NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

COMMUNICABLE DISEASES SURVEILLANCE BULLETIN

VOLUME 13. NO 4 NOVEMBER 2015

FOREWORD

The development of an HIV vaccine or the use of passive immunization to prevent HIV infection remains the best hope for an AIDS-free future. In this issue the potential of a modified RV144 HIV vaccine is discussed in the light of a Phase 1-2 trial currently underway in South Africa.

Cases of Acanthamoebic keratitis are rare in South Africa. Nevertheless, given the severity of infection, accurate and rapid diagnostic test results are expected to contribute significantly to potentially sight-saving clinical outcomes. The usefulness of a ribosomal DNA PCR assay for rapid diagnostic turnaround time is discussed in this issue.

Surveillance reports for this issue include the results of an investigation into an increase in pertussis cases in South Africa during the period July to September 2014. This report concludes that the increase in cases in this period is possibly related to disease periodicity. A comparison of mortalities among HIV positive MDR-TB patients on ARVs treated with regimens containing either moxifloxacin or ofloxacin in Gauteng Province, South Africa, during the period 2007 to 2012 concludes that mortality was similar in the moxifloxacin and ofloxacin treatment regimens although there was a delayed time-to-death in the moxifloxacin group.

This is the final issue for 2015 and we wish all our readers and contributors a safe and joyous holiday season.

Basil Brooke, Editor

CONTENTS

GEARING UP TO TEST ACTIVE AND PASSIVE IMMUNIZATION FOR HIV PREVENTION

Lynn Morris, Zanele Ditse, Nonhlanhla Mkhize, Penny Moore

HIV Virology section, Centre for HIV and STIs, NICD

Encouraged by the results of the RV144 HIV vaccine trial conducted in Thailand, the USA-based HIV vaccine Trials Network (HVTN) has embarked on an ambitious program to determine whether this vaccine regimen can reduce HIV infection rates in southern Africa. While the 31% efficacy seen in RV144 was modest, the hope is that with modifications this vaccine will show sufficient efficacy to warrant licensure and general use. These newer vaccines have been tailored to target HIV subtype C viruses that circulate in southern Africa and are currently being tested in HVTN 100. This is a small Phase 1-2 trial of 252 individuals, with plans to conduct a large scale efficacy trial if the data look promising.

The vaccine regimen comprises two components that aim to stimulate both arms of the immune response. For the cellular arm, the relatively harmless canarypox virus (called ALVAC) has been engineered to carry small pieces of HIV which trick the body into thinking it is under attack thereby inducing an immune response to HIV. Soluble protein antigens derived from the HIV envelope gp120 glycoprotein are used to elicit an antibody response to HIV. An immune correlates analysis of RV144 showed that individuals who developed antibodies to the variable region 1 and 2 (V1V2) of the gp120 vaccine had a reduced risk of HIV infection. Results from RV144 are being used to set criteria for a crucial go/no-go decision in HVTN 100. More than 90% of individuals who receive the vaccine must have binding antibodies to HIV antigens with at least 56% having V1V2 binding antibodies for the vaccine to proceed. Furthermore, at least 60% of vaccinees must have HIV-specific CD4+ T-cell responses.

This go/no-go decision will be made in the first quarter of 2016. If the criteria are met, then 5400 individuals will be enrolled into the HVTN 702 efficacy trial across 13 clinical sites in southern Africa including 6 sites in South Africa (Cape Town, eThekwini, Isipingo, Klerksdorp, Soshanguve and Soweto). Half will receive the vaccine while the other half will receive a placebo which does not contain any HIV antigens. Neither the participants nor the study co-ordinators will know who received the vaccine or placebo. This is called a double-blinded randomized clinical trial, or RCT, and is the “gold standard” for assessing whether a product works. In the case of HVTN 702, participants will be followed for up to 3 years for evidence of HIV infection. If there are significantly fewer infections in those who received the vaccine, then the vaccine will be considered efficacious. A vaccine able to reduce infection rates even by 50% will have a major impact on the HIV epidemic.

In parallel, the HVTN 703 or antibody-mediated protection (AMP) trial will also be conducted in southern Africa. This trial aims to test a related but different concept (see figure below), namely whether or not a pre-formed monoclonal antibody called VRC01 can prevent HIV infection following passive transfer. Unlike the V1V2 binding antibodies induced by vaccination that are thought to function through antibody-dependent cellular cytotoxicity (ADCC), VRC01 is a broadly neutralizing antibody that directly blocks infection of cells by a range of diverse viruses. Since no vaccine is yet able to elicit broadly neutralizing antibodies, this trial aims to provide important proof-of-concept that broadly neutralizing antibodies can prevent HIV infection in humans. In addition, this trial will provide insight into the dose of an

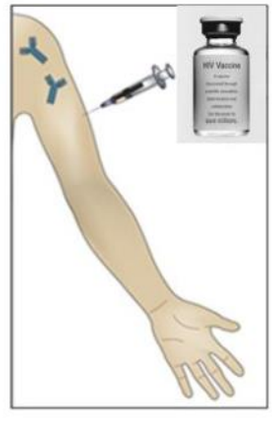
antibody that would be required for protection by vaccination.

The antibody VRC01 targets the CD4 binding site on the HIV envelope gp120 and is a highly conserved target. As such it shows excellent breadth, neutralizing more than 90% of global isolates including subtype C. It has previously been shown to be safe in humans and to prevent infection in monkeys. It has also been tested therapeutically in people infected with HIV and has been shown to reduce viral levels. However, the antibody was not effective in HIV-infected individuals whose virus was resistant to VRC01. This indicates that only viruses that are sensitive to VRC01 will be prevented from establishing HIV infection. Future trials may therefore need to consider the use of combinations of antibodies to cover the majority of HIV strains.

Participation in HVTN 703 will require that healthy HIV negative volunteers receive intravenous infusions every 2 months for 20 months (i.e. 10 infusions). A total of 1500 women at high risk of HIV infection will be enrolled into this study; 500 will receive VRC01 at the higher dose of 30 mg/kg body weight, 500 will receive 10 mg/kg and 500 will receive a placebo. This is also an RCT and vaccinees will be studied for over 2 years with HIV infection as an end-point. During the trial, volunteers will be counselled on safe sexual practises and provision of oral pre-exposure prophylaxis (PrEP) is under discussion. This trial is the first of its kind and is unprecedented in HIV prevention research.

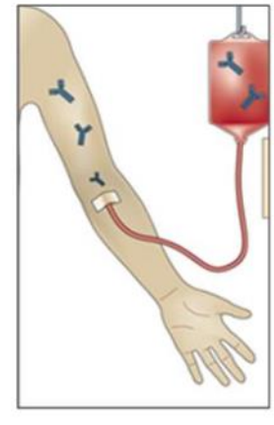
There is no doubt that the crisis caused by the HIV pandemic requires urgent and bold steps. While the roll-out of anti-retroviral therapy (ART) has greatly impacted the HIV epidemic, there are still 1.5 million deaths and 2 million new HIV infections around the world every year. In South Africa there are over 6 million people living with HIV of whom only 42% are being treated with ART. Issues of cost, access, toxicity and drug resistance make the use of ART to control the HIV epidemic unrealistic. The development of a vaccine or the use of passive immunization to prevent HIV infection remains our best hope for an AIDS-free future.

Active immunization



Vaccination to stimulate antibodies that correlate with a reduced risk of HIV infection. This is being tested in HVTN 702

Passive immunization



Pre-formed neutralizing antibody VRC01 is infused to provide protection against HIV infection. This is being tested in HVTN 703

GENOTYPING OF SOUTH AFRICAN *ACANTHAMOEBA* KERATITIS ISOLATES

Vivian Mothibe, Bhavani Poonsamy, Desiree du Plessis, John Frean

Centre for Opportunistic, Tropical and Hospital Infections, NICD

Background

Acanthamoebic keratitis (AK) is a rare but often severe, sight-threatening infection caused by certain free-living amoeba species (Figure 1).

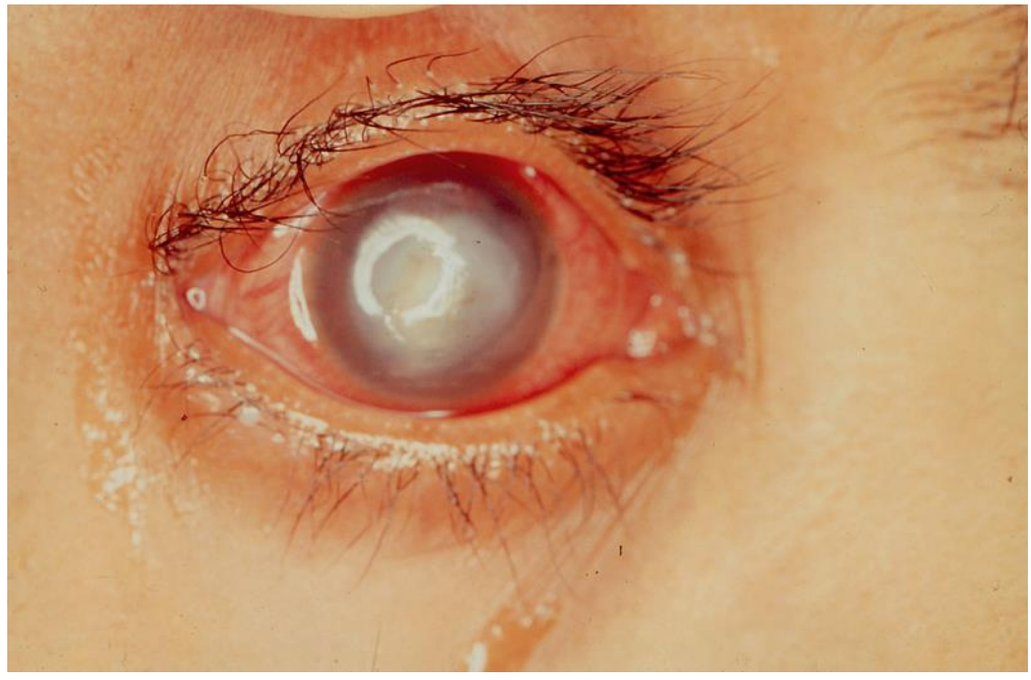


Figure 1: Acanthamoebic keratitis. Note the corneal infiltrate and the intense conjunctival reaction (image credit: Prof. T Carmichael)

Most free-living amoebae are non-pathogenic, but the genera *Acanthamoeba*, *Naegleria* and *Balamuthia* contain species that are potentially invasive. Free-living amoebae are ubiquitous in the environment. *Acanthamoeba* spp. can therefore be found in natural surface water bodies, both saline and fresh, and artificial environments like swimming pools, domestic and industrial water pipes, water tanks, air conditioners, cooling towers, drains, shower heads and taps. They occur in soil, compost, sewage, sediments, and in humans, on skin, and in the upper respiratory tract following inhalation of cysts.[1] Under unfavourable conditions the amoebae form resistant cysts that can survive in dry environments like dust and air, which can therefore be sources of infection. The cornea (particularly in those who wear contact lenses), and central nervous system (in the form of granulomatous amoebic encephalitis in immunocompromised persons) are the main target organs for invasive *Acanthamoeba* strains. Skin infections may also occur, and in immune-compromised patients, provide a route for haematogenous spread to the brain. People who handle contact lenses with unclean hands, who do not take proper care of contact lenses, or who wear them in inappropriate conditions or for excessive periods, are at risk for acanthamoebic keratitis. The infection is very

painful and progressive, and once deep corneal penetration and ulceration is established, vision is threatened. Aggressive, complicated and prolonged treatment is required, and sometimes only corneal transplant will save the eye. Prognosis is therefore dependent on the speed of accurate diagnosis and initiation of proper treatment. The traditional methods used for diagnosis are direct microscopic examination and culture of corneal material on non-nutrient agar seeded with *Escherichia coli*, which the amoebae phagocytose (Figure 2).

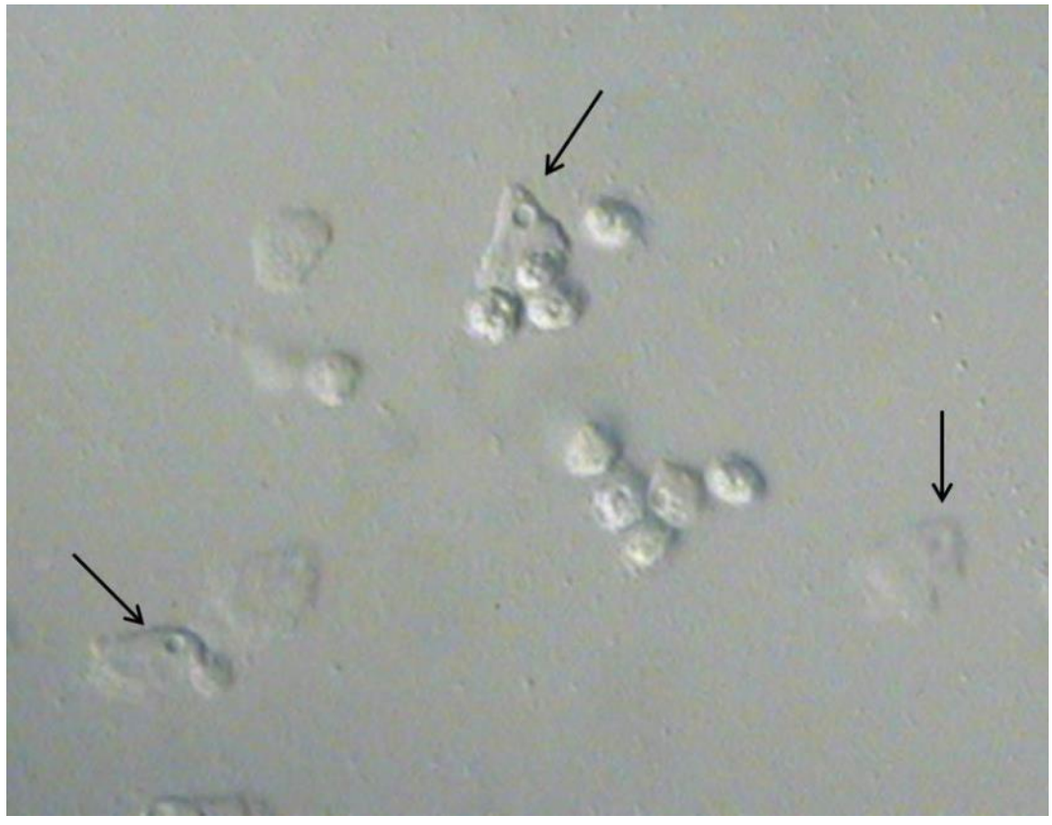


Figure 2: Agar plate culture showing *Acanthamoeba* trophozoites and their contractile vacuoles (arrows) and immature cysts (interference contrast, 400x).

Isolation of acanthamoebae from contact lenses and their containers sometimes provides indirect but highly suggestive corroborative evidence of the cause of keratitis. Morphological distinction between species is difficult and often unsatisfactory, so classification based on 18S ribosomal RNA gene sequences has allowed genotypic characterisation of strains of *Acanthamoeba* species. Seventeen genotypes have been distinguished, with most isolates associated with AK belonging to the T4 genotype, but T2a, T3, T5, T6, and T11 are also linked to AK. Certain genotypes (T1, T10, T12) are associated with granulomatous encephalitis, and no disease association has yet been found with the others.[1-4]

The aim of this study was to confirm the presence or absence of *Acanthamoeba* species in samples previously tested by culture and microscopy, using a molecular-based approach to determine the *Acanthamoeba* species genotypes of the positive samples.

Materials and methods

Forty-six archived patient samples, comprising 28 culture-

confirmed positive and 18 negative samples, collected between 1996 and 2014, were assayed. Among the positives, nine samples previously confirmed to be *Acanthamoeba* T4 genotype[5] were included as controls. A reference *A. castellanii* strain (ATCC 30010D) was also used. Stored samples were re-cultured and the genomic DNA was extracted manually using QIAamp DNA Mini kit (Qiagen, USA). Amplification of an approximately 500 bp 18S ribosomal gene sequence was done as previously described.[5] PCR products were electrophoresed on 2% agarose gel stained with SYBR safe DNA dye. Results were recorded on a UV gel documentation system. Amplicons of all positive isolates were sequenced (Inqaba Biotech, Pretoria) and compared to existing nucleotide sequences on the GenBank databases to determine the genotypes.

Results

DNA from 25 *Acanthamoeba* subcultures was amplified and sequenced, of which 24 were phylogenetically similar to genotypes previously deposited in GenBank. One sample did not align with any GenBank database sequence. Twenty-one isolates were identified as genotype T4 (of these, 13 were unnamed *Acanthamoeba* species and eight were *Acanthamoeba castellanii)* and three as genotypeT3 (unnamed species). Control samples were all T4 genotype, as expected.

Discussion and conclusion

A previous study showed that South African cases of AK were caused by the *Acanthamoeba* T4 genotype.[5] The results presented here support this finding. Accurate and rapid diagnostic test results will contribute to potentially sight-saving clinical outcomes. The much reduced turnaround time for PCR, compared to up to 10 days for culture, is clearly beneficial for clinical management. Additionally, PCR is highly sensitive and specific, and can be applied to other relevant clinical samples from cases of suspected acanthamoebic infections, such as skin and brain biopsies. To date, the only case of *Acanthamoeba* encephalitis reported in South Africa was a farm animal,[6] but it is possible that undiagnosed or misdiagnosed cases of this devastating condition occur amongst the country's large burden of HIV-related immune-deficient persons.

References

1. Khan NA. *Acanthamoeba* species. In: Khan NA (ed), 2008. Emerging Protozoal Pathogens. Abingdon: Taylor & Francis.
2. Boggild AK, Martin DS, Lee TY, Yu B, Low DE. Laboratory diagnosis of amoebic keratitis: comparison of four diagnostic methods for different types of clinical samples. *J Clin Microbiol* 2009; 47(5):1314-18.
3. Buerano CC, Trinidad AD, Fajardo LSN, *et al.* Isolation of *Acanthamoeba* genotype T4 from non-contact lens wearer from Philippines. *J Trop Med Public Health* 2014; 42(4):145-7.
4. Ledee DR, Iovieno A, Miller D, *et al*. Molecular identification of T4 and T5 genotypes in isolates from *Acanthamoeba* keratitis patients. *J Clin Microbiol* 2009; 47(5):1458-62.
5. Schroeder JM, Booton GC, Hay J, Niszl IA, Seal DV, Markus MB, Fuerst PA, Byers TJ. Use of subgenic ribosomal DNA PCR and sequencing for genus and genotype identification of *Acanthamoeba* keratitis and from sewage sludge. *J Clin Microbiol* 2001; 39(5):1903-11.
6. Van der Lugt JJ, van der Merwe HE. Amoebic meningoencephalitis in a sheep. *J S Afr Vet Assoc*1990; 61(1): 33-6.

AN INVESTIGATION OF A POTENTIAL INCREASE IN PERTUSSIS CASES IDENTIFIED THROUGH SENTINEL SURVEILLANCE IN SOUTH AFRICA, JULY 2012 – SEPTEMBER 2014

Hetani Ngobeni[1,2], Fahima Moosa[1,3,], Mignon du Plessis[1,3,4], Sibongile Walaza[1], Adam Cohen[5,6], Ebrahim Variava[7,8], Halima Dawood[9], Nicole Wolter[1,3,4], Anne von Gottberg[1,3,4] Cheryl Cohen[1,3]

[1]Centre for Respiratory Diseases and Meningitis, NICD

[2]Field Epidemiology Training Program, NICD

[3]Faculty of Health Sciences, University of the Witwatersrand, Johannesburg

[4]Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg

[5]Influenza Division, Centres for Disease Control and Prevention, Atlanta, Georgia, US

[6]Influenza Programme, Centers for Disease Control and Prevention, Pretoria

[7]Department of Medicine, Klerksdorp Tshepong Hospital Complex, Klerksdorp

[8]Pietermaritzburg Metropolitan Hospitals and University of KwaZulu-Natal, Pietermaritzburg

Introduction

It is estimated that *Bordetella pertussis* causes about 16 million cases and 195,000 deaths globally in children every year.[1] Children in developing countries are most affected, especially where vaccination coverage is low.[1] A global increase in pertussis cases has been noted in the last two decades.[2] This has been attributed to various factors including increased clinician awareness and improved diagnostics[2], decreased vaccination coverage, use of acellular pertussis (aP) vaccines instead of the previously used whole-cell vaccines and pathogen adaptation.[3] The relative contribution of these factors may differ between countries and is the subject of ongoing debate. The effectiveness of the aP vaccine has been found to wane after the last scheduled dose but immunity may be reactivated by a booster dose administered to older children.[4, 5]

Confirming a diagnosis of infection with *B. pertussis* in the laboratory is challenging as the organism is fastidious to culture. Although currently there is no satisfactory gold standard technique for laboratory confirmation of a pertussis infection, isolation of *B. pertussis* in culture has nearly 100% specificity.[2] Polymerase chain reaction (PCR) of *B. pertussis* is based on detection of the insertion sequence IS481.[6] Due to the high copy number (~200 copies) of IS481 in *B. pertussis,* the assay is susceptible to contamination and pseudo-outbreaks have been previously reported.[7] A culture-negative result does not exclude the diagnosis of pertussis as other factors such as previous immunization, receipt of antimicrobial therapy or testing late in the clinical course after several weeks of symptoms may affect the sensitivity of diagnosis.[8]

South Africa introduced whole-cell pertussis vaccines in 1950 which led to a marked decline in reported pertussis morbidity and mortality.[9] In order to achieve a triple vaccine comprising diphtheria, tetanus and pertussis (DTP), tetanus toxoid and diphtheria was added in May 1957.[9] The aP vaccines were introduced into the South African national immunisation programme - the Expanded Programme on Immunization (EPI) - in 2009 through a pentavalent combined vaccine.[10] Infants are

immunised with aP vaccines at 6, 10 and 14 weeks followed with a booster dose at 18 months.[11] The pentavalent combined vaccine includes: Bacille Calmette-Guérin (BCG), diphtheria, tetanus, acellular pertussis, inactivated polio vaccine, *Haemophilus influenza* type B and hepatitis B vaccine (DTaP-IPV/Hib/HBV)[12].

Data obtained from the National Institute for Communicable Diseases (NICD) pneumonia Severe Acute Respiratory Illness (SARI) surveillance programme, an active sentinel-site based surveillance programme for severe respiratory infection which was implemented in 2012, suggested an increase in pertussis cases from July-September 2014. The aim of this surveillance project was to conduct an investigation of patients identified though the SARI and Influenza-like Illness (ILI) surveillance programmes testing positive for pertussis from June 2012 to September 2014 in order to establish whether this was a pseudo-outbreak due to environmental and/or laboratory contamination or a true increase in disease. Furthermore, a comparison of the characteristics of patients during the period of increased case numbers against the baseline was conducted. Additionally, this project aimed to establish whether cases identified during the period of increased case numbers presented with classical pertussis symptoms.

Methods

In June 2012, the NICD began conducting enhanced surveillance for an expanded panel of respiratory pathogens (including *Bordetella* spp.) as part of the SARI programme at the Edendale and Klerksdorp-Tshepong Hospital Complex (KTHC) enhanced surveillance sites. In addition, the NICD initiated a programme of systematic ILI surveillance at public health clinics in the catchment area of the surveillance hospitals. Edendale Gateway clinic in Pietermaritzburg and Jouberton Clinic in Klerksdorp began systematically enrolling patients with ILI in June 2012.

The SARI and ILI programmes have been described previously.[12] In brief, dedicated surveillance officers screen all admissions and patients presenting for outpatient consultation Monday to Friday at SARI and ILI surveillance sites respectively. All patients meeting surveillance case definitions (Table 1) are approached for study enrolment and consenting patients are enrolled. Detailed epidemiologic data are collected through patient interview and medical record review and include demographic characteristics, clinical signs and symptoms, presence of underlying conditions, details of clinical management and outcome. Hospitalised patients are followed up until discharge or death to determine in-hospital outcome. Surveillance officers collect nasopharyngeal aspirates from children aged <5 years and nasopharyngeal and oropharyngeal swabs from individuals aged ≥5 years. In addition, induced sputum specimens are collected from all consenting hospitalised patients if not contraindicated.

Detection of *Bordetella* spp. was conducted using a previously published multiplex real-time PCR assay.[13] The assay detects the IS481 gene to determine the presence of *Bordetella* spp., the *pIS1001* gene for *B. parapertussis*, the *hIS1001* gene for *B. holmesii* and the pertussis toxin (*ptx*) gene to confirm *B. pertussis*.

Table 1: Case definitions by age criteria used in the influenza-like illness (ILI) and severe acute respiratory illness (SARI) surveillance systems.

Case	Age criteria	Case definition
ILI	All ages	2012-2014 • Acute fever of >38 degrees Celsius and/or self-reported fever within the last 3 days AND • Either cough or sore throat • The absence of other diagnoses 2014- • Acute fever of ≥38 degrees Celsius and/or self-reported fever within the last 10 days AND • Cough in the absence of other diagnoses
SARI	2days to < 3 months old	Any child with diagnosis of suspected sepsis or physician-diagnosed lower respiratory tract infection (LRTI) irrespective of signs and symptoms. Patient presenting within ≤10 days of the onset of illness
	3 months old to <5 years old	A child with physician-diagnosed acute lower respiratory tract infection (LRTI) including bronchiolitis, pneumonia, bronchitis and pleural effusion. Patient should be presenting within≤10 days of the onset of illness
	≥5 years old	Any person presenting with manifestations of acute lower respiratory infection with: sudden onset of fever (>38ºC) AND, cough or sore throat AND, shortness of breath, or difficulty breathing with or without clinical or radiographic findings of pneumonia), or tachypnea. Patient presenting within 7 days of the onset of illness From May 2014- changed to any person presenting with physician diagnosed LRTI with history of fever/documented fever (≥38^{0}C) and cough and onset within ≤10 days
Severe respiratory infection	All ages	Patient with clinical signs and symptoms meeting the case definitions for SARI or clinician-diagnosed LRTI irrespective of duration of symptoms and patients with suspected or confirmed TB

Investigation of increase in incidence

The baseline was defined as the period from June 2012 through June 2014 and the suspected outbreak defined as the period from July to September 2014. A *B. pertussis* case was defined as any individual with either an NP and/or sputum specimen with an IS481 Ct value <45 who presented during the period July 2012 to September 2014. In order to identify potential laboratory contamination all PCR controls, reagents and equipment were tested for *B. pertussis*. Environmental swabs from surfaces in specimen collection, vaccination and staff rooms from three facilities were collected and tested in order to identify possible environmental contamination with DNA. These surfaces included children's scales, immunization supplies cupboards, oral rehydration boxes, immunization chairs, surveillance officers' supplies cooler boxes, door handles to stock cabinet, isolation room basin, surveillance officer handwashing basin, data capture room basin, patient locker tap and biohazard bins.

For the retrospective case investigations clinical symptoms of pertussis were defined as one or more of the following: a cough of more than two weeks, paroxysmal cough, cough with inspiratory "whoop", post-tussive vomiting and apnoea or cyanosis in infants. A questionnaire was developed and face-to-face retrospective interviews were conducted with cases to determine the presence of typical pertussis symptoms at the time of illness as these were not specifically asked about in the SARI and ILI surveillance questionnaire. In addition to clinical presentation, data on case management, exposure contacts and post-exposure management of cases through the questionnaire were collected.

Chi-square tests were used for statistical analysis of data.

Results

Peaks of *B. pertussis cases* were noted during May-September among patients with both ILI and SARI in all the three years of surveillance (Figures 1 & 2). A mean of 5 *B. pertussis* cases per month from July-September were identified during the baseline period compared to 14 cases per month during the suspected outbreak period (Figure 2). No significant differences in the characteristics of individuals with SRI and ILI testing *B. pertussis* positive during baseline and suspected outbreak periods were identified (Table 2).

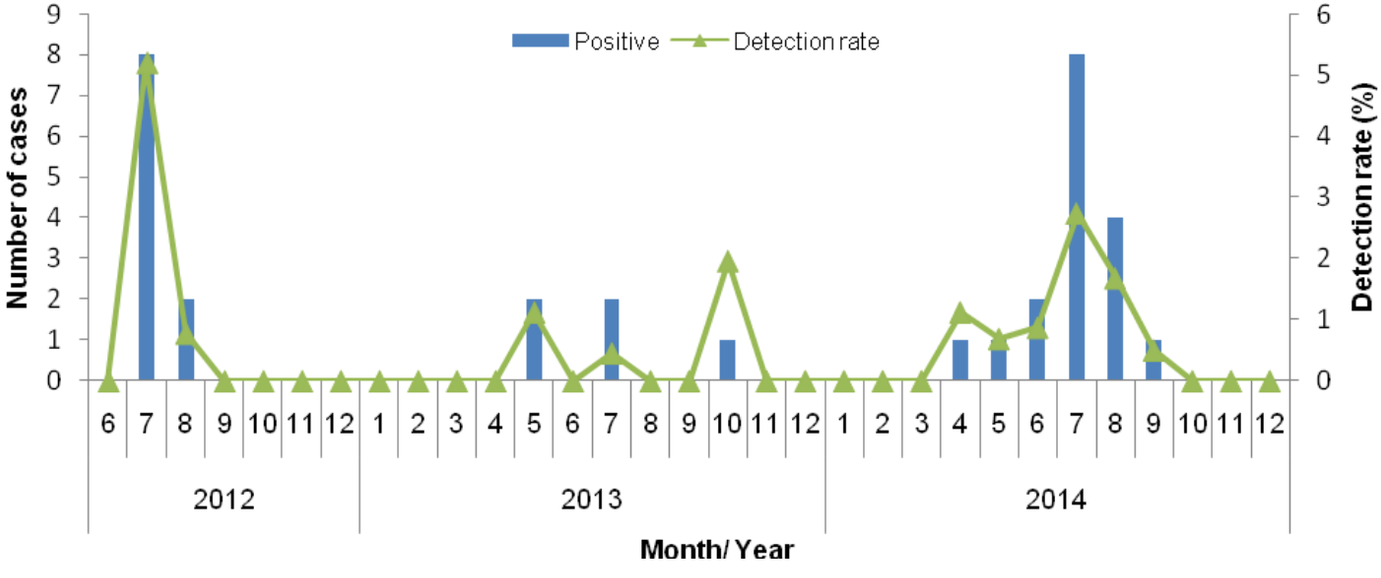


Figure 1: Numbers of positive cases and detection rate of *Bordetella pertussis* among influenza-like illness (ILI) cases in Klerksdorp and Pietermaritzburg, 2012-2014.

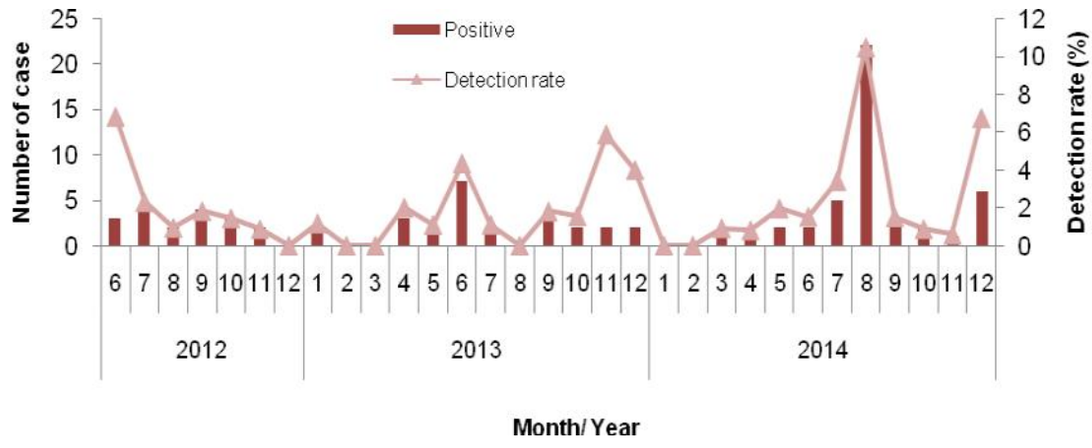


Figure 2: Numbers of positive cases and detection rate of *Bordetella pertussis* among severe acute respiratory illness (SARI) cases in Klerksdorp and Pietermaritzburg, 2012-2014.

Table 2: Comparison of characteristics of patients with influenza-like illness and severe acute respiratory illness testing positive for *Bordetella pertussis* during the baseline and suspected outbreak periods at the Edendale and Klerksdorp-Tshepong Hospital Complex sites, June 2012-Sept 2014

Characteristic		Influenza-like illness			Severe acute respiratory illness		
		Baseline* n/N (%)	Suspected Outbreak** n/N (%)	p-value	Baseline* n/N (%)	Suspected Outbreak** n/N (%)	p-value
Site				0.095			0.271
	Pietermaritzburg	10/19 (53)	3/13 (23)		22/49 (45)	13/39 (33)	
	Klerksdorp	9/19 (47)	10/13 (77)		27/49 (55)	26/39 (67)	
Age				0.575			0.506
	<4months	1 /19 (5)	0/13 (0)		6/49 (12)	3/39 (8)	
	4m - 1year	0/19 (0)	0/13 (0)		8/49 (16)	3/39 (8)	
	1-4y	4/19 (21)	5/13 (39)		4/49 (8)	4/39 (10)	
	5-24y	7/19 (37)	5/13 (39)		2/49 (4)	3/39 (8)	
	25-44y	7/19 (37)	3/13 (23)		17/49 (35)	11/39 (29)	
	45-64y	0/19 (0)	0/13 (0)		11/49 (22)	10/39 (26)	
	65+	0/19 (0)	0/13 (0)		1/49 (2)	4/39 (11)	
Sex				0.567			0.906
	Male	6/19 (31)	5/12 (42)		22/49 (45)	19/39 (46)	
HIV Status				0.191			0.514
	Infected	9/16 (56)	3/10 (30)		23/39 (59)	18/35 (51)	
Symptom duration				0.771			0.140
	<7 days	15/18 (83)	10/12 (83)		28/47 (60)	17/39 (44)	
Death							0.758
	Yes	0/19 (0)	0/13 (0)		4/48 (8)	4/39 (10)	

*Baseline: June 2012-June 2014
**Suspected outbreak: July–September 2014
n=Proportion of cases
N=Total number of cases

Of 32 environmental swabs taken, one (3%) surface from Tshepong hospital tested positive. This swab was taken from a basin in a data entry office used by nurses to wash their hands and also to store the ward rotation supplies cooler bag. Nurses did not take specimens or conduct any procedures in this room. Rooms where patient sample collection took place were not used for vaccination. All laboratory controls and reagents tested were negative for *B. pertussis.*

Epidemiological investigations to determine the presence of classical pertussis symptoms were conducted for 23 of 52 cases identified during the outbreak period (44%). All 23 showed at least one pertussis symptom, the commonest being history of cough more than 2 weeks (83%, 19/23), followed by paroxysmal cough (52%, 12/23). Apnoea was reported in both infants in this group (100%,2/2) and cyanosis in one of them (50%, 1/2). No epidemiologic links were identified between cases. Among children <5 years of age, vaccination data were missing for 30% of cases (7/23) in the baseline and 25% (4/16) in the suspected outbreak period. Of those with available data, 75% (12/16) in the baseline and 36% (4/12) in the suspected outbreak period were fully vaccinated according to the recommended schedule (p=0.027). Of the 23 interviewed cases 9% (2/23) were clinically suspected of pertussis by the attending physician. A mean of 5 (range: 1-7) household contacts for each case was found and none of the contacts reported receiving chemoprophylaxis.

Discussion

This investigation, including the environmental contamination results, suggests that the increase in pertussis cases during July-–September 2014 was unlikely a pseudo-outbreak or due to laboratory contamination. Rigorous laboratory and environmental disinfection practices minimized the likelihood of false-positive results from contaminated clinical specimens.

A seasonal increase in the incidence of pertussis cases was found throughout the surveillance period (2012-2014) with most cases occurring during South Africa's winter season of May to September. This correlation suggests true seasonality of pertussis disease in South Africa which may have, in part, contributed to the observed increase in case numbers that prompted this investigation. However, the observed increase in 2014 was greater than in previous years and could reflect underlying disease periodicity or other factors.[14]

These results are based on data that only go as far back as 2012 when the surveillance system was initiated. Without extensive baseline data it is difficult to determine whether this observed increase is due to pertussis epidemic peaks that occur every 3-5 years as described in other countries.[15] A retrospective review of case clinical presentation showed that cough of >2 weeks duration was the commonest symptom (present in 83% of individuals), consistent with previous findings in other settings.[16]

After the introduction of the acellular pertussis vaccine into the routine immunisation programme in 2009 in South Africa, the estimated coverage for the DTP vaccine according to the South African National Department of Health was 10.3% while UNICEF estimated the coverage to be at 69% in 2012.[17] Better quality data on vaccine coverage for South Africa are needed. The contribution of possible low vaccine coverage to the observed increase in pertussis incidence should be further explored.

A minority of cases identified were clinically suspected by the attending clinician, even though on review all cases had at least once classic pertussis symptom. The commonest symptom was chronic cough which is non-specific and clinicians may be more likely to consider other more common diagnoses such as tuberculosis. Contact follow-up with chemoprophylaxis was also not conducted for any cases, likely related to the low index of clinical suspicion and the fact that availability of surveillance results was delayed. It is important that clinicians consider pertussis in the differential diagnosis of patients with cough and submit specimens for testing. Suspected pertussis cases should be notified and contacts followed up. At sentinel sites, processes for transport, testing and reporting of pertussis results should be streamlined to ensure more rapid reporting of results to clinicians. Efforts are underway to implement this.

This study had several limitations. Cases may have been missed because the surveillance case definitions were not designed to specifically target pertussis for both ILI and SARI. Due to enrolment being restricted to weekdays, cases that presented over weekends were likely missed. Gaps in available data on the vaccination status of children have been identified which limited analysis. In addition, there was a delay in the collection of clinical data from cases which may have resulted in recall bias. It was not possible to collect environmental specimens from the Gateway clinic site. However, only 3 of the 52 cases in the outbreak period were identified from this site.

In conclusion, an increase in the incidence of pertussis cases in South Africa between July and September 2014 occurred. This increase does not appear to be due to environmental or laboratory contamination or a discrete outbreak as no epidemiologic links between cases was identified. The high proportion of children not fully vaccinated according to age should be explored further. Improved collection of complete vaccine histories in all children aged <5 years is needed. Recent data following the conclusion of this outbreak investigation shows a sustained increase in pertussis case numbers in 2015.[18] This suggests that the observed increase in case numbers may have been the start of a period of increased case incidence possibly related to disease periodicity. The possible contribution of waning immunity following the change to acellular pertussis vaccine in the routine immunisation programme since 2009 should be further investigated.

Acknowledgments

The authors wish to thank all the members of the CRDM and especially the SARI and ILI programme investigators.

References

1. CDC. Pertussis in other countries 2015 [updated 8 September 2015; cited 2015 7 October 2015]. Available from: http://www.cdc.gov/pertussis/countries.html. *Centers for Disease Control and Prevention* [Internet].
2. Fisman DN, Tang P, Hauck T, Richardson S, Drews SJ, Low DE, et al. Pertussis resurgence in Toronto, Canada: a population-based study including test-incidence feedback modeling. *BMC public health* 2011; 11: 694.
3. Mooi FR, van Loo IH, van Gent M, He Q, Bart MJ, Heuvelman KJ, et al. *Bordetella pertussis* strains with increased toxin production associated with pertussis resurgence. *Emerg Infect Dis.* 2009; 15(8):1206-13.
4. Winter K, Harriman K, Zipprich J, Schechter R, Talarico J, Watt J, et al. California pertussis epidemic, 2010. *J Pediatr.* 2012;161(6):1091-6.
5. Witt MA, Katz PH, Witt DJ. Unexpectedly limited duarability of immunity following acellular pertussis vaccination in preAdolescents in a North American outbreak. *Clin Infect Dis*. 2012 Jun; 54(12):1730-5.
6. Lievano FA, Reynolds MA, Waring AL, Ackelsberg J, Bisgard KM, Sanden GN, et al. Issues associated with and recommendations for using PCR to detect outbreaks of pertussis. *J Clin Microbiol*. 2002; 40(8):2801-5.
7. Tozzi AE, Celentano LP, Ciofi degli Atti ML, Salmaso S. Diagnosis and management of pertussis. *Can Med Assoc J*. 2005; 172(4):509-15.
8. Crowcroft NS, Pebody RG. Recent developments in pertussis. *The Lancet*. 2006; 367(9526):1926-36.
9. Strebel P, Hussey G, Metcalf C, Smith D, Hanslo D, Simpson J. An outbreak of whooping cough in a highly vaccinated urban community. *J Trop Pediatr* 1991; 37(2):71-6.
10. Madhi SA, Bamford L, Ngcobo N. Effectiveness of pneumococcal conjugate vaccine and rotavirus vaccine introduction into the South African public immunisation programme. *S Afr Med J*. 2014; 104(3):228.
11. Souter J. Vaccines administered at 6, 10 and 14 weeks. *Prof Nurs Today*. 2012; 16(2):24-8.
12. McAnerney JM, Cohen C, Moyes J, Besselaar TG, Buys A, Schoub BD, *et al*. Twenty-five years of outpatient influenza surveillance in South Africa, 1984-2008. *J Infect Dis*. 2012; 206(Suppl 1):S153-8.
13. Loeffelholz M. Towards improved accuracy of Bordetella pertussis nucleic acid amplification tests. *J Clin Microbiol*. 2012; 50(7):2186-90.
14. Broutin H, Viboud C, Grenfell BT, Miller MA, Rohani P. Impact of vaccination and birth rate on the epidemiology of pertussis: a comparative study in 64 countries. *Proc Biol Sci*. 2010; 277(1698):3239-45.
15. Broutin H, Guegan JF, Elguero E, Simondon F, Cazelles B. Large-scale comparative analysis of pertussis population dynamics: periodicity, synchrony, and impact of vaccination. *Am J Epidemiol*. 2005; 161(12):1159-67.
16. Senzilet L, Halperin SA, Spika JS, Alagaratnam M, Morris A, Smith B, et al.Pertussis is a frequent cause of prolonged cough illness in adults and adolescents. *Clin Infect Dis*. 2001 Jun 15; 32(12):1691-7.
17. Van den Heever J [Internet]. What is the South African immunisation coverage? 2012. [cited 2015 Oct 28]. Available from: https://www.google.co.za/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CBwQFjAAahUKEwid1vqVj-fIAhVB2RQKHR3aAQU&url=http%3A%2F%2Fwww.africacheck.org%2Fwp-content%2Fuploads%2F2012%2F11%2FS-Africans-Immunization-Coverage-ppt-EPI-Manager3.pdf&usg=AFQjCNEhtSBZ06kvqmEVTq0bIfXT8undgQ&sig2=FKWVFbay5PqKo4wIfc9EjQ
18. NICD. Increased detection of *Bordetella pertussis* in the pneumonia and influenza-like illness surveillance programmes. Communicable Diseases Communiqué; 14(6): 4-5.

COMPARING MORTALITY AMONG HIV POSITIVE MDR-TB PATIENTS ON ARVS TREATED WITH EITHER MOXIFLOXACIN OR OFLOXACIN CONTAINING REGIMEN, GAUTENG, 2007 - 2012

Hluphi Mpangane[1,2,3], Mbhekiseni Khumalo[4], Andries Dreyer[3], Alfred Musekiwa[2,5], Hendrik Koornhof[3], Carl Reddy[1,2], Nazir Ismail[1,3]

[1]Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa
[2]South African Field Epidemiology Training Programme, NICD, Johannesburg
[3]Centre for Tuberculosis, NICD, Johannesburg
[4]National Department of Health, Pretoria, South Africa
[5]Division of Global Health Protection, U.S. Centres for Disease Control and Prevention (CDC), Pretoria, South Africa

Background

Tuberculosis (TB) affects millions of people worldwide.[1] The WHO 2013 Global Tuberculosis report states that 8.6 million cases were diagnosed in 2012 of which 1.3 million deaths occurred.[1] Multidrug-resistant tuberculosis (MDR-TB) is defined as tuberculosis (TB) which shows resistance to both isoniazid and rifampicin, with or without resistance to other anti-TB drugs.[1,2] South Africa (SA) is ranked as the world's third highest TB-burden country and fifth as a DR-TB burden country.[1,2] Additionally, SA carries the burden of especially serious Human Immunodeficiency Virus (HIV) and TB epidemics.[3] Currently, about 6 million people are living with the HIV and 2.4 million are on anti-retroviral drugs in SA and, despite being the leader in global HIV research, these epidemics continue to worsen leading to increase morbidity and mortality.[3]

The management of MDR-TB in South Africa is based on guidelines of the National Tuberculosis Control Programme (NTCP) and Directly Observed Therapy Short course (DOTS) expansion and enhancement strategy, 2013.[2,4] The current standardised regimen for the management of patients diagnosed with MDR-TB who have not been exposed to second line drugs is initiated into two phases at all designated facilities.[2,5] The first phase, known as intensive or injectable phase, involves a six-month period of five drugs kanamycin (or amikacin) by injection, a fluoroquinolone (FQN), ethionamide, terizidone and pyrazinamide.[1,2,4,5] The second phase, known as the continuation phase, uses an oral regimen only and is based on the use of four drugs: FQN, ethionamide, terzidone and pyrazinamide for a minimum of 18 months after TB culture conversion.[1,2,4,5] The FQNs are a backbone of the MDR regimen and, in 2010, ofloxacin was replaced with moxifloxacin in SA.[2]

Fluoroquinolones are a family of broad spectrum, systemic antibacterial agents that are being used in second phase therapy.[6,7] Moxifloxacin and ofloxacin are FQNs that are associated with better outcomes in the treatment of MDR-TB than treatment with a regimen not containing an FQN.[8-10] Moxifloxacin is an 8-methoxy-fluoroquinolone with a long plasma half-life of 11 hours.[6,7] It shows potent bactericidal and sterilizing activity against *Mycobacterium tuberculosis* (*Mtb*) in murine studies. Based on murine models, moxifloxacin has the ability to clear sputum bacilli (an indication of clearance of *Mtb* from the lungs) more quickly than other second-line anti-TB drugs.[6,7] Ofloxacin is a synthetic broad-spectrum antimicrobial agent administered orally

or by injection.[9] Pharmacokinetically, ofloxacin, a fluorinated carboxyquinolone, has a steady-state concentration, which is attained after four oral doses.[8] Following multiple oral doses at steady-state administration, the half-lives are approximately 4-5 hours for ofloxacin and 20-25 hours for moxifloxacin.[8] FQNs are associated with an increased risk of tendinitis and tendon rupture.[6] Generally, patients tolerate these medications satisfactorily, but serious adverse events can develop.

The aim of this study was to compare the respective mortalities of HIV-positive MDR-TB patients on ARVs receiving regimens containing either moxifloxacin or ofloxacin at Sizwe Tropical Diseases Hospital over a five -year period spanning 2007- 2012.

Methods

Setting and participants

The cohort study is a retrospective record review of medical and laboratory records of patients treated at Sizwe Tropical Diseases Hospital in Gauteng Province during the period 2007-2012. Sizwe Hospital is a specialised TB institution responsible for the management of drug-resistant TB patients. The hospital receives patients from the six districts of Gauteng Province as well as referral cases from other provinces. All HIV positive adults (over 18 years) with a laboratory confirmed diagnosis of MDR-TB and on ART were included. Patients were stratified into those on a moxifloxacin or ofloxacin-containing regimen.

Data collection

Data on patients who met the study criteria were initially extracted from the MDR-TB registers. Case record forms (CRFs) were used to enter socio-demographic, clinical and outcome information. Analysis was limited to medical records that were available from the hospital archives. CRFs were captured on Epi Info7® and rechecked for accuracy. Data was then exported to Microsoft Excel for further statistical analysis.

Statistical methods

We conducted a descriptive analysis to summarize socio-demographic factors relating to the study population. Logistic regression was used to determine factors associated with death. Kaplan-Meier survival curves were used to pictographically describe time-to-death. All statistical analyses were performed using STATA version 13 and a two-sided p-value of less than 0.05 was considered statistically significant.

Ethics approval

The Health Science Research Ethics Committee of the University of Pretoria approved the study (approval number: 430/2014). Approval was also obtained from the Gauteng Provincial Department of Health and Sizwe Hospital.

Results

Over the 5 year study period the total number of MDR-TB patients treated at Sizwe hospital was 4 363. All HIV positive MDR-TB on ARVs who were treated exclusively with either ofloxacin or moxifloxacin regimen were retained leaving with 927 medical records. Out of these, 169 records could not be found at the hospital archive. A total of 758 patient records was analysed. The median age of the patients was 37.9 years (range 18-69 years) and 396 (52%) were males (data not shown).

Among the study population, 405 patients (53.4%) received a moxifloxacin-containing regimen and 353 patients (46.6%) received an ofloxacin-containing regime. A total of 189 (24.9%) patients were cured and 206 (27.0%) completed the treatment course. Two hundred (26.4%) defaulted treatment and 124 (16.4%) died. The proportion that died was 18.4% among those on ofloxacin containing regimen compared with 14.7%

of the moxifloxacin containing regimen. Using multivariable analysis, patients on an ofloxacin regimen appeared 17% more likely to die. However, this was not statistically significant (Table 1).

In terms of mortality, patients who had a baseline body weight between 50-70kg were less likely to die than those who weighed less than 50kg (OR= 0.44, 95% CI 0.26 -0.76, p=0.003). The odds of dying when the CD4 count was >50 cells/mm^3 was 46% less compared to those who had a CD4 count of <50 cells/mm^3 (OR= 0.44, 95%CI 0.26 - 0.76, p= 0.003). The risk of dying was considerably higher in patients who did not culture convert, compared to those who converted (OR= 58.36, 95% CI=22.79- 149.47, p=0.001). Patients who reported as unemployed had an 82% higher risk of dying compared to those who were employed (OR=1.82, 95% CI 1.05-3.14, p=0.032) (Table 1).

Based on a Kaplan-Meier survival curve, patients treated with a moxifloxacin containing-regimen had a delayed time-to-death as compared to those treated with an ofloxacin-containing regimen - most notably before week 10 (Figure 1).

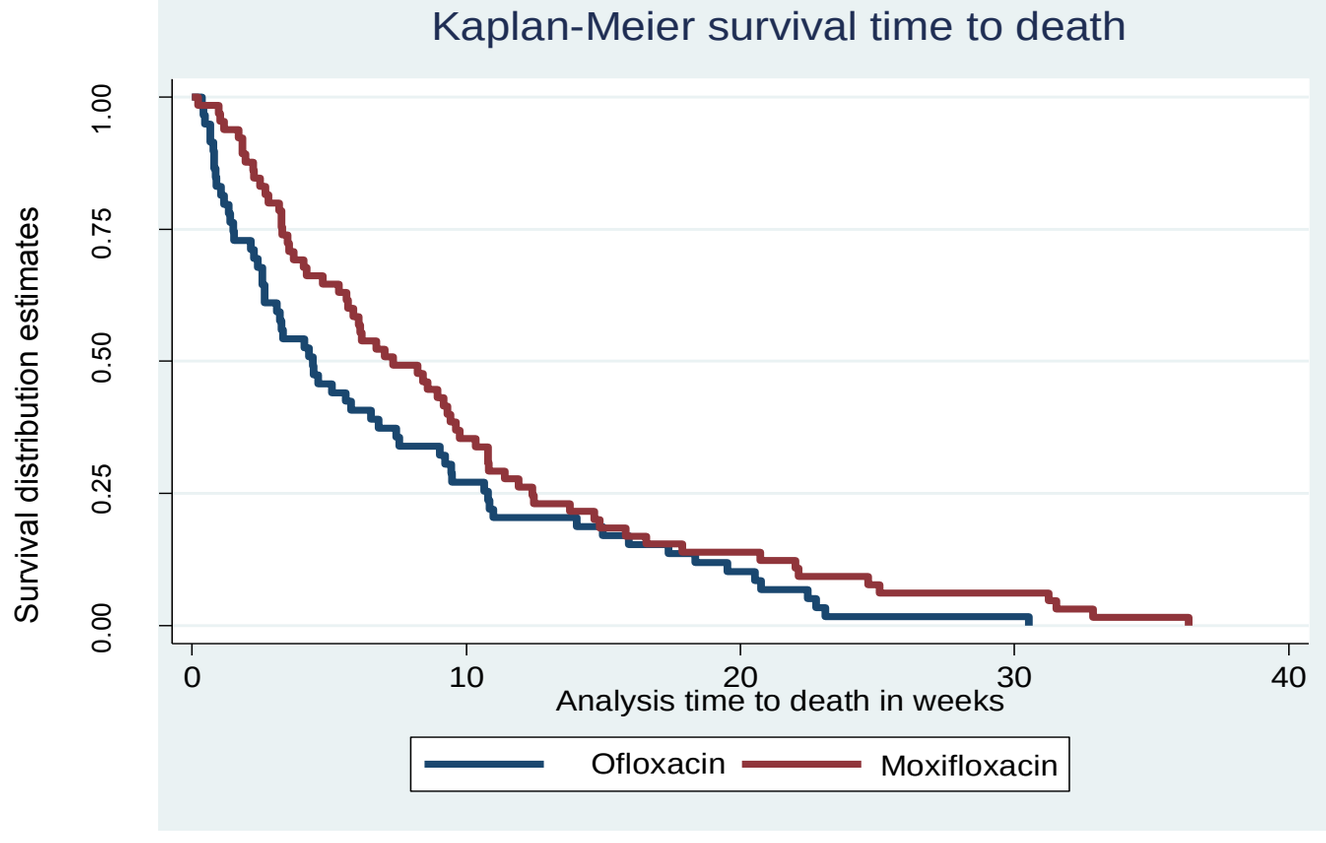


Figure 1: Time-to-death for patients treated with either moxifloxacin or ofloxacin containing regimen by time, among HIV positive MDR-TB patients at Sizwe Hospital, Johannesburg, South Africa, 2007 – 2012.

Table 1: Univariate and multivariate logistic regression model on mortality in Moxifloxacin and Ofloxacin treatment groups among HIV positive multidrug-resistant tuberculosis (MDR-TB) patients at Sizwe Hospital, Johannesburg, South Africa, 2007 – 2012.

Table		Univariate			Multivariate	
Variable	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Treatment regimen						
Moxifloxacin	1(Ref)			1(Ref)		
Ofloxacin	1.32	0.90 -1.95	0.154	1.17	0.71 - 1.93	0.54
Age category in years						
18-24	1(Ref)			1(Ref)		
25-34	1.84	0.69 - 4.90	0.225	2.011	0.51 - 6.74	0.258
35-44	1.44	0.54 - 3.85	0.471	1.65	0.49 - 5.59	0.419
45-54	1.33	0.47 - 3.77	0.591	1.18	0.32 - 4.35	0.803
55-64	0.81	0.79 - 3.69	0.79	0.13	0.01 - 2.05	0.148
65-74	omitted					
Gender				**NS**		
Female	1(Ref)					
Male	1.18	0.81 - 1.73	0.407			
Weight category [a]						
30 - 50 kg	1(Ref)			1(Ref)		
50 - 70 kg	0.56	0.37 - 0.84	0.005	0.44	0.26 - 0.76	0.003
70 - 90 kg	0.51	0.22 - 0.18	0.116	1.08	0.41 - 2.82	0.873
>90 kg	2.71	0.99 - 7.40	0.052	2.03	0.58 - 7.06	0.268
Cd4 count in cells/mm^3 [b]						
<50	1(Ref)			1(Ref)		
51-100	1.04	0.59 - 1.84	0.901	1.29	0.65 - 2..57	0.461
101-150	0.52	0.26 - 1.01	0.054	0.51	0.22 - 1.18	0.114
151-200	0.29	0.12 - 0.69	0.005	0.33	0.11 - 0.96	0.041
>250	0.32	0.18 - 0.58	0.001	0.46	0.23 - 0.92	0.028
Culture conversion						
Yes	1(Ref)			1(Ref)		
No	44.24	19.22 - 101.83	0.001	58.36	22.79 - 149.47	0.000
Patient category				**NS**		
New	1(Ref)					
Previously treated	0.99	0.67 - 1.47	0.962			
History of TB contact				**NS**		
None	1(Ref)					
Yes	1.12	0.73 - 1.71	0.612			
Adverse events				**NS**		
Not present	1(Ref)					
Present	1.22	0.63 - 2.37	0.547			
Alcohol intake				**NS**		
No	1(Ref)					
Yes	0.89	0.54 - 1.48	0.659			
Smoking				**NS**		
No	1(Ref)					
Yes	0.85	0.49 - 1.50	0.59			
Employment status						
Employed	1(Ref)			1(Ref)		
Unemployed	1.92	1.25 - 2.94	0.003	1.82	1.05 – 3.14	0.032
Infiltrates on Chest X-ray [c]				**NS**		
No	1(Ref)					
Yes	0.58	0.21 - 1.62	0.295			

Abbreviations: OR=Odds ratio; CI=Confidence interval; Ref=Reference; NS= Not significant
[a] Weight is defined as baseline weight as measured on admission by nurses
[b] Baseline CD4 count in cells/mm3 as confirmed by laboratory test on admission
[c] Infiltrates on chest x-ray was defined as chest x-ray taken on admission and interpreted by physician

Discussion

This study is the first to evaluate and report the outcomes of HIV positive MDR-TB on ARTs treated with regimens containing either moxifloxacin or ofloxacin in South Africa. Among the study cohort, mortality was not significantly different between the two study groups although time-to-death occurred earlier in those receiving an ofloxacin containing regimen. However, this was not clinically relevant as the difference in time-to-death was just a few weeks. *In vitro* studies from mice models have however shown that moxifloxacin is superior to other fluoroquinolones in in terms of outcome.[6] This disparity suggests that in clinical scenarios, factors other than drug choice are likely to be important.

Failure to convert was identified as a significant risk factor for mortality. In agreement with these findings, a study in KwaZulu-Natal Province showed an association between high mortality rate and culture non-conversion.[8] Reasons for this are unclear but possibilities could include undiagnosed additional drug resistance or other immunological deficiencies.

Patients with a CD4 count of less than 50 cells/mm^3 were three times more likely to die from MDR-TB compared to those with CD4 count greater than 250 cells/mm^3. This can be attributed to late ART initiation and late presentation for care. A previous policy on ART initiation was based on a threshold of <200 cells/mm^3, which may have contributed to the late initiation of ARTs.[10,11] Late presentation for care may also be attributed to centralised MDR-TB facilities in South Africa where patients may have had to wait for an available bed in order to be admitted for treatment initiation.[10] This situation should be addressed by changes in policy towards decentralization and deinstitutionalised treatment for MDR-TB.

A significantly higher mortality was observed in those weighing less than 50kg – a group presenting at an advanced stage of disease. The use of drugs containing 5 regimens as in this cohort is similar to a study conducted in Russia where these regimens reduced the incidence of mortality and treatment failure.[12] However, the use of a 5 or 6 drug regimen requires proper management of adverse events to ensure adherence to treatment especially when adults present with weight below 50kg. This is further complicated by the use of combination anti-retroviral regimens which, when combined, lead to a high pill burden inducing complex drug-drug interactions. Special attention in terms of individualised patient dosing with involvement of a pharmacist would be essential to provide safe and effective treatment and may mitigate some of the mortality risk.

This study had several limitations. Missing medical records, especially in the earlier years (2007-2009) meant that cases treated with ofloxacin were more likely to be missed. Furthermore, incomplete information about height measurement required for the calculation of the body mass index and unclear data recording for alcohol usage made it difficult to assess the significance of these variables. Final treatment outcomes of patients who were transferred from Sizwe Hospital were not available and could therefore not be included as a variable.

Conclusions

South Africa has amongst the highest TB and HIV prevalences in the world.[3,13] The converging dual epidemic of MDR-TB and HIV represents a growing threat to public health. Mortality was similar in both the moxifloxacin and ofloxacin treatment arms although there was a delay in time-to-death in the moxifloxacin group. Other factors such as degree of immune suppression and weight were shown to be very important factors influencing mortality beyond drug

selection. The use of moxifloxacin-containing regimens has been to be a good decision. However, special attention to individualised weight-based dosing and early management of MDR and HIV are essential to reduce mortality.

Acknowledgements

Sizwe Hospital staff are sincerely thanked for assisting with MDR-TB registers and retrieving patients' medical records. The South African Field Epidemiology Training Programme (SAFETP), especially Dr Lazarus Kuonza and Dr Seymour Williams, are thanked for their mentorship. Dorothy Southern is thanked for her input and guidance on scientific writing and for a critical review of this article.

References

1. World Health Organization. *Global tuberculosis report 2013. Geneva, Switzerland: WHO*. HTM/TB, 2013.
2. NDoH. *Management of Drug-Resistant Tuberculosis Policy guidelines abridged version January 2013*; National Department of Health, South Africa.
3. Rossouw T, Lessells RJ, de Oliveira T. *HIV & TB drug resistance & clinical management case book*. BioAfrica and SATuRN, 2013.
4. Scardigli A, Caminero JA. Management of drug-resistant tuberculosis. *Current Respiratory Care Reports*, 2013; *2*(4), 208-217.
5. Falzon D, Jaramillo E, Schünemann H, Arentz M, Bauer M, Bayona J, et al. WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. *European Respiratory Journal*, 2011;38(3):516-28.
6. Johnson J, Hadad D, Boom W, Daley C, Peloquin C, Eisenach K, et al. Early and extended early bactericidal activity of levofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. *The International Journal of Tuberculosis and Lung Disease*, 2006;10(6):605-12.
7. Miyazaki E, Miyazaki M, Chen JM, Chaisson RE, Bishai WR. Moxifloxacin (BAY12-8039), a new 8-methoxyquinolone, is active in a mouse model of tuberculosis. *Antimicrobial agents and chemotherapy,* 1999; 43(1):85-9.
8. Chigutsa E, Meredith S, Wiesner L, Padayatchi N, Harding J, Moodley P, et al. Population pharmacokinetics and pharmacodynamics of ofloxacin in South African patients with multidrug-resistant tuberculosis. *Antimicrobial agents and chemotherapy*, 2012; *56*(7): 3857-3863.
9. Farley JE, Ram M, Pan W, Waldman S, Cassel GH, Chaisson RE, et al. Outcomes of multi-drug resistant tuberculosis (MDR-TB) among a cohort of South African patients with high HIV prevalence. *PLoS One*, 2011; 6: e20436.
10. Narasimooloo R, Ross A. Delay in commencing treatment for MDR TB at a specialised TB treatment centre in KwaZulu-Natal. SAMJ: *South African Medical Journal,* 2012; 102(6), 360-363.
11. Badri M, Cleary S, Maartens G, Pitt J, Bekker LG, Orrell C, Wood R. When to initiate highly active antiretroviral therapy in sub-Saharan Africa? A South African cost-effectiveness study. *Antiviral Therapy*, 2006; 11(1), 63.
12. Velásquez, G. E., Becerra, M. C., Gelmanova, I. Y., Pasechnikov, A. D., Yedilbayev, A., Shin, S. S., et al. Improving outcomes for multidrug-resistant tuberculosis: Aggressive regimens prevent treatment failure and death. *Clinical Infectious Diseases*, 2014; 59(1), 9-15.
13. Middelkoop K, Mathema B, Myer L, Shashkina E, Whitelaw A, Kaplan G, et al. Transmission of tuberculosis in a South African community with a high prevalence of HIV infection. *Journal of Infectious Diseases*, 2015; 211 (1), 53-61.

Table 1: Provisional number of laboratory confirmed cases of diseases under surveillance reported to the NICD - South Africa, corresponding periods 1 January - 30 September 2014/2015*

Disease/Organism	1 January to 30 September, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Anthrax	2014	0	0	0	0	0	0	0	0	0	0
	2015	0	0	0	0	0	0	0	0	0	0
Botulism	2014	0	0	0	0	0	0	0	0	0	0
	2015	0	0	1	0	0	0	0	0	0	1
Cryptococcus spp.	2014	738	235	1422	1535	235	363	43	300	808	5679
	2015	504	180	826	1050	184	230	28	311	428	3741
Haemophilus influenzae, invasive disease, all serotypes	2014	26	15	81	44	0	17	4	5	80	272
	2015	14	8	84	29	2	6	1	3	89	236
Haemophilus influenzae, invasive disease, < 5 years											
Serotype b	2014	2	2	9	3	0	0	1	0	10	27
	2015	1	1	2	1	0	0	0	1	5	11
Serotypes a,c,d,e,f	2014	1	1	5	3	0	1	1	0	4	16
	2015	1	0	2	2	1	0	0	0	6	12
Non-typeable (unencapsulated)	2014	1	1	14	6	0	1	0	0	20	43
	2015	1	0	13	2	0	0	0	0	12	28
No isolate available for serotyping	2014	4	1	19	10	0	0	0	3	0	37
	2015	1	1	28	9	2	6	1	6	1	55
Measles	2014	1	2	6	3	0	1	6	1	2	22
	2015	2	1	2	2	0	0	3	1	4	15
Neisseria meningitidis, invasive disease	2014	31	4	43	15	0	2	0	2	46	143
	2015	24	9	38	18	1	3	1	3	27	124
Novel Influenza A virus infections	2014	0	0	0	0	0	0	0	0	0	0
	2015	0	0	0	0	0	0	0	0	0	0
Plague	2014	0	0	0	0	0	0	0	0	0	0
	2015	0	0	0	0	0	0	0	0	0	0
Rabies	2014	3	0	0	0	1	0	0	1	0	5
	2015	1	1	0	1	2	0	0	0	0	5
Salmonella typhi	2014	1	3	36	14	0	8	0	0	18	80
	2015	1	1	21	8	1	5	0	0	14	51
Streptococcus pneumoniae, invasive disease, all ages	2014	177	130	758	398	28	93	29	84	399	2096
	2015	165	107	754	272	72	65	20	77	473	2005
Streptococcus pneumoniae, invasive disease, < 5 years	2014	25	16	153	65	5	12	5	14	60	355
	2015	18	10	121	40	14	12	4	17	48	284
Vibrio cholerae O1	2014	0	0	2	0	0	0	0	0	0	2
	2015	0	0	0	0	0	0	0	0	0	0
Viral Haemorrhagic Fever (VHF)											
Crimean Congo Haemorrhagic Fever (CCHF)	2014	0	1	0	0	0	0	2	0	0	3
	2015	0	0	0	0	0	0	0	0	0	0
Other VHF (not CCHF)	2014	0	0	0	0	0	0	0	0	0	0
	2015	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.

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

0 = no cases reported

Table 2: Provisional laboratory indicators for NHLS and NICD, South Africa, corresponding periods 1 January - 30 September 2014/2015*

Programme and Indicator	1 Jan to 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	2014	37	18	61	56	35	34	8	17	31	297
	2015	59	12	50	54	42	39	5	9	23	293

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

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).

The Communicable Diseases Surveillance Bulletin is published by the National Institute for Communicable Diseases (NICD) of the National Health Laboratory Services (NHLS), Private Bag X4, Sandringham, 2131, Johannesburg, South Africa.

Suggested citation: [Authors' names or National Institute for Communicable Diseases (if no author)]. [Article title]. Communicable Diseases Surveillance Bulletin 2015; 13(4): [page numbers].