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FOREWORD

Pathogenic organisms and viruses are usually able to adapt to the presence of anti-pathogenic drugs by developing mechanisms of resistance that can result in control and treatment failure. The regular occurrence of drug resistance necessitates ongoing disease surveillance and outbreak readiness. In South Africa, the National Department of Health and the Centre for Tuberculosis of the National Institute for Communicable Diseases is currently conducting a country-wide survey to determine the prevalence and trends of multidrug-resistant TB (MDR-TB) in all nine provinces. The design and signifi- this survey is described in this issue. Also in this issue: details of an outbreak of carbapenem-resistant *Klebsiella* spp.(CRK) in a tertiary academic hospital in Cape Town are presented; transmitted HIV drug resistance statistics for the Gauteng and KwaZulu-Natal provinces for the period 2005 to 2010 are provided; and the significant decrease in the relative prevalence of syphilis over the past five years among patients presenting with genital ulceration at a health care facility in Johannesburg is described, as is the continuing local absence of macrolide resistance in the bacterium *Treponema pallidum*, the causative agent of syphilis.

This is the final issue for 2012 and we wish all our readers and contributors a safe and joyous holiday season.

Basil Brooke, Editor

CONTENTS

ANTI-TUBERCULOSIS DRUG RESISTANCE SURVEY FOR SOUTH AFRICA, 2012 – 2013

Chikwe Ihekweazu, Nazir Ismail, Hendrik Koornhof

Centre for Tuberculosis, NICD, NHLS

Introduction

Drug-resistant tuberculosis (TB), including multidrug-resistant TB (MDR-TB), is a major global health problem and poses a significant threat to TB control in South Africa. The need for reliable information on drug-resistant TB on a global scale, generated from programmes using standardised methodology and designed to be representative of a country or region, led the World Health Organization (WHO), together with the International Union Against Tuberculosis and Lung Disease (IUATLD), to start a laboratory-based anti-TB drug resistance surveillance project. The project was initiated in 1994 when a Supranational Reference Laboratory Network (SRLN) was assembled to ensure optimal performance of national reference laboratories participating in the global project. The ultimate objective of the global project was to establish competent reference laboratories to conduct quality-assured drug resistance surveillance on a prospective basis in national or sub-national area settings, obviating the need for periodic global initiatives. The four comprehensive Global WHO/IUATLD reports published in 1997, 2000, 2004 and 2008 cover drug susceptibility results from 35, 58, 77 and 81 countries and sub-national settings respectively and were based on

sputum smear-positive cases.[1-4] A fifth global report, published in 2010, concentrated on MDR-/XDR-TB (multidrug-resistant / extensively drug-resistant *Mycobacterium tuberculosis*) cases.[5] Data on newly-acquired TB cases (new cases) were clearly separated from those having received previous treatment (previously treated cases) in the post-1997 reports.

The first national survey of TB drug resistance in South Africa was performed in 2001/02 as part of the third Global Report.[3] This survey produced relatively low estimates of 0.9% - 2.6% of primary MDR-TB in the provinces. However, the gravity of the TB drug resistance situation in South Africa is potentially exacerbated by the persistently high prevalence of HIV infection which drives the TB epidemic as well as the emergence of extensively drug-resistant *Mycobacterium tuberculosis* (XDR-TB) strains. Given these circumstances, the National Department of Health (NDOH) and the Centre for Tuberculosis (CTB) of the National Institute for Communicable Diseases (NICD) decided to undertake a nationally representative survey to determine the prevalence and trends of MDR-TB in all nine of South Africa's provinces as compared to the eight provinces surveyed during 2001/02. This survey includes data on the HIV prevalence in culture-positive TB suspects. As an extension to the main survey, the types of MDR *M. tuberculosis*-complex strains circulating in the country will be established using data from a representative sub-population of TB suspects. The survey began in June 2012, and is being phased in with one province starting each month, with patients being enrolled for a maximum of one year.

Design of the current survey

Population-proportionate cluster sampling of survey sites is being used in the current survey. In order to determine the required sample size for the survey, the number of survey sites and the estimated number of smear-positive cases based on the total number of pulmonary TB cases detected in the previous year as well as a conservative estimate of the expected prevalence of rifampicin-resistant cases (lowest level of primary MDR-TB in any province [0.9%]) were taken into account. In addition, the proportions of previously treated TB suspects were included so as to ensure that the numbers of suspect cases entered into the survey were adequate and representative of TB in the country. Unlike previous surveys in which smear-positive cases were used for entry into the survey, patients entered into the current survey are consecutive TB suspects visiting clinic sites who are subsequently confirmed to be TB cases based on mycobacterial culture. Tuberculosis suspects rather than smear-positive cases are being used in the current survey in order to reduce the possibility of systematic exclusion of HIV-infected patients who often present with smear-negative TB.

Conducting the survey

Patients are eligible for inclusion in the survey if they are older than 18 years and present as a TB suspect according to World Health Organization (WHO) and International Union Against Tuberculosis and Lung Disease (IUATLD) definitions. Subject to informed consent, sputum specimens are collected from all consecutive TB suspects attending the facilities of the clusters selected for inclusion. The intake period of the survey will not exceed 12 months for each province. The number of clusters in each province was chosen so as to ensure that the duration of recruitment of survey suspects does not exceed 12 months at any of the selected sites. Where the case load per site is lower than the required number of patients with positive cultures per cluster, additional sites in close proximity to the low yield sites within the same sub-district are grouped together prior to sampling taking place. These sites begin enrolment on the same day. In all selected diagnostic sites, consecutive TB suspects giving sputum samples are included in the survey until the number required for one or more clusters

is reached.

Cultures from the sputum of all suspect cases in all participating clusters are obtained using the liquid medium-based *Mycobacterium* Growth Indicator Tube (MGIT) system (Becton-Dickinson, Sparks, Md, USA). Drug susceptibility testing using the MGIT 960 system is performed on all cultures from TB suspects against a total of 12 first- and second-line anti-TB agents. Thirteen drug concentrations are used in total because two concentrations of isoniazid are required to detect low- and high-level resistance to this drug respectively. Cases are categorised and recorded according to whether or not TB treatment had been received in the past. Sputum from all survey participants are also tested for HIV antibodies in order to provide information on TB-HIV co-infection rates in South Africa.

Representative study sites have been identified across the country and training of clinical and laboratory personnel has been undertaken to enable standardised processing of specimens. The phasing-in of the survey in all nine provinces will be completed during 2013 and the final results will be available in late 2013 or early 2014.

Significance of the survey

This is the first national drug resistance survey based on data obtained from sputum cultures and drug sensitivity tests of all TB suspects as opposed to the exclusive use of smear microscopy positive cases in previous Global WHO/IUATLD drug resistance surveys. Prevalence data on drug resistance amongst new and previously treated cases are key performance indicators of South Africa's TB control programme and will be used to inform future control strategies. Molecular typing of *M. tuberculosis* isolates obtained during this survey may delineate transmission patterns and clonal expansion and could also be used to identify infection control deficiencies.

Acknowledgements

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OUTBREAK OF CARBAPENEM-RESISTANT *KLEBSIELLA* SPECIES IN AN ACADEMIC HOSPITAL, WESTERN CAPE, MAY 2012.

Riyadh Manesen[1, 2], Colleen Bamford[3], Mariette Smith[3], Rachel Jacobson[3], Clinton Moodley[3], Seymour Williams[1, 4]

[1]South African Field Epidemiology and Laboratory Training Programme (SA-FELTP)
[2] School of Health Systems and Public Health, University of Pretoria
[3] Centre for Opportunistic, Tropical and Hospital Infections, NICD, NHLS
[4] Centers for Disease Control and Prevention, South Africa (CDC-SA)

Introduction

Enterobacteriaceae are a family of Gram negative, rod-shaped bacteria that include pathogenic enteric genera such as *Klebsiella* species. Intestinal colonization with Enterobacteriaceae produces a bacterial reservoir that may cause infection of the host and may be transmitted to others. Treatment of invasive infections requires the use of appropriate antibiotics, which may include carbapenems. Carbapenems are antimicrobials often used to treat extended-spectrum beta-lactamase-producing Enterobacteriaceae that show multiple resistances to antibiotics. However, the increase in carbapenem-resistant Enterobacteriaceae (CRE) is becoming a global concern, as CRE have developed resistance to most classes of antimicrobials. Infections and re-colonizations of patients with CRE are usually associated with high morbidity and mortality as well as increased hospitalization costs.[1,2]

On the 31st May 2012, the National Institute for Communicable Diseases (NICD) was informed about a cluster of cases involving carbapenem-resistant *Klebsiella pneumoniae* or *K. oxytoca* (CRK), which occurred in a shared haematology-oncology intensive care unit (public and private patients), housed within a tertiary academic hospital in Cape Town. This haematology-oncology ward serves as an isolation unit for highly immunocompromised patients. The South African Field Epidemiology and Laboratory Training Programme (SA-FELTP) was invited to assist with assessing the extent of CRK spread within the facility as well as to determine the common factors of transmission amongst cases. Active surveillance of patients in the haematology-oncology wards by means of stool or rectal swab screening had already been instituted, and infection prevention and control (IPC) measures had been intensified.

Materials and Methods

A case series review was conducted and demographic, clinical and laboratory information extracted using a standardized data collection tool. Contact staff (medical and nursing) working on the affected ward were screened by rectal swab for CRK and IPC inspection of the unit was undertaken.

Molecular epidemiology

Identification and susceptibility testing: Stool specimens or rectal swabs were plated onto McConkey agar containing 4 µg/ml gentamicin. Identification and susceptibility testing was performed on the Vitek2 (bioMèrieux, Marcy l'Etoile, France) using the AST-N133 card and interpreted according to Clinical Laboratory Standards Institute (CLSI) criteria. Eight isolates with reduced susceptibility to the carbapenems were identified; seven *K. pneumoniae* isolates and one *K. oxytoca* isolate. Carbapenemase production was detected in all 8 isolates using a Modified Hodge Test (MHT).

Antibiotic resistance gene screening: Polymerase Chain Reaction (PCR) assays were carried out on DNA extracted from cultured isolates, as well as on total DNA extracted from stool specimens. Assays were performed using primers designed to amplify the most commonly

described carbapenemase genes, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{SPM}$, *bla*$_{KPC}$, *bla*$_{GES}$ and *bla*$_{OXA-48-like}$. PCR amplicons of the expected size were obtained with the *bla*$_{OXA-48-like}$ primers (OXA48F 5'-CGTGTATTAGCCTTATCG-3'/ OXA48R 5'-CCTAGAAGTGGTTAGCG-3') for the eight samples culturing *Klebsiella* species with reduced carbapenem susceptibility, as well as the *bla*$_{OXA-48-like}$ positive control (gift from C. Corcoran, South Africa). PCR products from the eight strains were purified (QIAquick® PCR purification kit, QIAGEN, Germany)

Genetic relatedness: The genetic relationship of the isolates was investigated using pulsed-field gel electrophoresis (PFGE) according to a previously published protocol.[3] Total genomic DNA was digested, *in situ*, with XBaI (New England Biolabs, Inc, UK) and the resulting DNA fragments were separated using a 1% agarose gel in a CHEF-DRII GeneNavigator apparatus (GE Healthcare, Piscataway, USA) using an increasing pulse time of 5 - 60 seconds over 21 hours, at 200 V, in 0.5% TBE buffer maintained at 14°C. The resulting restriction profiles were analysed using GelCompar II version 5.1 (Applied Maths, St-Martens-Latem, Belgium). A dendrogram indicating the homology between the isolates was created using the Dice similarity coefficient. The band tolerance and optimizations were set at 1% and a similarity threshold of 80% and greater was used to define clusters.[4]

Results

Eight patients either infected (n=1) or colonised (n=7) with CRK were identified. Their demographic and clinical information is summarized in table 1. The median age was 48 years (range: 30-77 years) and half were male. Seven of the eight (87.5%) case patients were housed in the shared haematology-oncology ward during the outbreak period (April – May). These cases were hospitalized for treatment of hematologic cancers and were administered immunosuppressive drugs and multiple antibiotic therapies. One colonised case patient (case #6) was hospitalized in a separate intensive care unit (ICU) of the private hospital. None of the approximately 200 staff members screened for CRE were found to be colonized.

The occurrence of the CRK laboratory-identified cases over time is shown in figure 1. Case #1 was transferred from an outside institution and was administered multiple antibiotic treatment. CRK was detected on the same day for cases #1 and #2, whilst cases #3, #4 and #5 were detected on three successive days. Cases #6 and #7 were detected two days after case #5. All cases occurred over a period of 3 weeks from 12 to 24 May, during the initial period of active surveillance. The 8th case was identified on May 24th, 12 days after one of the patients (case #1) was moved to the private intensive care unit. Following detection of the first 2 cases, the ward was closed for admission of new patients and active screening of staff members for CRK was initiated. The ward was closed for thorough cleaning on the 30th May.

An IPC inspection of the haematology-oncology ward when the first cases were detected identified contact precaution (CP) breaches by healthcare staff. Follow-up discussions with these staff suggested an inadequate understanding of what contact precautions were required.

Sequencing revealed that all 8 strains had 100% homology to the *bla*$_{OXA-181}$ gene, a variant of *bla*$_{OXA-48}$OXA-48. The profiles of six *K. pneumoniae* isolates (cases 1, 5, 6, 2, 8 and 3) were indistinguishable (100% homology) and were assigned to Cluster A (figure 2). The single *K. oxytoca* isolate (case 7), was unrelated to cluster A, with only 64% homology, indicating horizontal gene transfer to other *Klebsiella* species.

Table 1. Demographic and clinical information of the 8 patients colonised or infected with carbapenem-resistant *Klebsiella* spp., April – May 2012.

I.D. #	Hosp	Residential address	sex	Age (yrs)	Diagnosis	Speci-men	Date isolated	Co-morbidity	Outcome
Case #1	Private	Cape Town	M	77	Multiple Myeloma	Blood	12/05/2012 (CrKp)	Hypertension, mitral valve replacement, (2003)	Deceased
Case #2	Public	Cape Town	M	36	Myelodysplasia	Stool	12/05/2012 (CrKp)	None	Inpatient isolation
Case #3	Private	Cape Town	M	44	Multiple myeloma	Stool	17/05/2012 (CrKp)	Asthmatic, Penicillin allergy	Discharged
Case #4	Private	Namibia	F	52	Acute lymphoblastic leukaemia (ALL)	Stool	18/05/2012 (CrKp)	Hepatitis (23/04/12)	Deceased (unrelated cause)
Case #5	Public	Cape Town	F	57	Multiple myeloma	Stool	19/05/2012 (CrKp)	None	Discharged
Case #6	Private	Cape Town	F	51	Ileo - rectal anasto-mosis	Tracheal aspirate	21/05/2012 (CrKp)	Intra-abdominal sepsis	Inpatient (ICU)
Case #7	Public	Cape Town	F	41	Thallassemia	Stool	21/05/2012 (CrKo)	Hypotensive	Discharged
Case #8	Public	Cape Town	M	30	Acute lymphoblastic leukaemia (ALL)	Stool, pus swab	24/05/2012 (CrKp)	None	Inpatient isolation

CrKp: carbapenem resistant *Klebsiella pneumonia;* **CrKo:** carbapenem resistant *Klebsiella Oxytoca*; Hosp = hospital classification.

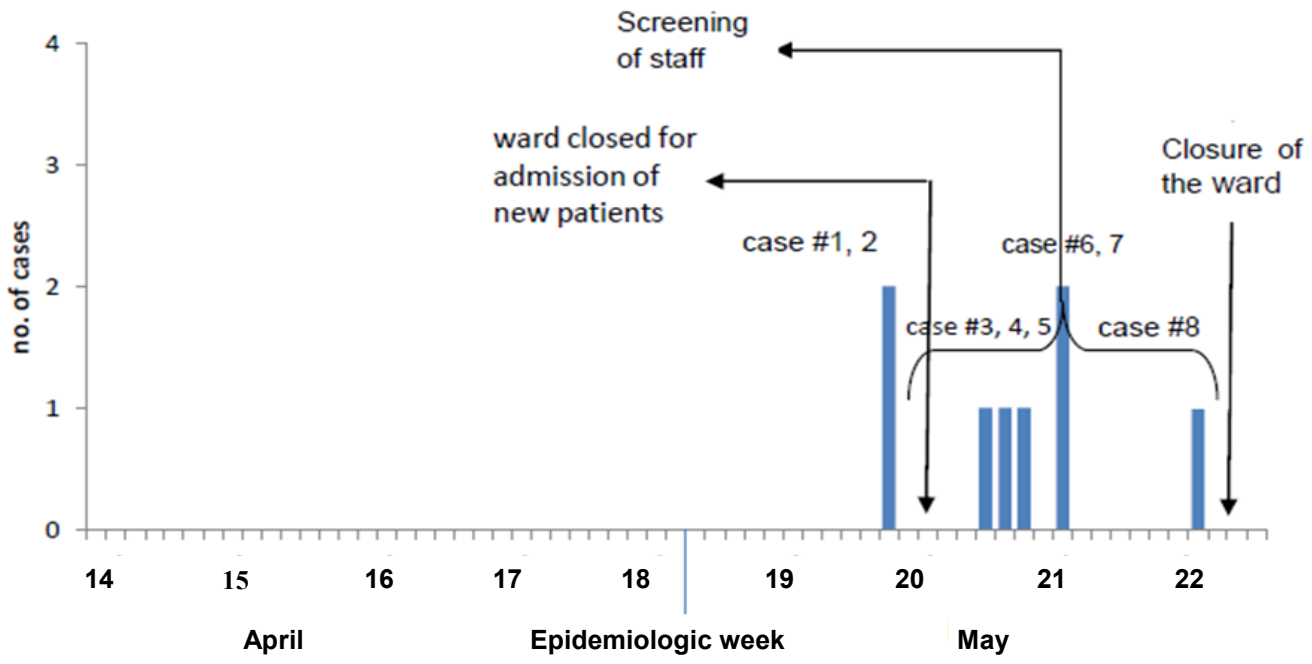

Figure 1. Epidemic curve for outbreak of carbapenem-resistant *Klebsiella* spp.colonised or infected cases, April – May 2012.

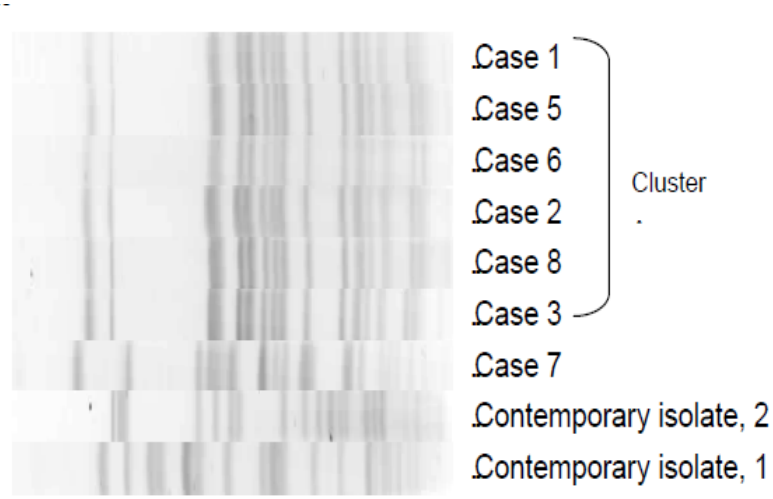

Figure 2. Dendrogram of carbapenem resistant *Klebsiella* isolates.

Discussion

Although there is no definitive proof, we hypothesize that case #1 probably acquired CRK intestinal colonization prior to the onset of bloodstream infection and is likely the index case of this outbreak. The IPC report described poor CP practices amongst care staff, which may account for the transmission of CRK between patients either via the hands of staff or contaminated equipment.

Transmission to case patient #6, who was never admitted to the haematology unit, may have occurred when case patient #1 deteriorated and was transferred to the ICU (private hospital) and placed in a bed adjacent to patient #6. As CRK was subsequently identified from the endotracheal specimen of case patient #6, transmission may have occurred as a result of poor ICP practices amongst care staff in the ICU. This assumption is supported by molecular typing, which showed that the CRK isolated from case patient #6 was indistinguishable from that of case patient #1.

This outbreak is especially important because two health-care institutions (public and private) were involved and CRK transmission via health-care staff probably played a key role in the spread of CRK within this facility. Limiting patient movements and implementing contact precautions for colonised patients appears to have suppressed the outbreak. Prompt closure of the ward for new admissions probably prevented further transmission within each of the facilities.

Based on this investigation we recommend the following:

- Intensify IPC awareness in the haematology-oncology ward with improved signage, etc. Regular monitoring should include all staff (temporary and full time) with possible patient contact.
- Discuss optimal nurse staffing to achieve adherence with IPC practices.
- Collect admission surveillance cultures for new patients to this ward with weekly follow-up. Consider surveillance cultures for all patients with previous admission to any local private hospitals since November 2011 and patients with previous admission to the affected ward in April- May 2012.
- Discuss (internally and at provincial level) the need for antibiotic stewardship and cooperation amongst providers about sharing antibiotic resistance information.

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SURVEILLANCE OF TRANSMITTED HIV-1 DRUG RESISTANCE IN GAUTENG AND KWAZULU-NATAL IN 2010

Gillian Hunt, Johanna Ledwaba, Adriaan Basson, Anna Salimo, Monalisa Kalimashe, Beverly Singh, Adrian Puren, Lynn Morris

Centre for HIV and Sexually Transmitted Infections, NICD, NHLS

Introduction

The emergence of HIV drug resistance is an inevitable outcome of rapid scale-up of population-based treatment antiretroviral therapy (ART) regimens. As part of its strategy for surveillance of drug resistance in resource-limited settings, the World Health Organization recommends surveillance for transmitted drug resistance (TDR) among individuals assumed to be recently infected, such as pregnant women.[1,2]

The Centre for HIV and Sexually Transmitted Infections (STI) has been performing TDR surveys in pregnant women since 2002, using specimens collected as part of the annual antenatal survey (ANSUR) conducted by South Africa's National Department of Health. In 2010, the national HIV prevalence estimate for Gauteng Province (GP) and KwaZulu-Natal (KZN) was 30.4% and 39.5% respectively.[3]

In this article updated transmitted HIV drug resistance statistics are provided for Gauteng and KwaZulu-Natal based on data from specimens collected as part of the 2010 ANSUR survey. Previously published results have indicated that while TDR remained low in Gauteng and KZN prior to 2009, these levels were nevertheless increasing in KZN.[4,5]

Specimen collection and testing

All participant specimens were selected from the GP and KZN ANSUR surveys conducted in 2010. HIV-1 positive specimens were selected for genotypic analysis according to inclusion criteria as defined by WHO guide-

lines for the classification of TDR: primigravid female age ≤21 years.[1] Genotypic resistance was defined as the presence of resistance mutations, using the Stanford Calibrated Population Resistance (CPR) algorithm Version 6.0.[6,7] Sequences were ordered according to date of collection and each prevalence category was assigned according to the recommended WHO method by which TDR prevalence is categorized as low (<5%), moderate (5-15%) or high (>15%).[2]

If surveillance drug resistance mutations (SDRM) were not present within the first 34 specimens assayed, prevalence was classified as <5%. If resistance was detected, then 47 sequences were evaluated and if the number of sequences with relevant resistance mutations was between 2 and 8, the prevalence of TDR was classified as 5-15%. Ethical approval for drug resistance testing was obtained from the University of the Witwatersrand Human Research Ethics Committee.

Demographic data from amplified specimens

All specimens selected from the surveys conducted in 2010 in GP and KZN were HIV-1 subtype C, except for one subtype A in KZN2010. The median age of women was 19 years. Resistance data from this survey are shown (in bold) in table 1 together with data from previous surveys conducted in these provinces.

Table 1: Transmitted HIV Drug Resistance Threshold Surveys performed in Gauteng and KwaZulu-Natal Provinces between 2005 and 2010.

Province	Year	Number sequences analyzed	Median Age (range)	HIV subtype	Number with mutations	Mutational patterns			Threshold level		
						PI	NRTI	NNRTI	PI	NRTI	NNRTI
Gauteng (GP)	2005	60	21 (18-22)	C	0				NC	<5%	<5%
	2006	53	20 (18-21)	C	0				<5%	<5%	<5%
	2007	56	20 (18-21)	C	2	M46I I85V	M184I		**5-15%**	<5%	<5%
	2008	60	20 (18-21)	C	0				<5%	<5%	<5%
	2009	47	19 (18-21)	C (1A)	1		M184V	Y188L	<5%	<5%	<5%
	2010	**58**	**19 (<21)**	**C**	**1**			**K103N**	**<5%**	**<5%**	**<5%**
KwaZulu-Natal (KZN)	2005	40	21 (18-24)	C	1			K101E Y181C	NC	<5%	NC
	2007	35	19 (18-22)	C	0				<5%	<5%	<5%
	2008	37	20 (18-24)	C (1B)	5	M46I M46I K219R K103N K103N	M184V	K103N	NC	NC	NC
	2009	47	19 (18-21)	C (1D)	3	K103N V106M K101P K103N			<5%	<5%	**5-15%**
	2010	**47**	**19 (<21)**	**C (1A)**	**4**		**T69D** **M41L** **M184V**	**K103N** **G190A** **K101E**	**<5%**	**5-15%**	**5-15%**

NRTI: Nucleoside Reverse Transcriptase Inhibitors; NNRTI: Non-nucleoside Reverse Transcriptase Inhibitors; PI: Protease Inhibitors
NC: Threshold level not classifiable due to insufficient number of available specimens. 2010 data shown in bold.

Classification of Threshold Survey (TS) sequence data

One hundred specimens were selected from the GP2010 survey, from which 58 sequences were obtained. Of the remainder, 6 specimens had insufficient volume for further processing, one could not be sequenced and 35 were not amplifiable by PCR for genotyping. In this survey, one specimen contained the Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI) SDRM mutation K103N. Consequently, the levels of TDR for each of the Protease Inhibitors (PI), Nucleoside Reverse Transcriptase Inhibitors (NRTI) and NNRTI drug classes were <5%.

Similarly, 100 specimens were selected from KZN2010, from which 47 sequences were obtained from the first 64 specimens tested. Four sequences had SDRM: 2 specimens had the NRTI mutations T69D and M41L, 1 specimen had the NRTI mutation M184V and the NNRTI mutations K103N and G190A, and 1 specimen had the NNRTI mutation K101E. There were no PI mutations detected in this subset. As a result, levels of TDR were classified as moderate (5-15%) for both the NRTI and NNRTI classes of drugs and low (<5%) for the PI class of drugs.

Discussion

Transmitted drug resistance surveillance was focused on specimens collected from Gauteng and KwaZulu-Natal because of the high HIV prevalence estimates in these provinces.[3] These data indicate that in Gauteng the levels of transmitted resistance remained low (<5%) for all drug classes in 2010. However, levels of transmitted resistance were moderate (5-15%) for the NNRTI and NRTI classes of drugs for the second successive year in KwaZulu-Natal.

The K103N mutation continues to be the commonest resistance mutation detected. This mutation is associated with TDR and is commonly found in patients failing first-line therapies in South Africa.[8-11] K103N and M184V occur rarely in untreated individuals,[7] and are selected by Nevirapine / Efavirenz and Lamivudine / Emtricitabine respectively, causing high-level resistance to these drugs. M41L is part of the multi-NRTI mutation complex and is associated with high levels of resistance to Stavudine and Zidovudine. T69 is a highly polymorphic residue, but the T69D and T69 insertion mutations have been associated with high levels of NRTI resistance. The NNRTI mutation G190A confers high level resistance to Nevirapine / Efavirenz whereas the K101E is associated with resistance to the second generation NNRTI Etravirine.

Transmitted resistance in resource-limited countries such as South Africa is not unexpected. Increased levels have been detected over the years in Europe and the USA[12,13] and more recently in Uganda where ART has been available for longer periods of time.[14] Our data suggest that increasing levels of transmission of NNRTI resistant virus are occurring in KZN. This data must be treated with caution and ongoing vigilance is required. In addition, systematic assessment of the ART delivery program, which may select drug resistant virus in populations receiving care, facilitating subsequent transmission to newly infected individuals, needs to be implemented.

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EPIDEMIOLOGICAL TRENDS AND MOLECULAR ANALYSIS OF PRIMARY SYPHILIS DETECTED IN PATIENTS ATTENDING A PUBLIC HEALTH CARE CLINIC IN JOHANNESBURG

Neetha Bhojraj Sewpershad, Etienne Müller, Venessa Maseko, Frans Radebe, David Lewis

Centre for HIV and Sexually Transmitted Infections, NICD, NHLS

Introduction

Syphilis is a sexually transmitted infection (STI) caused by the spirochete bacterium *Treponema pallidum.* It is a slowly progressing STI that has several stages and is difficult to diagnose clinically whilst early in its presentation. Diagnostic confirmation is either via blood tests or detection of spirochetes in a syphilitic ulcer (primary syphilis) by microscopy or polymerase chain reaction (PCR). Blood tests are divided into nontreponemal and treponemal tests. Nontreponemal tests such as rapid plasma reagin (RPR) tests are used initially. However, these tests are occasionally false positive and confirmation is required with a treponemal test, such as *Treponema pallidum* particle agglutination (TPPA). Treponemal antibody tests usually become positive 2-5 weeks after the initial infection and may be negative in 20-30% of primary syphilis cases. A low level of antibodies will stay in the blood for months or even years after the disease has been successfully treated.

Current South African treatment guidelines for early syphilis indicate the administration of intramuscular benzathine penicillin as a single 2.4 mega unit dose.[1] Patients who are allergic to penicillin may be given alternative therapy with either oral doxycycline or tetracycline, or one of the macrolide family, typically erythromycin or azithromycin. In South Africa, azithromycin is used as a periodic presumptive regimen to treat and prevent several STIs, including early asymptomatic syphilis infections, in female commercial sex workers. Clinical failure of azithromycin has been reported in different parts of the world. Reports of macrolide resistant *T. pallidum* strains highlight the importance of continued monitoring for resistance and treatment failure in countries where azithromycin is commonly administered for the treatment of syphilis.

This aims of this study were to describe trends in the relative prevalence of primary syphilis as a cause of the genital ulcer syndrome among patients presenting to a primary healthcare facility in Johannesburg, and to determine if the A2058G point mutation, which confers macrolide resistance, was present in *T. pallidum* identified in genital ulcer specimens.

Methods

Ethics approval was obtained for the study and all specimens tested were collected from participants who had consented to their specimens being used for future research. A total of 532 genital ulcer specimens were collected from patients attending a primary health care facility in Johannesburg during the period January to April annually between 2007 and 2011 as part of a sexually transmitted infections (STI) surveillance programme within Gauteng Province.

Ulcer specimens were tested for herpes simplex virus, *T. pallidum*, *Haemophilus ducreyi* and *Chlamydia trachomatis* L1-L3 by in-house real-time PCR assays. The relative proportions of syphilis among all ulcers and among the sub-group of ulcers where an STI

pathogen was detected were determined.

All *T. pallidum* positive samples were screened for the A2058G point mutation in the peptidyltransferase region of the 23S rRNA subunits using a rapid PCR-based restriction digest assay.[2] Syphilis sub-typing was performed on all positive samples to assess the level of heterogeneity among the *T. pallidum* strains.[3] This was based on two genes: the acidic repeat protein (*arp*) gene and the *T. pallidum* repeat (*tpr*) gene.[4]

Results

Sexually transmitted pathogens were detected in 347 ulcer specimens by an in-house real-time multiplex PCR assay. *Treponema pallidum* accounted for 22 of these which were analysed further. The overall relative prevalence of syphilis decreased to zero during the study period (figure 1). This decrease was more apparent among the sub-group of ulcers where an STI diagnosis had been made by molecular analysis.

DNA was only available for 16/22 *T. pallidum* strains from which restriction-digestion patterns were obtained. None of these samples contained the 23S rRNA gene point mutation (A2058G) that confers macrolide resistance.

All 16 confirmed *T. pallidum* specimens were positive for the *tpr* and *arp* assays and produced a full subtype in all cases. A total of 8 subtypes were identified in this population (figure 2).

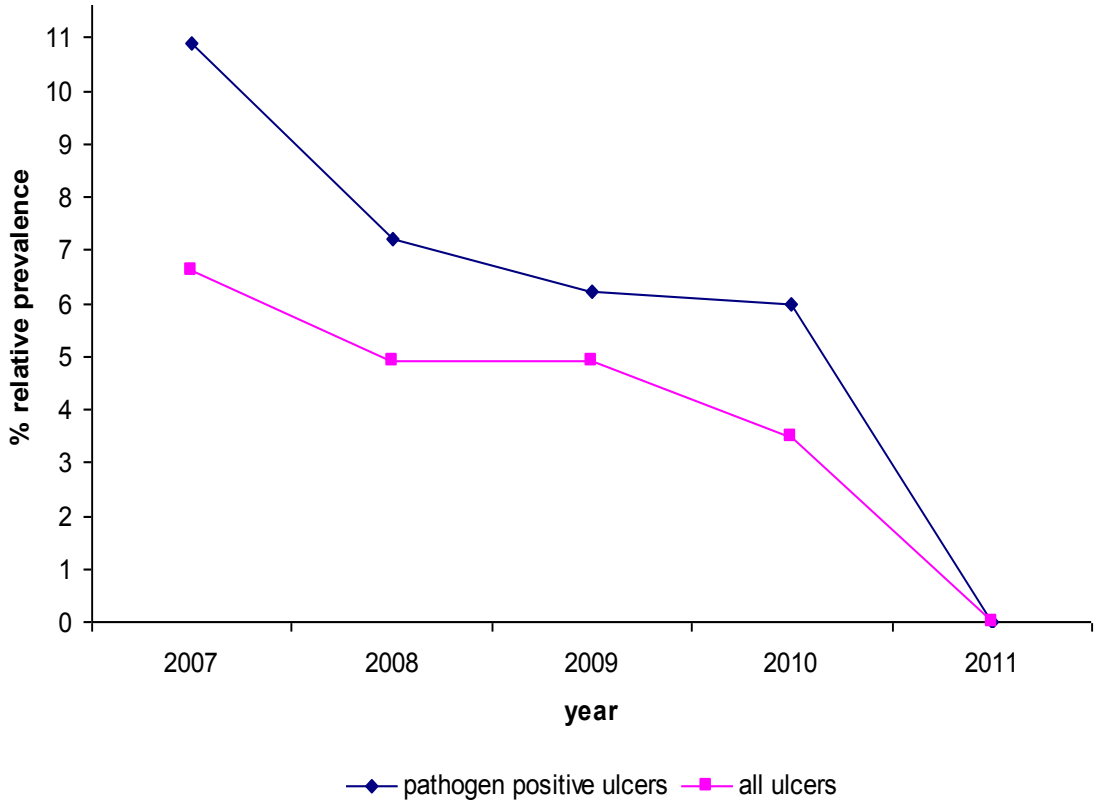

Figure 1: Relative prevalence of syphilitic ulcers from patients attending a public health care clinic in Johannesburg during the period 2007-2011.

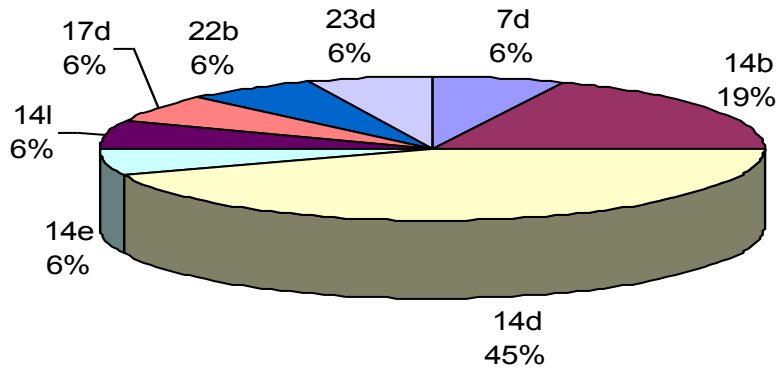

Figure 2: Subtype distribution of all 16 confirmed *T. pallidum* specimens collected from patients presenting with genital ulceration at a public health care clinic in Johannesburg.

Discussion

South Africa witnessed a decline in the national antenatal prevalence of syphilis from 11.2% in 1997 to 1.9% in 2008. This decline has been attributed to the impact of a decade of STI syndromic management at the primary healthcare level and to antenatal screening of pregnant women for syphilis. This decline is also apparent in the decreased relative prevalence of *T. pallidum* over the study period.

This study has furthered an understanding of the epidemiological trends and has contributed to the molecular typing of syphilis strains in South Africa. This is important because the emergence and dissemination of resistant strains could arise in southern Africa if macrolides are introduced as an alternative treatment option for syphilis or other STIs, such as chlamydial infection.

Using the proposed typing system, genetic heterogeneity at two different loci was demonstrated, allowing for the differentiation of all *T. pallidum* strains typed. This method of subtyping *T. pallidum* strains is reproducible and easy to perform and is currently the typing method of choice worldwide.

Conclusions

There has been a significant decrease in the relative prevalence of syphilis over the past five years among patients presenting with genital ulceration at the health care facility in Johannesburg. The most prevalent *T. pallidum* subtype was *14d* and the point mutation responsible for macrolide-resistance was not present among the *T. pallidum* DNA samples tested. Although macrolide resistance is unreported in Africa, it could emerge through drug selection pressure or importation in the future.

Acknowledgments

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Table 1: Provisional number of laboratory confirmed cases of diseases under surveillance reported to the NICD - South Africa, corresponding periods 1 January - 30 September 2011/2012*

Disease/Organism	1 Jan - 30 Sep, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Anthrax	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
Botulism	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
Cryptococcus spp.	2011	896	268	1396	805	364	486	41	356	347	4959
	2012	812	237	1411	1431	141	285	42	226	428	5013
Haemophilus influenzae, invasive disease, all serotypes	2011	26	21	117	54	5	19	11	6	72	331
	2012	28	14	82	27	1	7	4	4	61	228
Haemophilus influenzae, invasive disease, < 5 years											
Serotype b	2011	6	3	14	12	1	4	7	2	14	63
	2012	2	3	11	3	1	3	2	2	7	34
Serotypes a,c,d,e,f	2011	1	1	9	2	0	0	0	0	4	17
	2012	1	0	3	0	0	1	0	0	4	9
Non-typeable (unencapsulated)	2011	2	2	24	4	0	1	0	0	12	45
	2012	0	1	17	3	0	0	0	0	6	27
No isolate available for serotyping	2011	4	3	16	10	2	4	1	1	0	41
	2012	6	2	9	1	0	1	1	1	6	27
Measles	2011	1	2	34	23	1	1	8	6	6	82
	2012	0	1	7	6	1	0	0	1	1	17
Neisseria meningitidis, invasive disease	2011	33	20	111	24	7	14	6	5	38	258
	2012	30	9	67	20	2	3	0	7	37	175
Novel Influenza A virus infections	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
Plague	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
Rabies	2011	0	0	0	1	3	0	0	0	0	4
	2012	0	0	0	4	3	1	0	0	0	8
**Rubella	2011	199	23	399	183	318	255	44	213	79	1713
	2012	256	27	131	417	30	95	112	58	162	1288
Salmonella spp. (not typhi), invasive disease	2011	10	17	193	50	3	20	6	7	54	360
	2012	24	11	225	56	3	22	9	3	73	426
Salmonella spp. (not typhi), isolate from non-sterile site	2011	106	21	370	133	11	56	16	15	176	904
	2012	129	15	405	142	2	36	9	5	227	970
Salmonella typhi	2011	7	2	14	8	1	9	0	1	14	56
	2012	2	0	16	9	0	3	0	0	12	42
Shigella dysenteriae 1	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
Shigella spp. (Non Sd1)	2011	173	39	455	124	9	21	29	11	406	1267
	2012	193	43	447	171	3	19	18	5	309	1208
Streptococcus pneumoniae, invasive disease, all ages	2011	213	152	1021	355	44	128	47	112	378	2450
	2012	231	165	1036	440	50	122	32	95	320	2491
Streptococcus pneumoniae, invasive disease, < 5 years	2011	35	35	241	55	8	38	15	21	87	535
	2012	42	25	185	69	4	12	4	14	39	394
Vibrio cholerae O1	2011	0	0	0	0	1	0	0	0	0	1
	2012	0	0	0	0	0	0	0	0	0	0
Viral Haemorrhagic Fever (VHF)											
Crimean Congo Haemorrhagic Fever (CCHF)	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
***Other VHF (not CCHF)	2011	17	3	0	0	0	0	3	0	14	37
	2012	0	0	0	0	0	0	0	0	0	0

Footnotes

*Numbers are for cases of all ages unless otherwise specified. Data presented are provisional cases reported to date and are updated from figures reported in previous bulletins.

**Rubella cases are diagnosed from specimens submitted for suspected measles cases

***All cases for 2011 were confirmed as Rift Valley Fever

Provinces of South Africa: EC – Eastern Cape, FS – Free State, GA – Gauteng, KZ – KwaZulu-Natal, LP – Limpopo, MP – Mpumalanga, NC – Northern Cape, NW – North West, WC – Western Cape

U = unavailable, 0 = no cases reported

Table 2: Provisional laboratory indicators for NHLS and NICD, South Africa, corresponding periods 1 January - 30 September 2011/2012*

Programme and Indicator	1 Jan - 30 Sep, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Acute Flaccid Paralysis Surveillance											
Cases < 15 years of age from whom specimens received	2011	50	19	68	63	59	32	5	12	14	322
	2012	44	17	45	53	31	35	2	14	22	263

Footnotes

*Numbers are for all ages unless otherwise specified. Data presented are provisional numbers reported to date and are updated from figures reported in previous bulletins.
Provinces of South Africa: EC – Eastern Cape, FS – Free State, GA – Gauteng, KZ – KwaZulu-Natal, LP – Limpopo, MP – Mpumalanga, NC – Northern Cape, NW – North West, WC – Western Cape

U = unavailable, 0 = no cases reported

Monitoring for the presence of polio in a country is based on AFP (acute flaccid paralysis) surveillance – the hallmark clinical expression of paralytic poliomyelitis. The clinical case definition of AFP is an acute onset of flaccid paralysis or paresis in any child under 15 years of age. AFP is a statutory notifiable disease and requires that 2 adequate stool specimens are taken as soon as possible, 24 to 48 hours apart, but within 14 days after onset of paralysis, for isolation and characterisation of polio virus. The differential diagnosis of AFP is wide, the most common cause of which is Guillain-Barre Syndrome. The incidence of AFP in a population has been studied in a number of developing countries and WHO have determined, as a result of these studies, that the criterion for adequate surveillance of AFP is 2 cases per 100 000 population of children less than 15 years of age (it was formerly 1 per 100,000 but this was thought to be inadequately sensitive).

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