegations who had expressed interest in learning more about the SARGDDC and the SAFETP activities. Delegations included the Ethiopian Public Health Institute; the University of Indiana USA; the Defence Threat Reduction Agency and the United States Strategic Command Centre for Combating Weapons of Mass Destruction.

Dr Kuonza, a Senior Epidemiologist and SAFETP staff member, provided technical expertise on the Harmonisation of Surveillance Frameworks for Communicable Diseases in the SADC Region at a workshop held in the Seychelles in September 2015.

Dr Carl Reddy, the SAFET Programme Director, was part of a review team involved in the evaluation of the Colombia Field Epidemiology Programme, aimed at their accreditation for the Training Programme in Epidemiology and Public Health Interventions Network (TEPHINET). The lessons learnt are being used to advance the SAFETP application process for TEPHINET accreditation.

Dr Carl Reddy attended the Global Launch of the *No More Epidemics Campaign*. Speakers included Dr Nancy Knight, Country Director, Centers for Disease Control and Prevention, South Africa.

SAFETP continues to explore opportunities to expand the geographic footprint of the programme to increase the number of field epidemiology graduates. To this end the SAFETP staff met with delegates from the University of Limpopo; University of KwaZulu-Natal; and University of the Witwatersrand School of Public Health. The team also engaged with Prof. Thandi Puoane, an Emeritus Professor from the University of the Western Cape and a Non-Communicable Diseases Epidemiologist for FETPs in the Afro Region, to explore the expansion of competencies of the field epidemiologists to include non-communicable diseases.

Six SAFETP graduates were employed as the provincial epidemiologists for Limpopo, Gauteng, Free State, Eastern Cape, North West and Mpumalanga provinces. The SAFETP and Provincial Epidemiology Teams work closely in building epidemiological capacity in the respective provinces.

TEACHING AND TRAINING

In 2015 FETP had an intake cohort of ten first year and thirteen second year students. Three residents graduated in the year with a graduation attainment rate of 78%. The residents have participated in more than 15 outbreak investigations, conducted 21 large data base analyses and produced the following dissertations as part of their core learning activities:

1. Epidemiology of meningitis among adults in a province with a high HIV prevalence, South Africa, 2009 to 2013.
2. Cognitive and behavioural determinants of multiple sexual partnerships and condom use in South Africa: Results of a national survey.
3. Treatment outcomes for HIV positive MDR-TB patients on ARVs treated with moxifloxacin or ofloxacin containing regimen at Sizwe Tropical Disease Hospital, Gauteng and Witbank TB Specialised Hospital, Mpumalanga between June 2007 and June 2012.
4. Incidence and risk factors of tuberculosis smear non-conversion in Eden District Municipality, Western Cape Province, 2007–2013.
5. Risk factors associated with malaria mortality among laboratory confirmed cases in Tshwane district over a period of four years. A retrospective cohort study: 2011–2014.
6. Risk factors for tuberculosis infection in coal mines: Mpumalanga Province, South Africa, 2014.
7. Predictors of condom use among sexually active young women – National HIV Communication Survey, South Africa, 2012.
8. Exploring the knowledge, attitudes and practices (KAP) of healthcare providers on viral hepatitis notification in Gauteng, South Africa.
9. Transactional sex between male partners in Mpumalanga, South Africa 2012– 2015: Prevalence and association with HIV infection.
10. The epidemiology of caliciviruses in hospitalised children under five years of age in selected sentinel sites in South Africa, 2009–2013
11. Evaluating the performance of case definitions used for severe acute respiratory illness surveillance in identifying influenza infections in Edendale and Klerksdorp, South Africa, June 2010– December 2014.

Dr Lazarus is an honorary lecturer at the University of Pretoria and was responsible for the co-ordination and teaching of five modules for the Field Epidemiology Training Programme Track. In addition he delivered lectures in epidemiology at the Universities of the Witwatersrand and Stellenbosch.

SAFETP conducted a short course in Basic Epidemiology at the Inkosi Albert Luthuli Hospital for 23 participants from the KwaZulu-Natal DoH in May 2015. This short course offered an introduction to the basic concepts and methods of Applied Epidemiology, with a focus on outbreak detection, investigation and response. The second week of the short course was conducted in July and 14 work-related field projects were completed.

SAFETP Staff conducted short course training in Basic Epidemiology at the Eastern Cape Department of Health in August 2015. The training was attended by 25 participants from various districts within the Eastern Cape Province and offered an introduction to the concepts and methods of Applied Epidemiology. This was followed by a second week of training in October 2015. Eleven field projects were completed.

Ms Dorothy Southern, the SARGDDC scientific writer, facilitated a Scientific Writing Workshop for 11 first year residents in June 2015 before they were assigned to their field sites. She also facilitated a Grant Writing Short Course in February for 7 participants from the NICD.

Mr Alfred Musekiwa, the SARGDDC biostatistician, provided an Advanced Biostatistics training course for 11 SAFETP residents of the 2015 Cohort in May 2015.

The SAFETP training for field supervisors was hosted in August for 22 existing and potential supervisors. The objective of the training was to orientate supervisors on their roles and to identify challenges and opportunities to being a successful supervisor.

SARGDD facilitated National Health Information Repository and Data Warehouse Training in September at the NICD. The training was provided by Mr Kabelo Molepo from Health Information Systems and all 26 participants gained ongoing access to the DoH NHIRD database.

PROFESSIONAL DEVELOPMENT

Dr Lazarus Kuonza attended the Infectious Disease Modelling course at the London School of Hygiene and Tropical Medicine from 22 June–3 July 2015 with a sponsorship from the DTRA. The main objectives of the course were to introduce participants to the applications of mathematical modelling in understanding the transmission dynamics of infectious diseases. He presented lessons learnt at the NICD EPI forum on his return.

Dr Reddy attended the 8th Training Programme in Epidemiology and Public Health Interventions Network (TEPHINET) Global Scientific Conference held in Mexico City in September 2015. This was followed by the TEPHINET FETP Directors Meeting.

Dr Reddy participates in the Finance and Audit, Human Resources and Quality Assurance subcommittees of the African Field Epidemiology Network (AFENET) Board.

HONOURS

Ms Eva Mathatha won 3rd Best Presentation Award in the 2nd Snows Science Conference for Early Career Scientists Working in Africa, held in Golden Tulips Kumasi City Hotel, Ghana in May 2015, for her presentation on *Outbreak of Cholera in Diepsloot, Johannesburg, South Africa*.

Ms Lactatia Motsuku won the Best Poster Presentation Award at the FIDSSA Congress 2015, held at the Drakensberg, for her presentation *Foodborne illness outbreak at an intermediate school in North West Province, South Africa: October 2014*.

The MPH attainment rate has increased from 51% in 2011 to the current rate of 78%.

Two residents from the 2013 cohort Ms Akhona Tshangela and Ms Moira Beery were awarded the Masters in Public Health degree at the graduation ceremony that took place at the University of Pretoria in April 2015 and Dr Ngormbu Ballah graduated with his MPH at the graduation ceremony held in September 2015.

Ms Akhona Tshangela (Cohort 2013) accepted a position as a Field Epidemiologist with Africa CDC, based at the Africa Union in Addis Ababa, Ethiopia.

Mr Nevashan Govender (Cohort 2010) was appointed as Operations Manager of the Emergency Operation Centre (EOC) at the NICD.

RESEARCH OUTPUT

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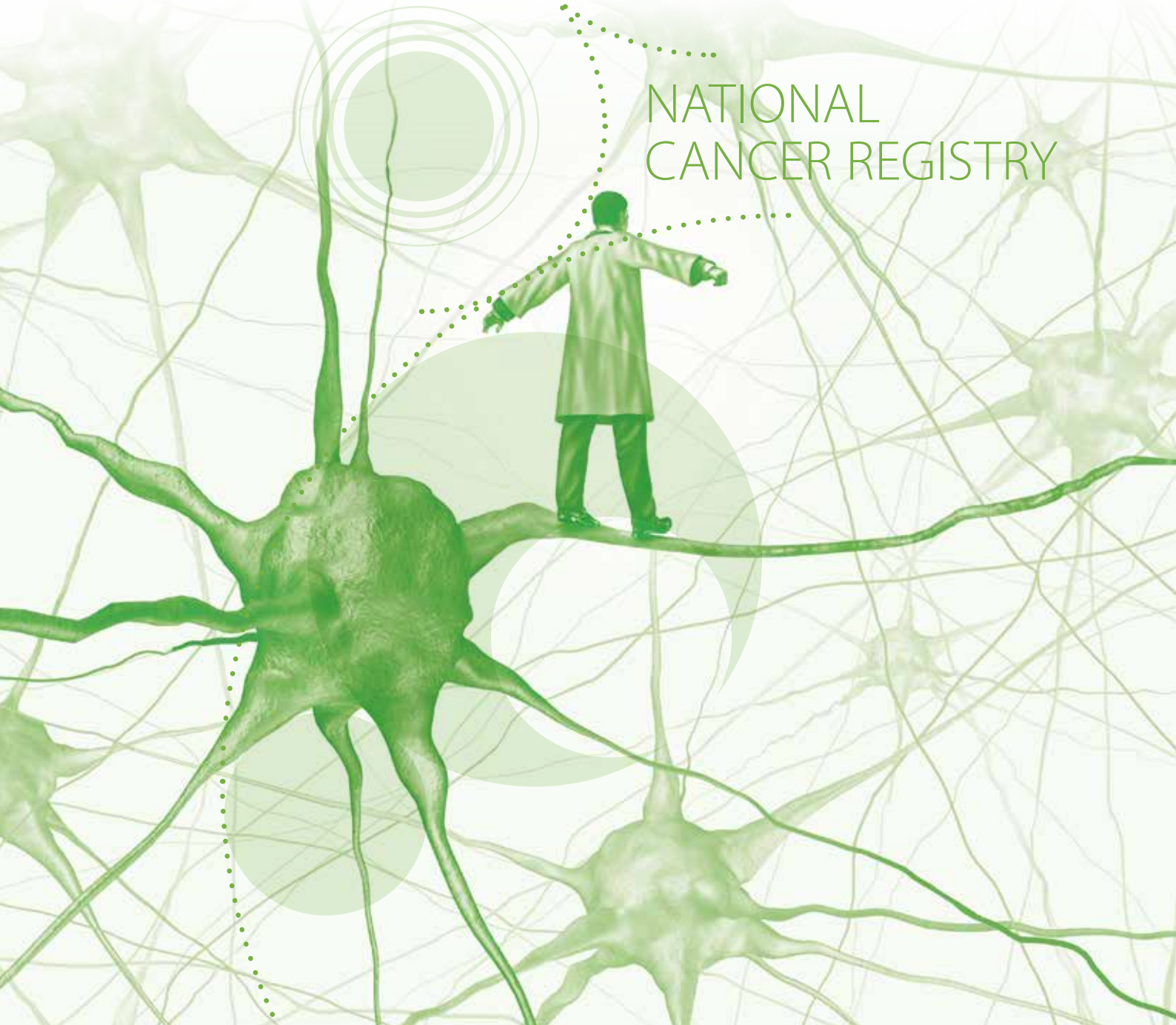
6. **Nicola Page**, Michelle J. Groomed, Tanya Murray, Sandrama Nadana, Rembuluwani Netshikweta, Karen H. Keddy, Bhavani Poonsamy, Jocelyn Moyes, Sibongile Walaza, Kathleen Kahn, Lazarus Kuonzaa, Maureen B. Taylor, Shabir A. Madhi, Cheryl Cohen. *Sapovirus prevalence in children less than five years of age hospitalised for diarrhoeal disease in South Africa, 2009–2013.* Journal of Clinical Virology 78 (2016) 82–88. Homepage: www.elsevier.com/locate/jcv

7. **Ballah NJ, Kuonza LR,** De Gita G, Musekiwa A, Williams S and Takuva S. Decline in syphilis seroprevalence among females of reproductive age in Northern Cape Province, South Africa, 2003–2012: utility of laboratory-based information. *Int J STD AIDS*. February 27, 2016; as doi:10.1177/0956462416636727

Presentations

Type	Number
International	16
National	9

NATIONAL CANCER REGISTRY



Head: Dr Elvira Singh

Cancer is projected to become a leading cause of morbidity and mortality in developing countries in the near future, with an increase from 6.1 million new cases in 2012 to 9.9 million new cases in 2030. Cancer surveillance information forms the cornerstone of health decision making for cancer services.

The National Cancer Registry (NCR) is South Africa's only source of national cancer incidence data and hosts the largest cancer study in Africa, namely the Johannesburg Cancer Case Control Study (JCS). The NCR was established as a pathology-based cancer surveillance system in 1986, and continues to report on laboratory-diagnosed cancer cases from public and private pathology laboratories in the country. Following the publication of Regulation 380 of the National Health Act in 2011, which made cancer a reportable condition, the NCR has initiated population-based cancer registration at its first sentinel site in South Africa. The NCR aims to be a resource of knowledge and expertise on cancer surveillance and cancer research for the South African Government and other relevant stakeholders, in order to assist in the planning of policies, programmes and research to support appropriate responses to cancer care challenges.

SURVEILLANCE

Pathology-based cancer surveillance

The NCR publishes cancer incidence for all cancers by race, gender and age, based on reports received from histology, cytology and haematology laboratories countrywide. In the year under review, unprecedented numbers of case reports were received, due to improved data collection, with over 75% of the cases reported to the NCR electronically. Generous grant funding from CANSA allowed for the employment of additional staff, increasing the number of coders from five to eight and allowing the bottleneck in data processing to be eased. Thus, cancer incidence estimates for 2010 were published and those for 2011 are currently being analysed. The lag in reporting is a common and accepted occurrence in cancer surveillance as a result of the labour intensive nature of cancer coding. However, plans are under way to develop an automated cancer coding software using natural language processing in order to expedite the cancer coding process. Cancer incidence estimates for 2010 changed little from the previous year, with breast and cervical cancer being the most common cancers amongst women according to age-standardised incidence rates, and prostate and lung cancers having the highest age-standardised incidence rates in men. It must be noted that this system represents the minimum number of cases in the country, as cancer patients who do not access pathology laboratories are not recorded in this surveillance system.

Population-based cancer surveillance (PBCR)

The population-based cancer registry complements the pathology-based registry as every case of cancer confirmed in the designated catchment area is recorded, regardless of the method of diagnosis. However, the limitation is that cancer incidence is calculated based on a sentinel site rather than the national population.

One surveillance officer was recruited in the financial year, allowing the NCR to begin data collection at Thelle Mogoerane Hospital and a private healthcare facility covering Vosloorus, Kathlehong and Thokoza, (population of 676 337 individuals). Between October 2015 and February 2016, 283 notifications were collected, captured and coded. The standard operating procedure for the population-based cancer registration site was finalised and implemented in October 2015.

RESEARCH PROJECTS

The epidemiology of HIV-related cancers in South Africa

NCR Researchers: *Dr M Sengayi, Dr E Singh*

Collaborators: *Prof. M Egger; Dr J Bohlius (Institute of Social and Preventive Medicine, University of Bern, Switzerland)*

Funding: *University of Bern, Switzerland*

The aim of this study was to characterise the epidemiology of HIV-related cancers in South Africa in terms of spectrum, prevalence and incidence of cancers in HIV positive people in the era of antiretroviral treatment in South Africa. Ascertainment of cancer in HIV cohorts was incomplete, and probabilistic record linkage was both feasible and essential for cancer ascertainment. The prevalence of cancer among HIV patients was 4%. Incidence of cancer in HIV-positive South Africans in the era of potent ART was high (overall incidence rate of 1 315/100 000 person years (95% CI 1 225-1 410)), particularly for AIDS-defining cancers and infection-related cancers. A systematic approach to cancer surveillance in HIV-positive people and the exploration and implementation of known cancer-specific prevention strategies in the HIV population is recommended. Among black adult cancer patients in Johannesburg, 33.7% tested positive for HIV; and over a third of those who were positive were unaware of their HIV status. Among AIDS-Kaposi's Sarcoma (KS) patients in Pretoria, survival improved in recent years as more patients accessed appropriate ART, chemotherapy and radiation therapy.

Prostate cancer

NCR Researcher: *Dr C Babb*

Collaborators: *Project 1; Prof. T Rebbeck (Harvard TH Chan School of Public Health and Dana Farber Cancer Institute), Dr P Fernandez (University of Stellenbosch, South Africa), Project 2; Prof. J Jacobson (University of Columbia, USA), Prof. M Haffejee (University of Witwatersrand, South Africa)*

Funding: *CANSA, NIH (U01), Herbert Irving Comprehensive Cancer Centre (USA)*

A CANSA funded project investigating 80 Single Nucleotide Polymorphisms (SNPs) in black men with prostate cancer was completed. These SNPs were previously found to be associated with prostate cancer by means of genome wide association studies, mainly in European and North American populations. The outcomes of the laboratory work were finalised and data analysis is currently under way.

The consortium MAD-CaP which includes the NCR has secured a NIH-U01 grant. It was awarded under the leadership of Prof. Rebbeck to investigate Genetics of Prostate Cancer in Africa. Six participant accrual centres in sub-Saharan Africa include: Accra (Ghana; two centres), Ibadan (Nigeria), Dakar (Senegal), Cape Town (South Africa), and Johannesburg (South Africa). The project's main aim is to do a genome wide association study to investigate genetic susceptibility to prostate cancer in men from Africa; and to discover novel prostate cancer loci and validate known loci in African men to provide new information about the genetic etiology of prostate cancer. It also aims to evaluate how population differentiation and the recent evolutionary history of African and African American populations inform the underlying reasons for the high rates of prostate cancer in Africans.

An additional study will document, via chart review, the prevalence of HIV and its association with demographic, clinical, and pathological characteristics of prostate cancer patients diagnosed in a private and public sector urology practice in Johannesburg, South Africa, in each of the last four years.

Hepatocellular Carcinoma

NCR Researchers: *Dr C Babb*

Collaborators: *Prof. A Kramvis (University of Witwatersrand)*

Funding: *CANSA, National Research Foundation, German Research Foundation (DFG)*

The aim of the study is to determine the presence of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and HIV infection or co-infection in a South African cohort, diagnosed with hepatocellular carcinoma. The study will evaluate the interactive or additive effect of co-infection with HBV/HCV/HIV in relation to hepatocellular carcinoma risk, taking into account potentially confounding and lifestyle factors including age, gender, alcohol consumption and smoking. Serum samples are being assessed by an MSc student for HBV and HCV infection (serology and genotyping).

Oesophageal cancer

NCR Researchers: *Dr C Babb*

Collaborators: *Prof. C Mathew (Kings College London, United Kingdom)*

Funding: *CANSA*

Initial studies into the genetic contribution to risk of oesophageal cancer suggest there may be substantial differences in the genetic determinants of susceptibility to oesophageal squamous cell carcinoma in the South African Black population when compared with other populations. However, a substantially larger case-control study, which includes cases and controls from other major South African black population groups, is required to confirm these interesting preliminary findings, and to form the basis of a more extensive investigation of genetic susceptibility to squamous cell carcinoma of the oesophagus. A PhD student has been registered to investigate genetic risk factors for oesophageal cancer in the South African black population. Prof. Mathew has received CANSA funding to work on the samples from the Johannesburg Cancer Study. This study has the potential to identify robust genetic markers of risk, and to use these, in combination with environmental risk factors, to construct risk profiles which could be used to identify high-risk individuals in the South African Black population.

Evolving risk factors for cancers in African population: Lifestyle, genetic susceptibility and cancer in South Africa

NCR Researchers: *Dr C Babb; Dr E Singh*

Collaborators: *Prof. D Bradshaw (MRC, South Africa); Prof. C Mathew (Kings College London, United Kingdom); Prof. F Sitas (MRC, South Africa); Prof. R Newton (University of York, UK and MRC/UVRI Research Unit on AIDS in Uganda), Dr T Waterboer (DKFZ, Germany)*

Funding: *SA MRC Newton Award*

The Johannesburg Cancer Case Control Study (JCS) has allowed for the collection of a unique dataset, the largest in Africa. The SA MRC/Newton award will utilise the risk factor questionnaires and samples collected in the JCS to obtain up-to-date, robust estimates on the role of key lifestyle factors such as tobacco, alcohol, hormonal contraceptives, sexual and reproductive history in leading and emerging cancers in the black adult South African population. The study will also assess the prevalence of more than 20 infectious agents among adults with cancers that are known or suspected to be caused by infection (e.g. cervix, head and neck, Kaposi's sarcoma, lymphomas, other genital cancers, liver, stomach). In addition, the study will investigate the extent to which inherited genetic variants contribute to the risk of breast, cervical and oesophageal cancers in a local setting. Systematic genome-wide screens in large sample sizes for these cancers will determine whether risk loci identified in non-African populations also contribute to African cancers; identify novel genetic risk factors specific to Africa; and facilitate fine-mapping of causal genes and variants. Finally, the study will combine the information collected to determine whether inherited genetic variants are associated with immune response to infection.

TEACHING AND TRAINING

The NCR is involved in teaching and training activities for undergraduate and postgraduate students through the Universities of the Witwatersrand and Pretoria. Supervision was provided to five postgraduate students and 2 DST-NRF interns were hosted, who both secured full time employment within the NHLS.

PROFESSIONAL DEVELOPMENT

The NCR staff presented at four conferences in the financial year under review.

HONOURS

Dr M Sengayi was awarded her PhD through the University of Bern, Switzerland.

RESEARCH OUTPUT

Establishment of a cancer surveillance programme: The South African experience

Singh E, Ruff P, Babb C, Sengayi M, Khoali L, Beery M, Underwood JM. *Lancet Oncology*. 2015; 16: e414-21

Cancer incidence in South Africa is largely under-reported because of a lack of nationwide cancer surveillance networks. The paper describes cancer surveillance activities in South Africa, and uses the International Agency for Research on Cancer (IARC) framework to propose the development of four population-based cancer registries in South Africa. It also provides an update on a cancer surveillance pilot programme in the Ekurhuleni Metropolitan District, and the successes and challenges in the implementation of the IARC framework in a local context. The development of a comprehensive cancer surveillance system in a middle-income country was studied, which might serve to assist other countries in establishing population-based cancer registries in a resource-constrained environment.

South African National Cancer Registry: Effect of withheld data from private health systems on cancer incidence estimates

Singh E, Underwood JM, Nattey C, Babb C, Sengayi M, Kellett P. *SAMJ*. 2015; **105**(2): 107-109.

This study aimed to estimate the impact of under-reported cancer data from private health laboratories using a linear regression analysis to project expected cancer cases for 2005–2007. The projected NCR case total varied from 53 407 (3.8% net increase from actual cases reported) in 2005 to 54 823 (3.7% net increase) in 2007. The projected number of reported cases from private laboratories in 2005 was 26 359 (19.7% net increase from actual cases reported), 27 012 (18.8% net increase) in 2006 and 27 666 (28.4% net increase) in 2007. While private healthcare reporting decreased by 28% from 2005 to 2007, this represented a minimal impact on overall cancer reporting (net decrease of <4%).

Childhood cancer incidence patterns by race, sex and age for 2000–2006: A report from the South African National Cancer Registry

Erdmann F, Kielkowski D, Schonfeld SJ, Kellett P, Stanulla M, Dickens C, Kaatsch P, Singh E, Scuz J. *Int J Cancer*. 2015 Jun 1; **136**(11): 2628–2639.

Higher childhood cancer incidence rates are generally reported for high income countries although high quality information on patterns of childhood cancer for low or middle income countries is limited, particularly in sub-Saharan Africa. For the first time, the childhood cancer data reported to the pathology report-based National Cancer Registry of South Africa from 2000–2006 was reported and compared to incidence data from Germany. The overall age-standardised incidence rate (ASR) for South Africa in 2000–2006 was 45.7 per million children. Substantial differences by cancer types within South Africa by racial group were observed; ASRs tended to be three- to four-fold higher in South African Whites compared to Blacks. ASRs among both Black and White South Africans were generally lower than those from Germany with the greatest differences observed between the Black population in South Africa and Germany, although there was marked variation between cancer types. More research is needed to understand the extent to which under-ascertainment and under-diagnosis of childhood cancers drives differences in observed rates.

Hematologic malignancies in South Africa 2000–2006: Analysis of data reported to the National Cancer Registry

Schonfeld SJ, Erdman F, Wiggel T, Singh E, Kellett P, Babb C, Schuz *J. Cancer Medicine* 2016.

Little is known about the incidence patterns of hematologic malignancies in sub-Saharan Africa, including South Africa. This study estimated incidence rates of pathology-confirmed adult cases of leukemia, myeloma and related diseases (myeloma), Hodgkin lymphoma (HL), and non-Hodgkin lymphoma (NHL) reported to the NCR between 2000 and 2006, by age, gender, and population group. Incidence rates of reported hematologic malignancies were generally 20–50% higher among males than females. Analysis suggested marked differences in the rates of reported hematologic malignancies by population group which were most pronounced when comparing the White versus Black population groups. This is the first country-wide report of the incidence of hematologic malignancies in South Africa.

HIV testing and burden of HIV in Black Cancer patients in Johannesburg South Africa: A cross sectional study

Sengayi M, Babb C, Egger M, Urban M. *H BMC Cancer* 2015; 15(144)

HIV infection is a known risk factor for cancer but little is known about HIV testing patterns and the burden of HIV infection in cancer patients. This was a cross-sectional analysis to identify predictors of prior HIV testing and to quantify the burden of HIV in black cancer patients in Johannesburg. A total of 5 436 cancer patients were tested for HIV of whom 1 833 [33.7% (95% CI=32.5-35.0)] were HIV-positive. Three-quarters of the patients had never been tested for HIV. The total prevalence of undiagnosed HIV infection was 11.5% (10.7-12.4) with 34% (32.0–36.3) of the 1 833 patients who tested HIV-positive being unaware of their infection. Men >49years and those residing in rural areas were less likely to have been previously tested for HIV. Men with at least a secondary education and those interviewed in recent years were likely to have had prior testing. Women >49years were less likely to have been previously tested for HIV. In women, having children <5years, hormonal contraceptive use, having at least a secondary education and recent year of interview were independently associated with previous HIV testing.

CONFERENCE PRESENTATION

The NCR presented research findings at four international conferences during the reporting period.

NATIONAL HEALTH
LABORATORY SERVICE

health
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