Neurodevelopmental delay among HIV-infected preschool children receiving antiretroviral therapy and healthy preschool children in Soweto, South Africa.

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Declaration

I declare that this manuscript is my own work, assisted only by the two co-authors and those referred to in the acknowledgements. The article was accepted for publication on 06/12/2011 by the journal "Psychology, Health and Medicine". It is being submitted in partial fulfillment of the requirements for the degree of Master of Science in Medicine (Part-time) (Coursework) at the University of the Witwatersrand. It has not been submitted to any other journals for publication or to any other University for examination.

Sarah Lowick

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.....4th.....day of...May.....2012

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Title

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Neurodevelopmental delay among HIV-infected preschool children receiving antiretroviral therapy and healthy preschool children in Soweto, South Africa.

Abstract

Neurodevelopmental delay has been documented in up to 97.5% of HIV-infected children in Soweto who were not yet on ART. With growing numbers of children in South Africa being successfully treated with antiretroviral treatment (ART), the effects of ART on neurocognitive functioning in children require investigation. The objective of this study was to determine the extent of neurodevelopmental delay in stable HIV-infected preschool children (aged 5-6 years) receiving ART and compare it to an apparently healthy (unconfirmed HIV-status) group of preschool children. Thirty HIV-infected preschool children (virologically and immunologically stable on ART for > 1 year) were conveniently sampled from 350 eligible children on ART at the Harriet Shezi Children's Clinic in Soweto, Johannesburg. The comparison group comprised thirty well-nourished preschool children attending the Lilian Ngoyi Primary Health Care Clinic in Soweto for routine immunisations. Each child was assessed using the Griffiths Mental Development Scales-Extended Revised Version (GMDS-ER), at a single point in time. The overall developmental z-scores on GMDS-ER were <-2 (indicating severe delay) in 27 (90%) children in the HIV-infected group compared to 23 (76%) in the comparison group (p=0.166). Mental handicap (overall GQ<70) was evident in 46.7% of children in the HIV-infected group compared to 10% in the comparison group (p= 0.002). There was a 7.88-fold increased likelihood of severe delay in the HIV infected group. The HIV-infected group and comparison group had significantly different (p=0.001) mean overall GQ scores of 70 (95% CI: 66.0-74.0) and 78 (95% CI: 75.6-80.5), respectively, with lower mean scores in the HIV-infected group in all individual

domains. Early initiation of ART in HIV-infected infants may improve cognitive functioning among this group, however, intervention strategies which optimize early cognitive development for all children in the area, need to be urgently considered.

Key words: HIV, child, neurodevelopment, cognitive, encephalopathy

Background

There are an estimated 2.5 million children under 15 years of age worldwide with HIV infection. Of these, 2.3 million live in Sub Saharan Africa (UNAIDS, 2010). Since April 2004, there has been a dramatic rollout of antiretroviral treatment (ART) in South Africa. In 2009 there were 86 270 children receiving ART in South Africa, which represented 54% of all children requiring ART (WHO/UNAIDS/UNICEF, 2010).

HIV is a neurotropic lentivirus that invades the central nervous system (CNS) soon after infection. Three pathways have been proposed whereby the virus crosses the blood-brain barrier and enters CNS parenchymal tissue: i) HIV may be carried into the brain by infected macrophages-monocytes and /or CD4 T lymphocytes (Trojan horse effect); ii) cell-free virus may pass from the vascular compartment into brain tissue between cerebral microvascular endothelial cells (CMVECs); iii) following direct HIV infection of the specialized endothelial cells of the blood-brain barrier or CMVECs, viral particles may be released into the cerebral compartment (L. Smith, Adnams,& Eley, 2008).

The virus in the CNS then infects various cell populations- macrophages, microglia, astrocytes and possibly neurons. Perivascular macrophages and microglia, are the only major cell types in the brain that express both CD4 and chemokine co-receptors, which are necessary for productive HIV infection. Astrocytes are infected via a CD4- independent mechanism. Viral interactions with astrocytes may play a significant role in paediatric HIV encephalopathy as foetal astrocytes may be particularly susceptible to HIV infection and the developing CNS is sensitive to alterations in astrocyte function. Productive infection of neurons has recently been shown to occur in children but neuronal loss is thought to be primarily an indirect effect of HIV infection.

Infected macrophages and microglia release viral proteins, pro-inflammatory cytokines, chemokines and nitric oxide which result in a cascade of neurotoxic events. Neuronal apoptosis may be activated by viral glycoproteins including gp120, tat, net, vpr and gp41 or a variety of host-derived mediators including cytokines, free radicals and excitatory amino acids. Brain regions such as the hippocampus, basal ganglia, and forebrain,which are susceptible to N-methyl-D-Aspartate-mediated excitotoxicity, are particularly vulnerable in HIV infection. There may be a neuroprotective role for NMDA-receptor antagonists in HIV infected patients and this is currently under investigation. There is evidence that HIV may inhibit neural progenitor cell proliferation, negatively affecting neurogenesis.

Compartmentalized viral populations have been demonstrated in both adult and paediatric studies where discordant drug resistance mutation patterns in blood and CSF have been documented. Properties of HIV CNS infection which can reduce the efficacy of antiretroviral treatment at the level of the CNS are firstly, the variable ability of antiretroviral drugs to cross the blood-brain barrier. Secondly, the relatively long lifespan of macrophages, microglia and astrocytes compared to CD4 T lymphocytes, which prevents fast elimination of the virus from the CNS. The viral load may, therefore be suppressed in the periphery but not in the CSF and ongoing CNS disease may occur (L. Smith et al., 2008; Van Rie, Harrington, Dow,& Robertson, 2007).

The consequence of the above is the development of neurologic and neurocognitive pathology. HIV affects the developing brain by causing static or progressive encephalopathy. HIV encephalopathy is described in the 1994 Centers for Disease Control and Prevention (CDC) classification of AIDS-defining events as: i) failure to attain or loss of developmental milestones or loss of intellectual ability; ii) impaired brain growth or acquired microcephaly;

iii) acquired symmetrical motor deficit. These occur in the absence of a concurrent infection other than HIV and persist for at least 2 months (CDC 1994).

Before antiretroviral therapy, natural history data suggested that HIV encephalopathy could be progressive or static. Progressive encephalopathy is characterized by the loss of acquired skills and is commonly seen in young children. Children with static encephalopathy gain new skills but continue to function on a level below the mean, on standardized testing (Foster, Biggs, Melvin, Walters, Tudor-Williams, & Lyall, 2006).

The radiological hallmarks of progressive encephalopathy include cortical atrophy and basal ganglia calcifications on computer tomography scans. White matter lesions and central atrophy are seen on magnetic resonance imaging. These radiological abnormalities are associated with more advanced disease (Van Rie, et al., 2007; Foster, et al., 2006).

Neurodevelopmental manifestations of HIV encephalopathy include global neurodevelopmental delay, overall impaired cognitive functioning, selective delays in executive function, language or visual spatial tasks and subtle differences in specific areas such as processing speed (Van Rie, et al., 2009).

This broad spectrum of clinical manifestations reflects the multiple factors that influence the impact of the virus in an individual child, including timing of infection, age of neurodevelopmental assessment and quality of the child's environment (Van Rie, et al., 2009). Delays in motor development, especially gross motor skills, most strongly differentiate HIV- infected from HIV-exposed children and are seen more frequently in infants than school-aged children. Delays in mental development occur later in infancy and most often present as a global cognitive deficit. Differences in specific areas of cognitive function have not been systematically observed in HIV-infected children. Delay in language

development, especially expressive language, commonly occurs in young HIV-infected children and often precedes the presence of abnormalities on neurological examination or CNS imaging. Studies on behavioural problems associated with HIV infection have given conflicting results. Higher rates of behavioural problems may be due to other biological and environmental factors rather than a direct result of HIV infection (Van Rie.et al., 2007)

Risk factors for HIV encephalopathy are advanced maternal disease, impaired maternal and child immune status, elevated CSF and plasma viral load, vertical transmission, intra-uterine infection and treatment availability (Van Rie, et al., 2007).

The clinical spectrum of neurodevelopmental pathology, ranges from cerebral palsy and mental handicap to subtle learning or behaviour deficits (Sherr, Mueller, & Varrall, 2009; R. Smith et al., 2006; Van Rie, Harrington, Dow, & Robertson, 2007). Rates of encephalopathy in the era prior to ART are consistently high (Abubakar, Van Baar, Van de Vijver, Holding, & Newton, 2008; Baillieu & Potterton, 2008; Van Rie, et al., 2007). Neurodevelopmental delay has been documented in up to 97.5% of children in Soweto who were not yet on ART (Baillieu & Potterton, 2008).

Since the introduction of ART, studies from well-resourced countries have reported a dramatic decline in PHE (Chiriboga, Fleishman, Champion, Gaye-Robinson, & Abrams, 2005; Sanchez-Ramon et al., 2003). However, there are few studies on the effects of ART on neurocognitive development in Sub Saharan Africa. Findings have been variable and early initiation of ART appears to be critical (Feucht U, 2009; Laughton et al., 2009; L. Smith, Adnams, & Eley, 2008; Van Rie, et al., 2009; Van Rie, et al., 2007; Van Rie, Mupuala, & Dow, 2008). Two South African cohort studies found high pre-ART levels of neurocognitive delay with no improvement following six months of ART (Feucht U, 2009; L. Smith, et al., 2008). Conversely, improvements were reported by a study in the Democratic of Congo after

6- 12 months of ART (Van Rie, et al., 2009; Van Rie, et al., 2008). The Children with HIV Early Antiretroviral Therapy (CHER) trial demonstrated that early initiation of ART in South African children resulted in favourable, short-term neurodevelopmental gains, decreased infant mortality and disease progression, in comparison to later initiation of ART (Laughton, et al., 2009). As a result, updated WHO and South African Department of Health recommendations advise that all infants (<12 months old and <24 months respectively) be started on ART, regardless of CD4 count or WHO staging (SANAC, 2010; WHO, 2010).

The prevalence of intellectual disability among children in general has been reported as 35.5 and 44 per 1000 in two South African studies (Christianson et al., 2002; Katzenellenbogen, Joubert, Rendall, & Coetzee, 1995). These figures represent those with more severe developmental disability and do not include children with subtle cognitive impairments. Recent research on early childhood development in resource-limited countries has raised concern about loss of intellectual potential due to poverty, poor health and nutrition and under stimulating home environments (Grantham-McGregor et al., 2007). There are few data on this phenomenon in South African children.

This cross-sectional, comparative study assessed the neurodevelopmental functioning in two groups of preschool children (aged 5-6 years) in Soweto using the Griffiths Mental Development Scales-Extended Revised Version (GMDS-ER):- an HIV-infected group, successfully treated on ART for at least one year and an apparently healthy comparison group.

Methods

Study Population and Design

Participants for this cross-sectional comparative study were enrolled from two sites. The HIV-infected children were enrolled from the Harriet Shezi Children's Clinic (HSCC) between July 2009 and March 2010. This clinic is the largest paediatric HIV management and treatment site in South Africa and is based at Chris Hani Baragwanath Hospital (CHBH); a large tertiary hospital serving the population of Soweto and surrounding areas of Johannesburg, South Africa. At the time of this study there were 5109 patients attending the clinic with 3376 on ART; of these about 350, aged 5-6 years were eligible for enrolment. Convenience sampling was used to enrol thirty HIV-infected preschool children during a routine clinic appointment at HSCC. Clinic attendees' files were screened for eligibility and subjects were enrolled if they met study entry criteria. A maximum of two subjects were enrolled and tested on any one day. Eligible children were born after January 2004 (around the time that ART became widely available in South Africa), clinically stable with no intercurrent illness, had CD4%>15% and were virologically suppressed (VL<50copies/ml). All had received ART for more than one year. Children were excluded if there was a history of perinatal complications, head injury or genetic or syndromal disorders.

The comparison group consisted of children attending immunisation clinic at Lilian Ngoyi Primary Healthcare Clinic (LNPHC) between February 2010 and April 2010. This clinic is situated adjacent to CHB Hospital. Convenience sampling was also used to enrol the 30 apparently healthy children, aged 5-6 years, from LNPHC during routine immunization or growth monitoring visits. Attendees were screened and eligible if they had no history of neurological impairment, acute or chronic illness, admissions in the past year, symptoms of

HIV, positive HIV Elisa or PCR test and their growth parameters ranged from the 3rd to 97th percentile on the WHO growth chart. This comparison group was chosen as a sample of "apparently" healthy children from the community. Healthy children were not routinely tested for HIV at the immunisation clinics at the time; although this is more widely recommended now. Those who were undernourished or ill were excluded and referred for further investigation and HIV testing.

Measurements

All participants were recruited and tested between July 2009 and March 2010. Informed consent and assent was received from all caregivers and children, respectively.

A standard questionnaire was used to elicit demographic data from the primary caregivers in both groups. A simplified asset-based survey was used to determine socioeconomic status (SES). Primary caregivers were asked whether they owned a car, microwave, computer, fridge, radio or television; owning more than three items resulted in classification in a higher SES group and owning less than four items, a lower SES group. Disease and treatment data were collected for the HIV-infected group through interview questionnaires and a review of the clinic files. Children had received ART according to the national treatment guidelines at the time.(National-Department-of-Health, 2004).

The developmental functioning of all participants was assessed at the time of enrolment using the GMDS-ER (Luiz D, 2006). The scales measure development trends which are significant for intelligence according to age group; 0-2 years or 2-8 years and cross-cultural construct validity has been documented (Luiz, Foxcroft, & Stewart, 2001). For this study, the updated 2006 version of the scales for 2-8 year olds was used. This scale examines the following six domains of functioning which are individually scored and from which an overall score is

determined: Locomotor (gross motor functioning); Personal-Social (social functioning); Hearing-Speech (receptive and expressive language ability); Eye-Hand (fine motor functioning); Performance (visual spatial ability) and Practical Reasoning (logical thinking and mathematical ability).

The Griffiths Mental Development Scales were developed in 1954 in Great Britain by Dr Ruth Griffiths for use with children from birth to 2 years of age. In 1970 they were expanded to include children from birth to 8 years of age and the sixth subscale (Practical Reasoning) was added for the older age group. The development of the Scales was based on detailed systematic observation and recording of children's behaviour-in their natural environments-at home, at play, in the streets and in the school playground. Material for test items emerged from these observations. Previous and existing tests and test methods (Buhler, Gesell, McCarthy, and Shirley) were taken into account and included. The Scales fulfilled stringent statistical requirements of reliability and validity. The Scales address the requirements of both special needs and normally developing children. The Scales were based on the study of trends that appeared significant for mental growth and the interrelations between the basic avenues of learning (such as physiological/locomotor, eye/hand, voice/hearing) which develop with rhythm in time and space and are influenced also by environmental and social factors. The items are diverse, tapping the main aspects of child development. They are placed in order of gradually increasing difficulty and are based on natural activities such as walking, talking and playing. Play is an experience that is common to all cultures. Thus items in the GMDS should be common to many different cultures and can potentially be used in children from a variety of cultural backgrounds. The Scales are widely used internationally in both clinical practice and research and were first introduced in South Africa in 1977.

Revision of the GMDS began with the Scales from Birth to Two Years. This was complete in 1996. The revision of the Extended Scales from 2-8 years began in 1996 and was completed

by 2006. A trend of gradually increasing IQ scores has been noted in the United Kingdom over recent years in most standardized tests. Reasons for this upward trend in GQ or IQ include improvements in the educational system, improved nutrition, better health conditions, increased dissemination of information and early exposure to advanced technology. It is, therefore imperative to revise and regularly update norms of standardized tests. In addition the GMDS-ER has improved the scoring standards and test reliability and reviewed and updated all test items.

The original GMDS (1970) 2-8 year was scored using age-equivalents, general (GQ) and sub quotients (SQ) and percentiles, while the updated version (2006) is scored using z-scores, age-equivalents, and percentiles. Although it has been translated into Afrikaans and Xhosa, the most commonly used languages in this area were Zulu, Sotho and English. Most participants and caregivers spoke English; however a translator (clinic sister or lay counsellor) was necessary for a few assessments (<5 per site). The test was administered and scored by the investigator, who has nine years' experience as a registered Griffith's user with the Association for Research of Infant and Child Development.

Results of the GMDS-ER assessment were analysed and scored using the Griffiths Mental Development Scales –Extended Revised: Two to Eight Years: Administration and Analysis Manual (Luiz D, 2006). The primary analysis included individual z-score calculations in each of the domains and overall; a z-score of <-2 on any of the domains indicated significant neurodevelopmental delay.

The primary analysis revealed large proportions of children with overall and sub quotient scores in the <-2 z-score range. In order to better describe this group, as z-scores of -3 and -4 cannot be used, a secondary analysis using general quotient (GQ) and sub quotient (SQ) scores, from GMDS (1970) was undertaken. GQ and SQ scores were calculated by obtaining

the raw score and age equivalent for each domain and overall in the GMDS-ER analysis manual. The age equivalent score was then divided by the chronological age to yield a GQ overall and SQ for each domain. This was done following consultation with experienced colleagues (personal communication- L. Jacklin, B. Laughton, L. Stroud, N. Van Zyl, November 2010). This scoring method has been used by Van Heerden and Van Rooyen in South Africa (Van Heerden, 2007; Van Rooyen, 2005).

General quotient (GQ) and sub quotient (SQ) scores of <70 were indicative of severe neurodevelopmental delay or mental handicap. Mean scores (calculated from the GQ and SQ values) were compared between study groups and also categorised into strata according to clinical significance- mental handicap (<70), borderline (70-79), low average (80-89), average (90-109) and high average (110-119). The secondary analysis enabled a more detailed evaluation and comparison of the children falling within the <-2 z-score range.

Ethics Clearance

Ethical clearance for the study was obtained from the Human Research Ethics Committee of the University of the Witwatersrand.

Data Analysis

Neurodevelopmental z-scores (GMDS-ER) were categorised into developmentally delayed (<-2 standard deviations) or not delayed (≥-2 standard deviations). Statistically significant differences in proportions of delayed children per domain and overall between the HIV-infected and comparison group were compared using either χ^2 tests or Fisher's exact tests (where expected frequencies were <5). Overall GQ and individual domain SQ means (GMDS 1970) were calculated and compared using either the two-tailed t-test (equal variances) or the Welch test (unequal variances). Proportions of each group with GQ or SQ

<70 (mental handicap) were compared between groups using either χ^2 tests or Fisher's exact tests.

Odds ratios were used to determine the strength of the association between HIV-status and neurodevelopmental delay per domain. All analyses were performed using Stata® 11 (StataCorp LP, USA).

Results

Demographic and clinical characteristics of participants

In total, 60 children aged 55-75 months were enrolled into the study; 30 HIV-infected and 30 healthy comparison children. There were no significant differences ($p>0.05$) in the median ages, gender, socioeconomic status and maternal or paternal orphan status between the groups. The primary caregiver was one or both parents in 21 (70%) children in the HIV group and 28 (93.3%) in the comparison group (Table 1).

Of the 30 HIV-infected children, 29 (97%) were at WHO stage III or IV at the time of starting antiretroviral therapy (ART) with 70% having been treated for either pulmonary or extra pulmonary tuberculosis in the past. HIV encephalopathy (delay or regression of milestones; acquired microcephaly or pyramidal tract signs) was diagnosed in five children (16.67%) and an additional five children (16.67%) had severe failure to thrive prior to starting ART. The mean weight-for-age z-score was -5.02 prior to starting ART and -1.01 at the time of testing ($p<0.00$). The median age of starting ART was 24 months (IQR 15-40.5) and the median duration of ART was 40 months (IQR 23.5-47.5). Twenty children were initiated on a regimen of lamivudine, stavudine and lopinavir/ritonavir. Of these, six subsequently had single drug substitutions due to side-effects. Lamivudine, stavudine and efavirenz were initiated and maintained in nine children (≥3 years at ART initiation). The

remaining child received lamivudine/stavudine/nevirapine. The median CD4% increased from 11.95% (IQR 9.23-18.7) prior to starting ART to 33.90% (IQR 28.05-37.05) at the time of conducting the neurodevelopmental tests ($p<0.000$). The median viral load prior to ART was 34 000 copies/ml (IQR 35 500-1 275 000) with all children virally suppressed at the time of enrolment onto this study.

Results of Developmental testing

The primary analysis of GMDS-ER z-scores revealed that 27 (90%) and 23 (76.7%) children in the HIV-infected and comparison group, respectively, had overall z-scores <-2 (Table 2). The HIV-infected group had consistently higher proportions of children with z-scores <-2 for all domains and overall. The comparison group performed better than the HIV-infected group in the locomotor, personal-social and eye-hand domains ($p<0.05$). The greatest disparity between groups was found in the locomotor domain where 6.7% of the comparison group and 46.7% of the HIV-infected showed delay. The hearing-speech domain demonstrated the least percentage difference between groups and was also the worst area of functioning, where 90% of the HIV-infected and 83.3% of the comparison group had z-scores <-2.

The secondary analysis compared general and sub quotients to further elucidate the proportion of children with severe neurodevelopmental delay (mental handicap) in each of the groups. The HIV-infected and comparison groups' overall mean GQ scores were 70 and 78, respectively ($p<0.05$) with significant differences between the two groups found in all domains (Table 3) (Figure 1). Table 4 provides a detailed breakdown of the proportions of children, per group, at different levels of functioning, derived from mean GQ scores. Almost half of HIV-infected children (n=14; 46.7%) had severe delay (GQ<70) compared to three children (10%) in the comparison group ($p<0.05$). However, a large proportion (87%) of the comparison group was classified as functioning in the borderline or below average range.

Although the comparison group performed far below expected levels, their overall GQ and SQ scores were consistently higher than those of the HIV-infected group (Table 3).

When comparing the proportions of children with GQ scores of <70, there was a 7-fold increase in the likelihood of having severe neurodevelopmental delay in the HIV-infected group (OR=7.88; CI: 1.96–31.68) compared to the comparison group (Table 5). The two groups differed significantly in all domains except locomotor, hearing-speech and performance, when comparing proportions with SQ <70. In the locomotor domain 7 children in the HIV-infected group and 1 child in the comparison group had a SQ <70 (p 0.052). The z-score analysis shows the greatest disparity between groups in the locomotor domain –14 children in HIV-infected group versus 2 in the comparison group (p 0.000) (Figure 2). This apparent contradiction is a result of a number of factors-firstly, the choice of < 70 as a cut-off point. This was based on the clinical significance of this value. GQ values below 70 are internationally classified as mental handicap and children in this category usually require special schooling. Secondly, the Fischer exact test, rather than the Chi-squared test (p 0.023), was used to analyse the locomotor SQs, as the expected cell size was calculated as 4. The z-scores were analysed using the Chi-squared test. The third factor that contributes to the statistical paradox is the small sample size, as evidenced by the wide confidence intervals. A larger sample would probably have resulted in more consistent results. In the hearing-speech and the performance domains, both groups performed poorly with 25 (83.3%) of the HIV-infected and 20 (66.7%) of the comparison group having SQs <70 in the language domain (p>0.05). Similarly, 19 (63.3%) of the HIV infected and 12 (40%) of the comparison group had SQs of <70 in the performance domain (p>0.05) (Table 5).

Discussion

This exploratory study raises concerns about the early developmental wellbeing of preschool children in the Soweto area. As anticipated HIV-infected children fared worse on neurodevelopmental testing than their healthy counterparts although both fell far short of the norms for the standardized British population (Luiz D, 2006).

Despite successful ART, 90% of the HIV-infected children had significant delay. These results are consistent with two other South African studies which showed high levels of persistent neurocognitive impairment on ART (Feucht U, 2009; L. Smith, et al., 2008). The ongoing impairment may be attributed to the direct neurotoxic effects of HIV infection, resulting in permanent structural central nervous system (CNS) damage prior to the initiation of ART or incomplete penetration of the blood-brain barrier by antiretroviral agents (Lindsey, Malee, Brouwers, & Hughes, 2007; Van Rie, et al., 2007). Even though, all children in this study received high CNS-penetrating regimens, Patel et al. recently demonstrated that high versus low CNS-penetrating regimens increase survival in those with HIV encephalopathy but do not significantly reduce the risk of developing HIV encephalopathy (Patel et al., 2009). In addition, environmental stressors, malnutrition, stunted growth, iron deficiency anaemia and recurrent infections, which occur more commonly in the presence of HIV disease, may be impeding healthy neurodevelopment (Van Rie, et al., 2007).

The CHER study demonstrated improved development in those initiated on ART early versus those initiated after three months of age (Laughton, et al., 2009). This prospective study continues to examine the longer term effects of early initiation of ART on neurocognitive functioning (Laughton, et al., 2009). No children in our study population were started before three months of age, which might explain their poor performance.

More than three quarters of the apparently healthy, well-nourished comparison children also had significant neurocognitive delay. However, asymptomatic HIV infection or HIV exposure was not excluded, and whether this may account for some of the poor performance in the comparison group is unknown.

Although the GMDS-ER is widely used in South Africa for clinical and research purposes, it has not been validated here. The earlier test has been extensively studied in South Africa and results correlate closely with the British population in a number of studies (Allan, 1988; Bhamjee, 1991; Luiz, et al., 2001; Mothuloe V, 1994; Stewart, 1997; Tukulu, 1996). A positive correlation was found between general quotient and school performance (Mothuloe V, 1994) and between the Junior South African Intelligence Scales (JSAIS) and the GMDS range (Luiz, et al., 2001). There have been two studies comparing the performance of normal South African children (4-7 years and 5-6 years) to British children using the GMDS-ER (Van Heerden, 2007; Van Rooyen, 2005). Van Rooyen found no significant differences in overall GQs, while Van Heerden did report superior performance of the British population when analyzing both age groups together. Both studies found similar subscale differences. The British standardized sample performed better in the hearing-speech and practical reasoning subscales, while the South African sample were slightly superior in the locomotor and personal-social areas. There were significant differences between the groups in the language, eye-hand and practical reasoning domains.

Cultural, language, socioeconomic and educational factors differ from the test reference population and also vary greatly within South Africa itself. This was the rationale for selecting a community sample control group for comparison with the HIV group. Standardized tests are few and those available have been validated for specific ages, racial, language and cultural groups (Van Heerden, 2007). The GMDS-ER is a comprehensive psychometrically sound measure of a child's abilities and has the potential to be used across

diverse population groups in South Africa. Our results must, however, be cautiously interpreted. This study highlights the need for standardization of the GMDS-ER across the multicultural South African population.

Despite these limitations, the poor performance of the healthy children does raise concern, especially in view of South Africa's poor educational record (Kanjee, 2005. ; Mullis, Martin, Kennedy, & Foy, 2007; WEF, 2010) and mounting global concerns around delays in early childhood development in resource-limited settings. The Lancet ran a series of articles in 2007 calling for increased awareness and action on the crisis of early childhood development in developing nations (Engle et al., 2007; Grantham-McGregor, et al., 2007; Walker et al., 2007). Extensive research reveals that conservatively, 200 million children under five years of age fail to reach their full potential in cognitive and socio-emotional development (Grantham-McGregor, et al., 2007). The authors cite four main causes for this: malnutrition leading to stunting; iodine and iron deficiency and inadequate stimulation. Other risk factors include malaria, HIV, violence, maternal depression, diarrhoea and exposure to heavy metals (Walker, et al., 2007).

The findings of our study are supported by others conducted in South Africa, using the GMDS in healthy children. A Cape Town study followed 187 children from birth to five years of age. Family stability, higher maternal education and income were positively associated with developmental profile (Molteno et al., 1991). In another study of five year olds in Cape Town, poorer cognitive functioning in lower socioeconomic groups was demonstrated (Allan, 1988). A control group of Grade one learners in a Cape Town Foetal Alcohol Syndrome study performed lower than expected (Adnams et al., 2001).

Three South African studies using the Griffiths 0-2 year 1996 scale have found neurodevelopment in the first and early second year to be similar or superior to the British

population (Amod Z, 2007; Cockcroft, Amod, & Soellaart, 2008; Perez et al., 2005). A deterioration of functioning has been shown between 11 and 21 months in children from low socio-economic circumstances in Cape Town (Laughton et al., 2010). The effects of SES may become more marked as the child develops (Allan, 1988). This research indicates the possibility of developmental decline during the early years of childhood in disadvantaged children.

High rates of cognitive developmental impairment were found in a Kinshasa study in healthy control subjects-51.1% with moderate or severe delay. The children were all under six years of age, with both parents alive and HIV uninfected, but living in an impoverished area. There were substantial improvements in cognitive functioning on repeat testing one year after parents received developmental education and the children received basic healthcare (Van Rie, et al., 2009; Van Rie, et al., 2008).

The HIV-infected group performs consistently lower than the comparison group in all areas. Mean GQ and SQ comparisons reveal significant differences in all domains and overall between the HIV- and comparison groups (Table 3). However, the developmental profile patterns of both groups are very similar as demonstrated in Figure 1. Both groups function best in the Personal-Social and Locomotor domains and worst in the Hearing-Speech and Performance areas. There was no statistical difference between groups in the latter two domains on z-score and SQ <70 analysis, as both groups were impaired in these areas (Table 2 and Table 5). The low language scores are consistent with the findings of other studies in HIV-infected children (Wolters, Brouwers, Civitello, & Moss, 1997). The language domain is most indicative of higher order cognition and delays have been associated with maternal depression (Laughton, et al., 2010), a low level of maternal education and low SES (Molteno, et al., 1991). Van Heerden and Van Rooyen have reported on developmental profile patterns

in normal South African children on the GMDS-ER. Both studies showed better functioning in the Locomotor and Personal-Social domains and poorer performance in Hearing-Speech and Practical Reasoning. Our findings are similar except in regard to the Performance and Practical Reasoning domains, where Performance was weaker than Practical Reasoning.

The environmental factors that compromise development in young children are extreme in HIV-infected/affected homes. These include parental death, illness and depression, multiple caregivers and poverty. In our study one HIV-infected child in five, was a maternal orphan and 30% were not cared for by either parent. It is therefore essential that HIV-infected parents access ART for their own health and for the sake of their children's physical and neurodevelopmental wellbeing.

The limitations to this study include: small sample size resulting in wide confidence intervals, convenience sampling, cross-sectional design and language barriers, which may have resulted in information bias. Our results must be cautiously interpreted as HIV infection and exposure were not excluded in the comparison group and we did not have data on parental education level in either group. In addition, the GMDS-ER has not, as yet been validated for the South African population.

Our findings, although preliminary, paint a bleak picture of the neurodevelopmental functioning of HIV-infected children and of preschool children in Soweto in general. This exploratory research highlights the need for increased awareness about neurocognitive problems in children in the community. There is a need for larger studies, to examine factors associated with developmental delay, in order to identify those children most at risk. Preventive and intervention strategies must be implemented to reduce HIV in children, initiate early ART and maintain the health of HIV-infected parents. Despite its limitations,

our study reinforces the urgent need to develop intervention strategies to optimise early childhood development for all children in Soweto and South Africa.

Neurodevelopmental delay among HIV-infected preschool children receiving antiretroviral therapy and healthy preschool children in Soweto, South Africa.

Table 1: Demographic characteristics of participants

	HIV-infected group	Comparison group	p-values
Number of participants	30	30	
Median Age in months (IQR)	63 (60 - 67.8)	61.5 (60 -72)	0.44†
Males	13 (43.3%)	15 (50%)	0.605*
Females	17 (56.6%)	15 (50%)	
Maternal orphans	6 (20%)	1 (3.3%)	0.103#
Paternal orphans	3 (10%)	3 (10%)	0.460#
Primary caregiver			
Parent	21 (70%)	28 (93.3%)	0.020*
Mother only	14 (46.7%)	19 (63.3%)	(parent vs.
Father only	2 (6.7%)	0	non-parent)
Both parents	5 (16.7%)	9 (30%)	
Non-parent	9 (30%)	2 (6.7%)	
Grandmother	3 (10%)	1 (3.3%)	
Aunt	1 (3.3%)	1 (3.3%)	
Other	5 (16.7%)	0	
Socio-economic status			
Low (≤ 3 assets)	17 (56.7%)	18 (60%)	0.793*
High (> 3 assets)	13 (43.3)	12 (40%)	

†Wilcoxon rank-sum (Mann- Whitney) test

*Chi-squared test

#Fisher's exact test

Table 2: Proportion of children with z-scores < -2 standard deviations per domain – primary analysis

	HIV-infected group N=30 (n (%))	Comparison group N=30 (n (%))	p-value	Odds Ratio (CI)
Total Overall	27 (90%)	23 (76.7%)	0.166*	2.74 (0.63-11.82)
Locomotor Domain	14 (46.7%)	2 (6.7%)	0.000*	12.25 (2.46-60.91)
Personal-Social Domain	8 (26.7%)	1 (3.3%)	0.026**	10.55 (1.23-90.65)
Hearing-Speech Domain	27 (90%)	25 (83.3%)	0.706**	1.8 (0.39-8.32)
Eye-Hand Domain	15 (50%)	7 (23.3%)	0.032*	3.29 (1.08-9.95)
Performance Domain	19 (63.3%)	12 (40%)	0.071*	2.59 (0.91-7.34)
Practical Reasoning Domain	21 (70%)	15 (50%)	0.114*	2.33 (0.81-6.73)

*Chi-squared test

**Fisher's exact test

Table 3: Comparison of means between groups on GMDS-secondary analysis

	HIV-infected group N=30 Mean (95% confidence interval)	Comparison group n=30 Mean (95% confidence interval)	p-value*
General Quotient	70 (66.0-74.0)	78.0 (75.6-80.5)	0.001
Locomotor Domain	75.7 (71.7-79.7)	82.7 (80.2-85.2)	0.004
Personal-Social Domain	76.8 (72.4-81.2)	85.8 (82.6-88.9)	0.001
Hearing-Speech Domain	60.6 (56.7-64.5)	66.9 (64.0-70.0)	0.011
Eye-Hand Domain	77.3 (72.8-81.8)	82.8 (80.3-85.3)	0.036
Performance Domain	62.3 (55.5-69.1)	73.1 (67.5-78.6)	0.014
Practical-Reasoning Domain	68.2 (64.2-72.3)	75.4 (72.4-78.4)	0.005

*Welch test

Table 4: Classification of children according to levels of neurodevelopmental functioning - secondary analysis

	HIV-infected group N=30 (n (%))	Comparison group N=30 (n (%))
Severe Delay (GQ<70)	14 (46.7%)	3 (10%)
Borderline Delay (GQ 70-79)	11 (36.7%)	13 (43.3%)
Low Average Functioning (GQ 80-89)	4 (13.3%)	13 (43.3%)
Average Functioning (GQ 90-109)	1 (3.3%)	1 (3.3%)
High Average To Superior Functioning (GQ≥110)	0 (0%)	0 (0%)

GQ – General Quotient

Table 5: Comparison of proportions of children in each group with severe delay (general or sub quotients < 70) – secondary analysis

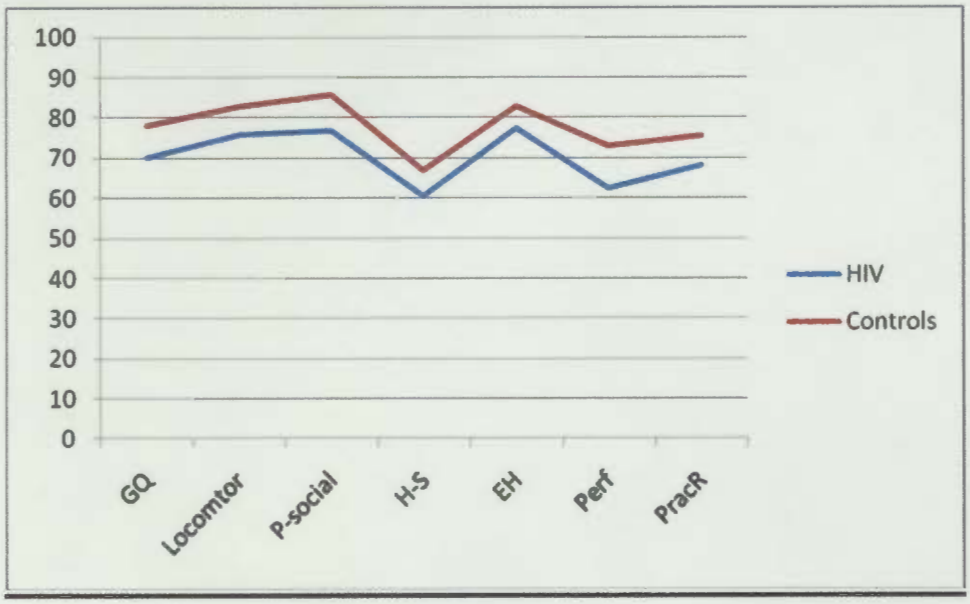
	HIV-infected Group N=30 (n(%))	Comparison Group N=30 (n (%))	p-value	Odds Ratio (CI)
General Quotient	14 (46.67%)	3 (10%)	0.002*	7.88 (1.96-31.68)
Locomotor Domain	7 (23.3%)	1 (3.33%)	0.052**	8.82 (1.01-76.96)
Personal -Social Domain	8 (26.7%)	0 (0%)	0.005**	–
Hearing -Speech Domain	25 (83.3%)	20 (66.7%)	0.222*	2.14(0.62-7.39)
Eye-Hand Domain	9 (30%)	2 (6.7%)	0.020*	6 (1.17-30.72)
Performance Domain	19 (63.33%)	12 (40%)	0.071*	2.59(0.91-7.34)
Practical-Reasoning Domain	18 (60%)	7 (23.33%)	0.004*	4.92(1.61-15.07)

*Chi-squared test

**Fisher's exact test

Figure 1.

Comparison of mean GQ and SQ values in HIV-infected and comparison groups.



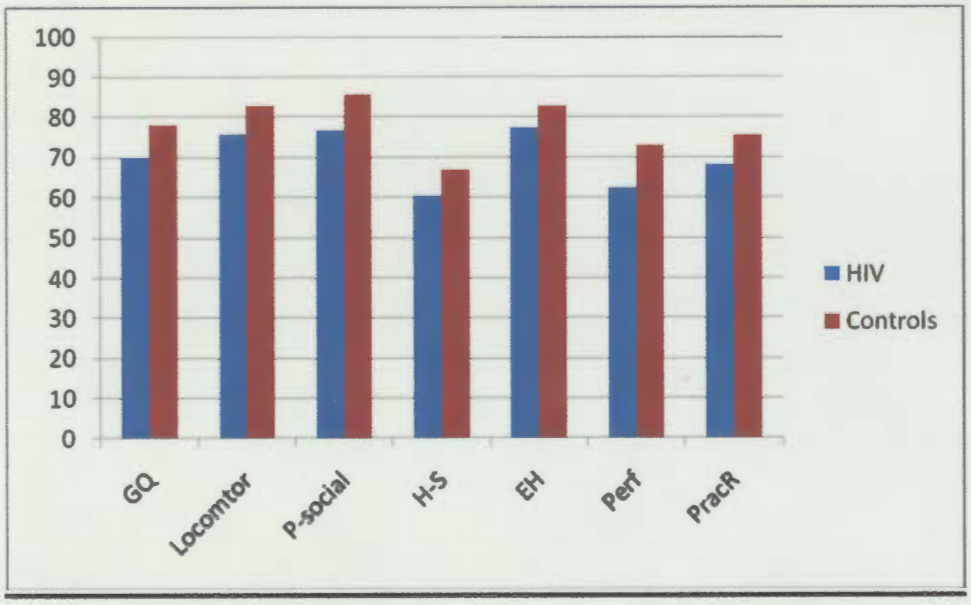


Figure 2

Locomotor Z-score Stata analysis table

	Comparison group	HIV-infected group	Total
z-score ≥-2			
Number	28	16	44
Percentage	93.33	53.33	73.33
z-score <-2			
Number	2	14	16
Percentage	6.67	46.67	26.67
Total			
Number	30	30	60
Percentage	100.00	100.00	100.00

Pearson chi2(1) = 12.2727 Pr = 0.000
Fisher's exact = 0.001
1-sided Fisher's exact = 0.000

Locomotor Sub quotient (SQ) Stata analysis table.

	Comparison group	HIV-infected group	Total
SQ Locomotor ≥ 70			
Number	29	23	52
Percentage	96.67	76.67	86.67
SQ Locomotor <70			
Number	1	7	8
Percentage	3.33	23.33	13.33
Total			
Number	30	30	60
Percentage	100.00	100.00	100.00

Pearson chi2(1) = 5.1923 Pr = 0.023
Fisher's exact = 0.052
1-sided Fisher's exact = 0.026

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19-Sep-2011

Dear Dr. Lowick:

Manuscript ID PHM-2011-07-0329, word count 3000 entitled "Neurodevelopmental delay among HIV-infected preschool children receiving antiretroviral therapy and healthy preschool children in Soweto, South Africa." which you submitted to Psychology, Health & Medicine, has been reviewed. The comments of the referee(s) are included at the bottom of this letter.

After careful review of the manuscript and the reviewer comments, I would like to invite you to revise and resubmit your manuscript for reconsideration. Please take the reviewer comments into account and set out your responses to us in detail.

To revise your manuscript, log into http://mc.manuscriptcentral.com/ac-phm-vcy and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please do not track changes on the new file. Once the revised manuscript is prepared, you can upload it and submit it through your Author Centre.

IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

When submitting your revised manuscript, you should respond to the comments made by the referee(s) in the space provided during the revision submission process. In order to expedite the processing of the revised manuscript, please include each reviewer comment in this space and respond to each individually. Please be as detailed as possible and ensure your response is anonymous.

To facilitate timely publication of manuscripts submitted to Psychology, Health & Medicine, your revised manuscript should be uploaded as soon as possible. The revision period will expire two months after this email, so please do contact us if you feel unable to resubmit within this timescale. If it is not possible for you to submit your revision in a reasonable amount of time, we may have to consider your paper as a new submission.

Please note that this is not a guarantee of publication. Revisions will be sent through a secondary review process before a final decision is made.

Once again, thank you for submitting your manuscript to Psychology, Health & Medicine and I look forward to receiving your revision.

Sincerely,
Professor Lorraine Sherr
Editor

Referee(s)' Comments to Author:
Referee: 1
Comments to the Author
The study is interesting, but there are methodological limitations:
a) the health status of the control group
b) validation of the scale population
c) education level of parents
Not clear statistical analysis and a detailed description of the results

Referee: 2
Comments to the Author
This paper addresses a very important topic in HIV infection, which is the presence of HIV in a brain in development. The project has some main problems that difficult a more strong conclusion, the scale used GMDS-ER was not validate for the population in the study, the influence of cultural and demographic in neuropychological is very well known and specific norms for a specific population are required. Other way these results could not be interpretated appropriately. This population has other confounding factor, the language, the test used was not translated for the language more prevalent in this area so it was necessary translators, this can lead a misinterpretations. The control group was not tested for HIV. Why these participants were not tested for HIV was not exposed. An appropriate control group for a HIV study must at least be HIV negative; this probably had great influence in the results of this group. Could these children be tested now? Although, these results, with appropriate discussions, could be seen as preliminary results of the evaluation of neurodevelopmental delay among HIV infected children and further investigations are required.
This paper, in my opinion, can be published, although some modifications must be done:
1. The control group must be tested for HIV
2. All the confound factors and weaknesses of the paper must be extensively discussed.

06-Dec-2011

Dear Dr. Lowick,

Re. PHM-2011-07-0329.R1 Neurodevelopmental delay among HIV-infected preschool children receiving antiretroviral therapy and healthy preschool children in Soweto, South Africa.

Thank you for submitting the above manuscript, word count 3198, to be considered for the journal.

The paper has been sent out for review by independent referees and I am pleased to inform you that it has been accepted for publication and will shortly be sent for onward processing, after which we aim to publish your manuscript in 6-10 months.

Please do ensure that once you receive a copyright form from the publishers it is signed and returned immediately, as failure to do so will delay publication of your article.

Thank you again for your interest in the journal and fine contribution.

Sincerely,
Professor Lorraine Sherr
Editor

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Sarah Lowick

CLEARANCE CERTIFICATE	**M090426**
PROJECT	Neurodevelopmetanl Delay among HIV-Infected Preschool Children Receiving Antiretroviral Therapy and Healthy Preschool Children in Soweto, South Africa (new title)
INVESTIGATORS	Dr Sarah Lowick.
DEPARTMENT	Department of Paediatrics
DATE CONSIDERED	09.04.29
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 16/01/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr T Meyers

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

UNIVERSITY OF THE WITWATERSRAND
FACULTY OF HEALTH SCIENCES
SCHOOL OF PUBLIC HEALTH
RESEARCH REPORT

PROJECT TITLE

TEMPORAL AND SPATIAL CHANGES IN THE *TUBERCULOSIS* BURDEN AND LABORATORY SERVICES OF THE EASTERN CAPE

SIBUSISO MKWANANZI

A research report submitted to the Faculty of Health Sciences,
University of the Witwatersrand,
in partial fulfillment of the requirements for the degree of
Master of Science in Medicine in Epidemiology and Biostatistics

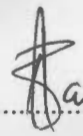
Johannesburg, South Africa

October 2012

Financial support for this MSc (Epidemiology and Biostatistics) was made available by the Gauteng Department of Health, to whom I am most grateful.

DECLARATION:

I, Sibusiso Mkwananzi declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine in Epidemiology and Biostatistics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



..... [Signature of candidate]

Full Name: SIBUSISO MKWANANZI

....12th.....day of .....OCTOBER......, 2012.

DEDICATION

In honour of my parents

Dr. J. B. Mkwananzi & Dr. L. M Resha-Mkwananzi.

ABSTRACT:

Background: The incidence of *tuberculosis* in sub-Saharan Africa and South Africa, according to the Medical Research Council (MRC) was ≈350 and 720 cases per 100 000 population respectively in 2001. In South Africa *tuberculosis* has reached epidemic levels, largely due to high levels of Human Immunodeficiency Syndrome (HIV) as well as the emergence of resistant strains. The Eastern Cape is one of the poorest provinces; this has led to treatment challenges. Previous studies have established *tuberculosis* burden and described perceptions and knowledge in communities. No previous studies have tracked laboratory functioning, prevalence of sensitive and resistant *tuberculosis*, applied Geographic Information Systems (GIS), nor attempted to relate the burden of *tuberculosis* to laboratory functioning. This study aims to understand *tuberculosis* patterns, including resistant *tuberculosis* areas and laboratory related risk factors. The study objectives include assessing tuberculosis temporal plus distribution trends, laboratory functioning as well as socio-demographic and laboratory factors associated with the disease. This will assist health managers to prevent further disease spread in the area.

Methodology: An analytical cross-sectional study was conducted using National Health Laboratory Services (NHLS) data from 2004 to 2009 of the Eastern Cape and Statistics South Africa population data. The Chi-squared test was used to identify significant differences between the two *tuberculosis* groups- (sensitive *tuberculosis* and resistant *tuberculosis*). A Chi-squared test for trend was used to confirm the presence of significant temporal trend. The Kulldorff M scan statistic was used for spatial and space-time analyses to identify statistically significant geographic clustering of *tuberculosis* at the district and yearly level. Finally association between pulmonary *tuberculosis* and socio-demographic factors as well as laboratory functioning were assessed using a univariate and multivariable logistic regression

approach in three different models where the dependent variables were any *tuberculosis*, resistant *tuberculosis* and XDR *tuberculosis* independently.

Findings: The incidence proportions of any, sensitive, multi-drug resistant and extensively drug resistant *tuberculosis* were all found to have significantly increased over time. In addition an increase in any and resistant *tuberculosis* incidence rates occurred in most districts across the time span and generally districts containing or near large cities had higher incidence rates. Spatial and space-time clustering analysis identified sub-districts with excess *tuberculosis* both in space and space-time. Primary clusters were confirmed to involve sub-districts in proximity or possession of large cities and generally occurred in the latter years between 2007 and 2009. The laboratory microscopy, culture and polymerase chain reaction testing loads all increased over time indicating an increase of suspects over time in the province. Provincial laboratory efficiency varied among the different tests; with the microscopy and polymerase chain reaction tests' efficiency increasing, but the culture test efficiency decreasing. The study finally found that gender (p-value:<0.001), increasing age (p-value:<0.001) and laboratory inefficiency (p-value:<0.001) were all independently associated to any *tuberculosis* and resistant *tuberculosis*. The study found that the independently associated factors of extensively drug resistant *tuberculosis* were female sex (p-value:<0.001), age groups 0-4 years (p-value:<0.001), 15-19 years (p-value:0.079) and 35-44 years (p-value:0.003) as well as laboratory inefficiency (p-value:<0.001).

Interpretation and recommendations: The Eastern Cape's increase in *tuberculosis* incidence rates from 2004 to 2009 suggests that control measure efforts such as educational prevention campaigns, case finding and curbing of treatment defaulters need to be upscaled in this province urgently. Laboratory loads increased over time from 2004 to 2009 in the

Eastern Cape. In addition to this a strong independent association of laboratory inefficiency with *tuberculosis* was found which highlights the critical need for optimal functioning laboratory services to ensure control of *tuberculosis* in this province. Laboratory efficiency and load need to be evaluated regularly in order to improve and maintain sufficient standards. High rates of tuberculosis were observed in or near large cities which has been attributed to urban poverty. Collaboration of numerous government departments (health, environment, housing, urban planning, social welfare etc) is needed to improve the living conditions of the poor in urban areas. This will then address the detrimental effects of urbanization and decrease the tuberculosis incidence rates associated with large cities. High risk socio-demographic and geographic groups were identified through spatial analysis and regression analysis. These groups need to be targeted directly with educational campaigns and routine voluntary testing. Finally in light of the current national *tuberculosis* policy it is recommended that:

-regular monitoring of external quality assurance systems at laboratories be conducted to ensure regular optimum functioning of laboratories.

-frequent surveys of sensitive and drug-resistant *tuberculosis* be made mandatory to guarantee current data on *tuberculosis* levels that can be compared to registry data.

-patients that submitted sputum samples, but did not fetch results be followed up and informed of their status at their residences

-education, influence and persuasion mechanisms be used to make the general public aware of the importance of having regular tuberculosis check-ups

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ABBREVIATIONS AND ACRONYMS

DBMS	: Database management systems
DOTS	: Directly Observed Therapy Short-course
GIS	: Geographic Information Systems
GPS	: Global Positioning System
HIV	: Human Immunodeficiency Syndrome
MDR-TB	: Multi-drug resistant *tuberculosis*
MGIT	: Mycobacteria Growth Indicator Tube
NICD	: National Institute of Communicable Disease
NHLS	: National Health Laboratory Service
PCR	: Polymerase Chain Reaction
RR	: Relative risk
TB	: *Tuberculosis*
XDR-TB	: Extensively drug resistant *tuberculosis*

NOMENCLATURE:

Temporal changes- changes in the *tuberculosis* number and proportion of cases occurring over time

Spatial changes-differences in the *tuberculosis* burden of different districts in the Eastern Cape

Pulmonary *Tuberculosis*- laboratory confirmed *tuberculosis* affecting the lungs of an individual.

Any *Tuberculosis*- pulmonary *tuberculosis* that incorporates sensitive *tuberculosis* as well as resistant *tuberculosis* (MDR *TB* and XDR *TB)*

Multi-drug resistant *tuberculosis*- an isolate of *Mycobacterium tuberculosis* that is resistant to at least rifampicin and isoniazide

Extensively drug resistant *tuberculosis* - an isolate of *Mycobacterium tuberculosis* that is found to be MDR-*TB*, which is also resistant to any fluroquinolones and at least 1 of the 3 injectable second-line drugs (capreomycin, kanamycin or amikacin)

Resistant pulmonary *tuberculosis*- includes both multi-drug resistant *tuberculosis* and extensively drug resistant *tuberculosis*

Test time-the duration of time from registration of a specimen to the diagnostic tests' review date.

Laboratory efficiency- refers to the efficiency of a laboratory calculated through dividing its annual average microscopy test time with the province's annual average microscopy test time.

Laboratory load- Level of use of *tuberculosis* diagnostic services based on the number of total suspected *tuberculosis* cases being tested at a certain laboratory

CHAPTER ONE:

This chapter attempts to conceptualise and introduce the main aspects of this study. The background first gives an outline of the current tuberculosis patterns and the past use of Geographic Information Systems *(GIS) both globally and in South Africa. The literature review highlights findings of previous studies concerning the trends of tuberculosis over time, GIS use in studying tuberculosis, risk factors of tuberculosis and tuberculosis laboratory services. The chapter ends by formulating the problem statement, justification and study objectives of the study.*

1.0 INTRODUCTION

1.1 BACKGROUND

1.1.1 Tuberculosis

Tuberculosis accounts for 2.5% of the global burden of disease (1). Worldwide there are an estimated 9.27 million (141 cases per 100000 population) new *tuberculosis* cases every year and the incidence change rate is 1% per year (2, 3). Of these new cases, it is estimated that 1 million (11%) occur in children younger than 15 years of age and 75% of cases occur among the economically productive age group (1).

In developed countries, there has been a significant incidence decline in the past ten years, but this is not the case in the developing world where cases of infected people are still escalating (2). Developed countries such as the United States, Western Europe, Canada, Japan and Australia have low rates(less than 25 cases per 100000 population) of *tuberculosis* and only account for 5% of *tuberculosis* cases globally (4, 5). Intermediate rates (26-100 cases/100000) occur in Central and South America, Eastern Europe and Northern Africa (4). About 95% of *tuberculosis* cases occur in developing countries (6). *Tuberculosis* rates are high (more than 100 cases per 100000 population) in most

developing countries and 22 of these high burden countries are responsible for 80% of the entire global burden (7). In 2007 the five highest ranking *tuberculosis* incidence rates countries were India (2.0 million), China (1.3 million), Indonesia (0.53 million), Nigeria (0.46 million) and South Africa (0.46 million) (4).

The average *tuberculosis* incidence rate in sub-Saharan Africa is estimated at 350 cases per 100000 population. In select areas of Southeast Asia and in sub-Saharan Africa *tuberculosis* is being driven by the human immunodeficiency virus (HIV) epidemic (8). This is because 10% of patients with HIV will develop active *tuberculosis* per year; whereas 10% of people without HIV will develop *tuberculosis* throughout their entire lifetime (8). In Asia 24% of all *tuberculosis* cases are co-infected with HIV compared with 38% in sub-Saharan Africa (6).

Tuberculosis was the seventh major cause of death and the world's second most common cause of death from an infectious disease in 2007 (1, 6). In the past century *tuberculosis* has killed 100 million people, despite there being treatment for the disease for 50 years (6). Globally an estimated 2 million people die annually from *tuberculosis* annually, which is roughly equivalent to a *tuberculosis* death every 15 seconds (1, 6). In the entire African region, *tuberculosis* kills 1500 people every day constituting 500000 deaths annually (9).

1.1.2 Tuberculosis burden in South Africa

South Africa has a *tuberculosis* incidence of 720 per 100000 population according to the Medical Research Council (MRC) (10, 11). This incidence is 60 times higher than that seen in the industrialised world and 73% of South Africa's *tuberculosis* cases are co-infected with HIV (10). *Tuberculosis* was declared to be a national crisis in 2005 (12). In 2008, 112000 people died from this disease in South Africa alone (10).

1.1.3 Usefulness of spatial and spatial-temporal analysis

Geographic Information Systems (GIS) and spatial analysis are effective tools that are increasingly being used as essential instruments for planning and evaluating health challenges and interventions. GIS has been applied successfully in epidemiological studies, disease control, infectious disease transmission studies and identification of environmental risk factors. Spatial analysis in public health is used for the visual mapping of disease to show the areas of high and low prevalence, modelling disease spread and monitoring health interventions (13). The majority of the world's *tuberculosis* burden is realised in Africa needing spatial analysis to provide timely information on the course of disease and health interventions in order to implement correct health actions (14). In Africa there are cost, resource and infrastructural constraints making it crucial to use health funds as optimally as possible. (14). The *tuberculosis* burden increases in the absence of well established and efficient health systems. Spatial and spatial-temporal analysis can aid in such situations by guiding officials to place intervention implementation and service delivery locations where the need is greatest (14). With *tuberculosis* this can be translated into planning for the correct number and distribution of treatment points.

1.2 LITERATURE REVIEW

1.2.1 Tuberculosis trends over time

Studies show that temporal trends of *tuberculosis* are divided into two main categories viz. those of developed regions and of developing regions (15). In developed settings (such as those shown by the United States, Canada, Western Europe, Japan and Australia) *tuberculosis* generally exhibited steady or falling prevalence and incidence rates over the past twenty years. Developing regions namely sub-Saharan Africa, India, China and the islands of Southeast Asia however had increasing trends since the 1980's (15).

In sub-Saharan Africa *tuberculosis* temporal increase is generally attributed to the Human Immunodeficiency Virus (HIV) epidemic which acts as a co-factor of transmission and disease (15, 16). In certain African countries, *tuberculosis* notification rates doubled over a ten year period during the 1990's due to the co-evolution of these epidemics.

1.2.2 Tuberculosis and GIS

In countries outside of Africa, GIS has been used frequently in *tuberculosis* research. In the United States, a study was done to identify the geographical areas where *tuberculosis* transmission was occurring in order to establish the high-risk groups by linking GIS technology with molecular surveillance (17). In Thailand, GIS was used to assess geographical clustering of multidrug-resistant *tuberculosis* cases in a Hmong refugee camp (18).

Most African *tuberculosis* studies using GIS have been conducted in South Africa. GIS was used to map *tuberculosis* cases in the Western Cape, analyse the distribution of treatment points in rural KwaZulu-Natal and investigate childhood *tuberculosis* in two urban communities in Cape Town (14).

1.2.3 Risk factors of tuberculosis

Numerous studies have been conducted throughout the world to establish the risk factors of *tuberculosis*. Studies done in Japan identified low educational attainment, old age and poverty with increased risk and found high incident clusters in the coal mining sector, industrial and urban areas (15, 19). In Brazil higher *tuberculosis* rates were found to occur in state capitals or metropolitan areas (20). In the Gambia, studies revealed that *tuberculosis* occurred more in people that were permanent residents of the area while in Madagascar *tuberculosis* distribution was associated mainly with the number of people having low adherence to treatment and the number of households with more than one case

(21, 22). In South Africa, particularly in the Western Cape, it has been shown that increased *tuberculosis* prevalence is independently associated with high levels of community income inequality, unemployment, overcrowding and poverty (23, 24).

Studies have shown that *tuberculosis* prevalence, infection rate, incidence and mortality can all be decreased in a developing country, especially in the absence of human immunodeficiency virus. When HIV is prevalent, as is the case in most sub-Saharan African countries, *tuberculosis* incidence will eventually rise due to co-infection. As a result *tuberculosis* control needs to be comprehensive and done in conjunction with control of the concurrent HIV epidemic in such settings (25, 26).

1.2.4 Tuberculosis laboratory services

The role of the laboratory with regards to pulmonary *tuberculosis* control was declared as a vital function in diagnosis and treatment management (27). A laboratory's level of efficiency contributes to the time taken for diagnosis and treatment of a patient. Delay of diagnosis refers to the length of pre-treatment period of pulmonary *tuberculosis* (28). A study in England showed that a delay in treatment causes the infection of other people in the community (29) . Various studies have also found that diagnosis delay decreases the likelihood of favourable patient treatment outcomes, in particular chronic disease, more complications and mortality (28, 30, 31). A systematic review of diagnosis and treatment delay conducted by Storla et al in 2008 revealed that delay was due to lack of specific *tuberculosis* services at health facilities and this caused repeated administration of incorrect antibiotics to patients (32). Development of explicit protocols and *tuberculosis* programmes within health centres improved diagnosis and treatment time (32). Another review of time delays in diagnosis suggested that the shortest delays were in China (25 days) while the longest were in Tanzania (185 days) (33). It concluded that all the diagnosis delays were considerable and this contributed to *tuberculosis* transmission

dynamics, prevention and control strategies (33). Factors shown to be associated with the diagnosis delays are disease severity, access to health services, the level of expertise of health personnel and an individual's perception of *tuberculosis* (34).

1.3 PROBLEM STATEMENT:

Approximately one-third of the world's population is living with *tuberculosis* which translates to more than 2 billion people being infected with this disease (4). Sub-Saharan Africa has the highest *tuberculosis* incidence rates (6). South Africa has a *tuberculosis* burden that is more than double the rest of Africa (10, 35).The Eastern Cape only obtained substantial improvements in health infrastructure and co-ordination following independence (36). The Directly Observed Therapy Short-course (DOTS) program, though functioning since 1995, has had little effect and *tuberculosis* continues to be a key health challenge in the province. In 2006, the Eastern Cape's *tuberculosis* incidence rate (705 cases per 100 000) was at par with the national average (722 per 100 000) yet the cure rate was lagging behind the national average, ranking as the fourth lowest (35, 37). In light of the above it is imperative that temporal and distribution trends of *tuberculosis* be investigated in the area in order to establish whether the *tuberculosis* incidence is rising or falling over time as well as to establish the areas that are more affected by the disease. Laboratory performance will be investigated to ascertain whether there are any diagnostic challenges or delays occurring in the province. Finally regression analysis will assist in determining the independently associated factors of *tuberculosis* in the province to assist in the formulation of relevantly targeted programmes.

1.4 JUSTIFICATION FOR THE STUDY:

The burden and control of *tuberculosis* is a global health concern. In South Africa the disease has reached epidemic levels according to the World Health Organization. This has

been propelled by the HIV prevalence as well as the emergence of *tuberculosis* resistant strains. The Eastern Cape is one of the poorest South African provinces, having a history of limited resources and large numbers of people living in poverty as well as relying on government assistance. The province has therefore found it difficult to treat and manage the increasing *tuberculosis* burden. Previous studies in the area have concentrated on establishing the levels of *tuberculosis* as well as describing perceptions and knowledge of the community. There have been no previous studies trying to track the changes in the *tuberculosis* burden over time in this province. No studies have been conducted in this province assessing spatial-temporal patterns between the different districts. In addition no studies have attempted to relate the burden of resistant pulmonary *tuberculosis* over time and in various districts to laboratory diagnostic efficiency and the load in the Eastern Cape. Answering these questions will help improve and understand dynamic *tuberculosis* patterns and assist health managers to control and prevent further disease spread in the province.

1.5 STUDY OBJECTIVES:

1. To determine the changes in the incidence proportion of sensitive and resistant *tuberculosis* in the Eastern Cape from 2004 to 2009.

2. To determine the changes in laboratory load and laboratory efficiency in the districts of the Eastern Cape from 2004 to 2009

3. To determine the distribution and changes in the distribution over time (2004-2009) of sensitive and resistant *tuberculosis* in districts of the Eastern Cape.

4. To establish the association between socio-demographic factors, laboratory efficiency as well as laboratory load and pulmonary *tuberculosis* incidence proportion in the Eastern Cape over the study period.

CHAPTER TWO

This chapter first describes the study setting, design, participants and data used in the study. It then details the analytical techniques that were used in the study to address the overall and specific objectives.

2.0 MATERIALS AND METHODS

2.1 STUDY DESIGN

An analytical cross-sectional study was conducted using data from the National Health Laboratory Service's (NHLS) database for the Eastern Cape from 2004 to 2009. This was an appropriate design because pre-existing data of both exposures and outcomes were collected simultaneously through routine laboratory surveillance of *tuberculosis*. In addition this study design was appropriate in establishing the exposure variables (socio-demographic and laboratory test factors) that were associated with the outcome variable (pulmonary *tuberculosis* prevalence) in the Eastern Cape.

2.2 STUDY AREA

The study area is the Eastern Cape province of South Africa. It is the 2nd. largest province encompassing an area of 169 580km^2 and stretches to border with the Western Cape in the west, Kwa-Zulu Natal in the far northeast, Free State in the north, the Northern Cape in the northwest, Lesotho in the northeast and the Indian ocean on the east and southeast (38). There are currently 6.9 million people living in the province and 65% of them live in rural areas (38). The Eastern Cape is comprised of approximately 87.6% black Africans, 7.5% coloureds, 4.7% whites and 0.3% Asians.

2.3 STUDY POPULATION:

The study population comprised suspects that had sputum specimens taken after presenting at health institutions or screening stations with *tuberculosis*-like symptoms in the Eastern Cape from 2004 to 2009.

2.4 INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria:

All age groups

Patients with laboratory confirmed pulmonary *tuberculosis*

Exclusion Criteria:

Patients with missing laboratory data

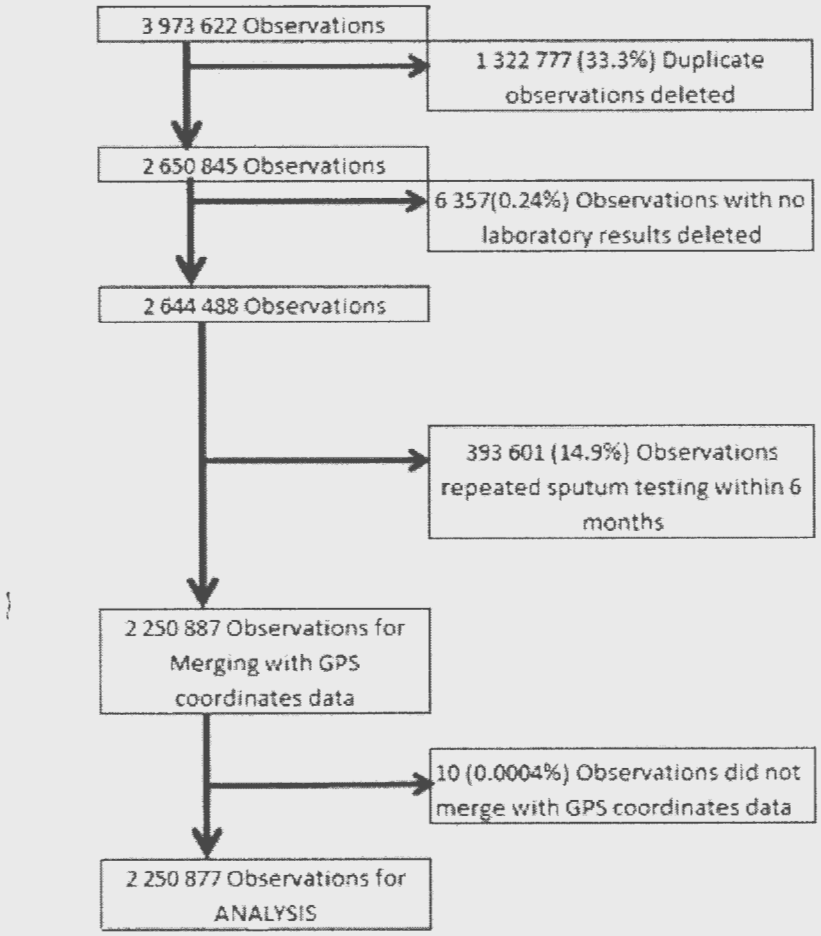
Patients without pulmonary *tuberculosis* including those with extra-pulmonary *tuberculosis*

2.5 STUDY PARTICIPANTS

The primary study's participants consisted of suspected cases of *tuberculosis* that had sputum specimens taken. Suspected cases had *tuberculosis*-like symptoms including coughing for more than 2 weeks [most important symptom] and possible haemoptysis, dyspnoea, malaise, weight loss, night sweats or chest pain. This encompassed a total of 3 973 622 observations which was the total number of sputum samples collected from 2004 to 2009 (See figure 2.1). Of these sputum samples 1 322 777 (33.3%) samples were duplicates from people that had given two or more samples and were therefore deleted. From the remaining 2 650 845 observations, 6 357 observations were deleted due to missing laboratory results. Participants that had repeated sputum testing within six months were then also deleted leaving a total of 2 250 887 observations to merge with the Global Positioning System (GPS) data. Ten observations could not be matched with corresponding

GPS co-ordinates for the clinics at which they tested and therefore the final number of observations included in the study for data analysis was 2 250 877.

Figure 2.1: Flow chart of study participants



2.6 MEASUREMENT AND DATA SOURCES

2.6.1 DATA SOURCE AND SOFTWARE UTILISED

This secondary data analysis utilised routine laboratory surveillance data collected by various laboratories in the Eastern Cape and stored in a central repository database of the National Health Laboratory Services (NHLS). The National Institute of Communicable Diseases (NICD) gave permission to use the data. The NHLS datasets were all entered using Excel 2003. Data was extracted from the database to STATA using Database management systems (DBMS) copy software. STATA version 11 was used to clean, code, merge and statistically analyse all the data. The program SaTScan was used to do the spatial analysis through the utilisation of a shape file for all the Eastern Cape clinics, count

data for the *tuberculosis* observed data and population census data to relate information to the various sub-district populations.

The study variables used can be divided into four main categories viz. those explaining basic demographic features of the population, those identifying source and referral health institutions, those linked with identification of the infectious agent and those detailing resistance characteristics of isolated bacteria.

2.6.2 STUDY DENOMINATORS

The study had two types of denominators. Firstly, the total number of participants tested in each category of data classification was used as a denominator to describe the study participants. Proportions of participants with various characteristics were obtained for the entire study population as well as by disease state and resistance. This then showed the proportion of participants who had various characteristics in the overall study population; in the participants with and without *tuberculosis* and in the participants with and without resistant *tuberculosis*. The second set of denominators used in the study was population data which was used as a denominator for the incidence proportion calculations as well as for spatial analysis. Population data was obtained by extracting data from Statistics South Africa's (Stats SA's) website pages (39, 40). Information obtained from the 2001 census and 2007 community survey as well as the estimates of the province's mid-year population numbers for 2003, 2004, 2005, 2006, 2008 and 2009 was utilised (39, 40). The estimated provincial population data were then broken down into district population data through being divided according to district contribution of provincial population. The percentage of district contribution to the provincial population was based on the district percentage contributions of the 2007 community survey conducted by Statistics South Africa.

2.7 DATA PROCESSING METHODS AND DATA ANALYSIS PLANS

2.7.1 DESCRIPTIVE STATISTICS

2.7.1.1 Characteristics

The study population's characteristics were summarised in tabular form to show participants throughout the Eastern Cape across all the years, differences in disease status and resistance variations. The Chi-squared test was used to establish whether differences found between groups were statistically significant or not.

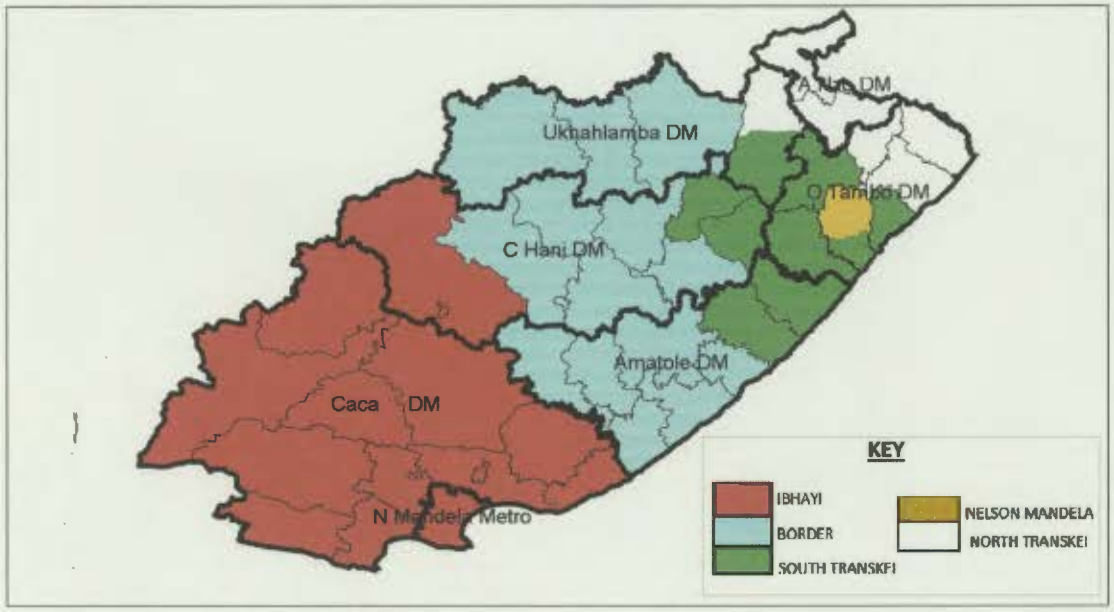
2.7.1.2 Laboratory testing trends

The laboratory load and test times for the entire province were plotted over time for three major test categories: microscopy, culture and polymerase chain reaction (PCR). These tests were analysed due to all sputum specimens going through the process of microscopy and confirmatory diagnosis depending on positive culture or PCR results. Susceptibility test time was analysed at laboratory level because very few laboratories offered the investigation. The susceptibility test time variable was obtained by averaging the sum of various individual sensitivity tests' times, with the assumption that tests are done successively and not simultaneously.

In addition to analysis at provincial level, microscopy load and test times were also analysed at the NHLS branch level. This was chosen over the district level due to clinics from various districts sending sputum specimens to a specific laboratory, not necessarily within the same district, to do necessary tests for diagnosis and classification i.e. testing laboratories were in a different district to the clinics where the patient presented. Different laboratories were grouped into various branches which were completely independent of district zoning.

The laboratories used by the Eastern Cape health system are grouped into six branches being the Border, Ibhayi, Nelson Mandela, North Transkei, South Transkei and External branches see figure 2.2. As seen in the figure below laboratory branches overlap district municipal borders and the Nelson Mandela branch encompasses only a part of a sub-district.

Figure 2.2: Map of the laboratory branches in the Eastern Cape Province



The microscopy load in the various branches was compared by year using a contingency table (looking at the specific number of suspected specimens over time). Line graphs were also created to visualise differences in laboratory load for each branch over time.

2.7.1.3 Trends of tuberculosis frequency and distribution

The annual trend of *tuberculosis* incidence was then assessed from 2004 to 2009 by sensitive *tuberculosis*, resistant *tuberculosis* and extensively drug resistant (XDR) *tuberculosis* using contingency tables. Population data of the Eastern Cape was used as a denominator for the incidence proportion calculations. Line graphs were constructed to show changes in the incidence proportion of *tuberculosis* cases over the study period.

Statistical significance of these yearly trends was assessed using Chi-squared test for trend and a p-value < 0.1 was used as the cut-off.

Differences in the incidence proportion of *tuberculosis* cases in the districts of the Eastern Cape were shown for the entire study period using bar charts for pulmonary *tuberculosis*, sensitive *tuberculosis*, resistant *tuberculosis*, Multi-drug resistant (MDR) *tuberculosis* and XDR *tuberculosis* and a line graph was used to show the district changes for each year for pulmonary *tuberculosis*. This line graph made it possible to see incidence proportion differences among all the districts at any single point in time.

2.7.2 INFERENTIAL ANALYSIS

2.7.2.1 Spatial and Spatial-temporal Analyses

The Kulldorff scan statistic was used for spatial and space-time analyses. This scan statistic is a spatial method for identifying and assessing disease case surpluses. It utilises a circular or cylindrical window to detect clusters spatially and in time. Circular windows are used for spatial analysis and cylindrical windows for spatial-temporal analysis. The windows are then assessed statistically to establish the likelihood of the observed number of cases being able to occupy the size of window created or not, with reference to the entire study area's number of cases and corresponding window sizes (41). In this way the program eventually identifies areas where the observed numbers of cases are so high that they are clustered showing that the geographical area or time period is inherently prone to the disease for some reason. The method is said to avoid pre-selection bias due to looking for clusters objectively throughout the study population and area (41).

The SaTScan program was used to establish all primary, secondary and tertiary clusters in sub-districts of the Eastern Cape. Denominators were based on the 2001 census and 2007 community survey data extracted from the Stats SA website (39, 40) and were broken

down to sub-district level based on the findings of the 2007 community survey (40). All SaTScan analyses used population counts to calculate the expected number of cases (42). Expected counts were calculated for each sub-district through population counts being stratified by age groups to estimate a weighted average for the study period. The natural log of this weighted average was entered as the offset variable in a Poisson regression. For sub-districts having zero population for a certain age group, a population of one was used. A Poisson regression was first used to estimate the expected age-adjusted counts with age being the independent variable and number of cases within each sub-district and age group being the dependent variables. The expected counts were aggregated across age groups within each tract and multiplied by 1000 for the population file (consisting of expected number of cases) for spatial analyses (41). It was assumed that incident *tuberculosis* follows a Poisson distribution (43). According to the null hypothesis, the probability of a case being diagnosed in a particular location is equal throughout the province, adjusting for the density of the population. Poisson regression was therefore applied to compare the expected *tuberculosis* counts to the observed *tuberculosis* counts with resultant relative risk (RR) and p-value being the essential output. In all analyses, the number of Monte Carlo replications was set to 19,999 (44).

Purely spatial analysis uses data from the entire study period and with this establishes whether the geographic variations in *tuberculosis* (sensitive, resistant and both together) prevalence are simply random or statistical deviations from randomness, analyzed in a single model. In addition to this the areas where there are statistical excesses or deficits are located. All significant clusters (p-value< 0.1) of *tuberculosis*, resistant *tuberculosis* and XDR-*TB* were recorded. The space-time analyses discovered clusters that were significant for smaller time frames (yearly) up to and including the entire span of the study period.

Space-time analysis establishes whether the yearly geographic variations are random or statistically significant deviations from randomness, where specific year's statistically significant excesses and deficits lie and establishes whether significant deviations are temporary or persistent. The maximum spatial cluster size was adjusted to identify optimal detection of both excesses and deficits in space and space-time.

2.7.2.2 Risk factors of *tuberculosis*

The association between socio-demographic factors, laboratory efficiency and three different dependent variables (viz. any *tuberculosis*, resistant *tuberculosis* and XDR *tuberculosis*) was assessed for the Eastern Cape using three different logistic regression models.

Independent variables: Age recoded categorically, Gender and Laboratory efficiency.

The evaluation of laboratory efficiency was analysed through using microscopy efficiency as a proxy measure as this was the only test where data was present for more that 50% of the laboratories throughout the period. The microscopy efficiency of each laboratory was established through calculating the province's average microscopy test time for each year and then dividing the laboratory's annual average microscopy test time by the province's annual average test time.

A univariate logistic regression analysis was first run for the three different binary outcomes. Variables found to be significantly associated ($p<0.05$) or marginally associated ($p<0.15$) were selected for inclusion into the equivalent multivariable logistic regression analysis along with any confounders. From the multivariate logistic regression model all main exposure variables with a resultant p-value of less than 0.05 were considered to be independently associated with the dependent variable in question.

CHAPTER THREE

This chapter covers the results of the data analysis conducted and encompasses both the descriptive and inferential data analyses.

3.0 RESULTS

3.1 DESCRIPTIVE ANALYSIS RESULTS

3.1.1 Demographic characteristics of study participants

This section summarises the demographic features of the study participants. Characteristics of all the participants are first detailed and then the characteristics are compared by the various TB outcomes detailed earlier (any, resistant and XDR tuberculosis).

Table 3.1: Breakdown of characteristics of people suspected of TB: Eastern Cape, 2004-2009.

Disease State	
No Tuberculosis	2 160 842 (96%)
Tuberculosis	86 689 (4%)
Sex	
Male	1 035 403 (46%)
Female	1 170 456 (52%)
Unknown	45 018 (2%)
Age	
Minimum	1 day old
Maximum	107 years old
Median	38 years old
Interquartile Range	26 -53 years old
Age Categorised	
0-4	22 509 (1%)
5-9	45 018 (2%)
10-14	112 543 (5%)
15-19	135 053 (6%)
20-34	630 246 (28%)
35-44	450 175 (20%)
45-54	337 632 (15%)
55+	517 701 (23%)
District	
Alfred Nzo	157 561 (7%)
Amathole	517 702 (23%)
Cacadu	180 070 (8%)
Chris Hani	292 614 (13%)
Nelson Mandela	472 684 (21%)
OR Tambo	495 193 (22%)
Ukhahlambi	135 053 (6%)

Table 3.1 describes the characteristics of the study participants. In general the number of participants without *tuberculosis* far exceeded those with the disease. A total of 2 160 842 (96%) participants had no *tuberculosis* and 86 689 (4%) participants had *tuberculosis*.

There were 1 035 403 (46%) males, 1 170 456 (52%) females and 45 018 (2%) participants whose sex was unknown. The participants' ages ranged from 1 day old to 107 years old and the median age was 38 years old with an Interquartile range of 26 to 53 years of age. When age was categorised into eight categories the largest category was found to be the 20-34 year olds consisting of 28% of the participants, followed by the 55+ age category with 23% of participants and the 35-44 years category with 20% of participants. The fewest number of participants (22 509- 1%) belonged to the 0-4 year old age category. The majority of participants originated from the most developed districts of the province being Amathole (23%), OR Tambo (22%) and Nelson Mandela (21%). Fewer participants were from Chris Hani (13%), Cacadu (8%), Alfred Nzo (7%) and UKhahlamba (6%) districts.

Table 3.2: Socio-demographic characteristics and incidence (cases per 100 000) of *tuberculosis* n=2 250 877:2004-2009*

	No Tuberculosis: n=2 164 188	Tuberculosis: n=86 689	Tuberculosis Incidence	P-VALUE
	n(%)	n(%)	cases (95% CI)	
CATEGORICAL VARIABLES				
Sex				<0.001
Male	973 884 (45%)	46 812 (54%)	238 (226;250)	
Female	1 147 020 (53%)	38 143 (44%)	177 (168;186)	
Unknown	43 284 (2%)	1 734 (2%)		
Age Categorised				<0.001
0-4	21 642 (1%)	867 (1%)	19 (18; 20)	
5-9	43 284 (2%)	867 (1%)	19 (18; 20)	
10-14	108 209 (5%)	867 (1%)	17 (16, 18)	
15-19	129 851 (6%)	3 467 (4%)	68 (65; 71)	
20-34	605 973 (28%)	33 809 (39%)	343 (326; 360)	
35-44	432 838 (20%)	23 406 (27%)	620 (589; 651)	
45-54	324 628 (15%)	13 003 (15%)	399 (379; 419)	
55+	497 763 (23%)	10 403 (12%)	212 (201; 223)	
District				<0.001
Alfred Nzo	151 493 (7%)	1 734 (2%)	46 (44; 48)	
Amathole	497 763 (23%)	14 737 (17%)	135 (128; 142)	
Cacadu	173 135 (8%)	10 403 (12%)	411 (390; 432)	
Chris Hani	281 344 (13%)	5 201 (6%)	106 (101; 111)	
Nelson Mandela	432 838 (20%)	38 143 (44%)	556 (528; 584)	
OR Tambo	497 763 (23%)	13 003 (15%)	108 (103; 113)	
Ukhahlambi	129 851 (6%)	3 467 (4%)	179 (170; 188)	

*Pearson Chi-square test used to test all variables

The socio-demographic characteristics of participants are compared by disease state in table 3.2. The percentages given are column percentages therefore per variable the percentages in each column will add up to 100% e.g. among participants without

tuberculosis 45% were male, 53% were female and the sex was unknown for 2%. The two groups (*tuberculosis* positive and *tuberculosis* negative individuals) were found to be significantly different in all characteristics tested being sex, age categorised and residential district, shown through the chi-squared tests having significant p-values of less than 0.05. The participants without *tuberculosis* were significantly older at a median age of 39 years, consisted of more females than males and were predominantly from the Amathole, OR Tambo and Nelson Mandela districts. Individuals with *tuberculosis* were significantly younger with a median age of 36 years, consisted of more males and were predominantly from the Nelson Mandela district.

Tuberculosis incidence by the various characteristics is also recorded in table 3.2. According to this, *tuberculosis* occurred mostly amongst males, the 35-44, 45-54 and 20-34 year age groups as well as in the Nelson Mandela metropolitan and Cacadu district.

Table3.3: Characteristics of TB positive participants by resistance: Eastern Cape, 2004-2009*

VARIABLE	TB n=82 101	Resistant TB n=4 588	P-VALUE
	n(%)	n(%)	
Sex			<0.001
Male	44 750 (55.50)	2 371 (51.68)	
Female	35 877(44.50)	2 127 (48.32)	
Age Categorised			<0.001
0-4	1 037 (1.26)	34 (0.74)	
5-9	451 (0.55)	32 (0.70)	
10-14	798 (0.97)	38 (0.83)	
15-19	3 270 (3.98)	184 (4.01)	
20-34	31 163 (37.96)	1 758 (38.32)	
35-44	21 266 (25.90)	1 225 (26.70)	
45-54	12 457 (15.17)	705 (15.37)	
55+	9 568 (11.65)	430 (9.37)	
District			<0.001
Alfred Nzo	1 290(1.57)	93 (2.03)	
Amathole	12 798 (15.81)	1 323 (28.84)	
Cacadu	9 283 (11.31)	358 (7.80)	
Chris Hani	5 156 (6.28)	181 (3.95)	
Nelson Mandela	35 302 (43.00)	1 515 (33.02)	
OR Tambo	11 859 (14.44)	840 (18.31)	
Ukhahlambi	3 316 (4.04)	142 (3.10)	

*Pearson Chi-square test used to test all variables

Table 3.3 presents the differences in characteristics between participants with sensitive *tuberculosis* and those with resistant *tuberculosis*. Participants with sensitive *tuberculosis* outnumbered those with resistant *tuberculosis* greatly. The two groups were statistically different in all characteristics tested for, as seen from the p-values of the Chi-squared tests. Similarities were present however in both groups: There were more males than females, majority of participants were older than 20 years old (with the 20-34 and 35-44 year old age groups comprising the most cases) and participants were predominantly from the Nelson Mandela Metropolitan, followed by the Amathole district then the OR Tambo district which are the three most populated and developed districts in the Eastern Cape.

3.1.2 Trends of tuberculosis frequency and distribution

3.1.2.1 Tuberculosis frequency over time

This section describes the changes in *tuberculosis* frequency over time in the Eastern Cape from 2004 to 2009. The frequency is first shown as the number of cases that occurred followed by the estimated incidence by year and resistance. Graphical depictions of these trends are also presented for easier visualization.

Table 3.4: *Tuberculosis* (any, sensitive, resistant) frequency by year: Eastern Cape, 2004-2009

Year	Tuberculosis	Sensitive Tuberculosis	Resistant Tuberculosis	XDR Tuberculosis
2004	7411	7092	319	0
2005	9689	9211	478	0
2006	13119	12361	758	0
2007	16095	15043	1052	0
2008	20209	19255	954	66
2009	20166	19139	1027	107

Table 3.4 shows the annual changes in the *tuberculosis* frequency overall and by resistance patterns throughout the study period. A 2.7-fold increase of all *tuberculosis* occurred from 2004 to 2009.There was a slight decrease from 2008 to 2009, but this was not statistically significant. Resistant *tuberculosis* cases rose three-fold from 319 in 2004 to 1027 in 2009.

Though absent initially from 2004 to 2007, 66 cases of XDR *tuberculosis* emerged in the Eastern Cape in 2008 and this increased to 107 cases in 2009.

Figure 3.1: Incidence of any *tuberculosis* by year: Eastern Cape, 2004-2009

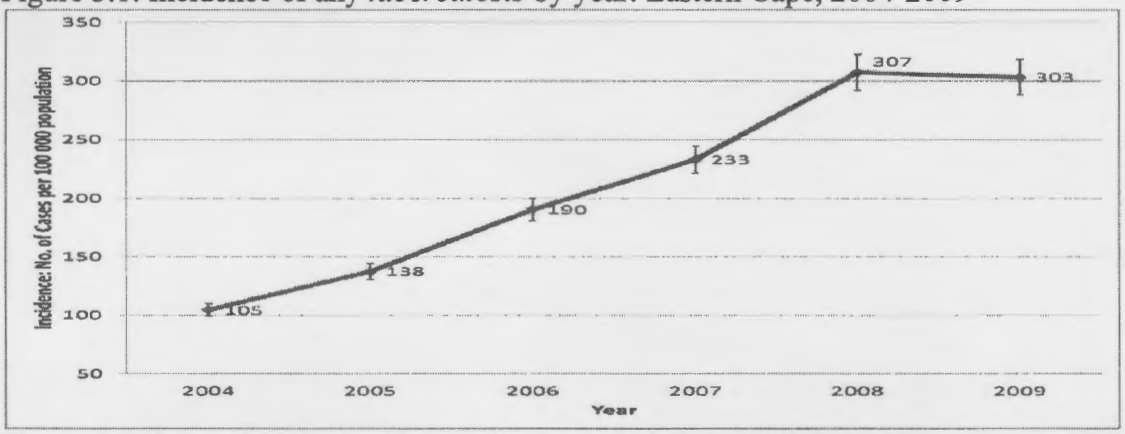
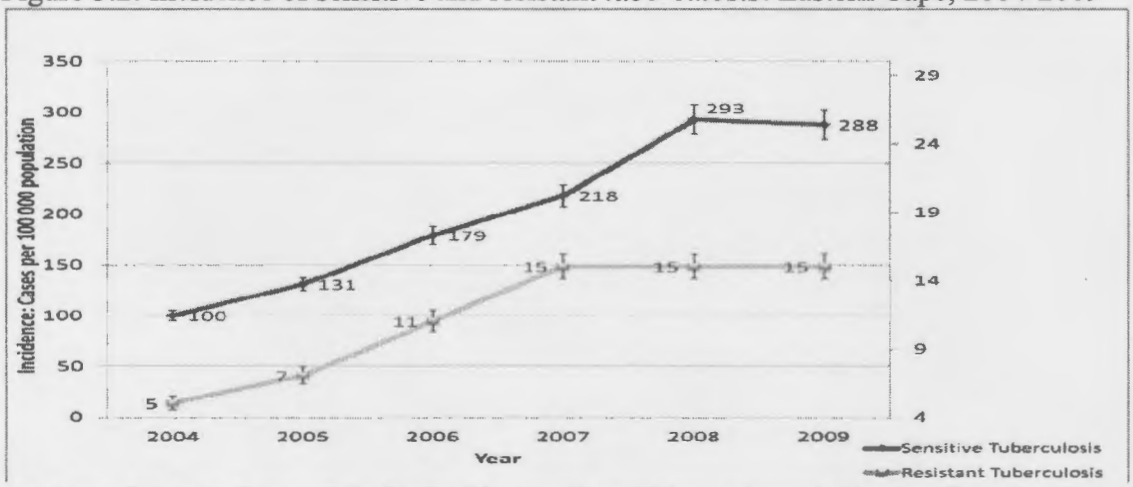


Figure 3.1 shows that the incidence of *tuberculosis* increased from 105 cases per 100 000 population in 2004 to 303 cases per 100 000 population in 2009. The test for linear temporal trends indicates highly statistically significant increase in all *tuberculosis* from 2004 to 2009 ($p<0.001$).

Figure 3.2: Incidence of sensitive and resistant *tuberculosis*: Eastern Cape, 2004-2009



As seen above in figure 3.2, the incidence of sensitive *tuberculosis* significantly increased from 100 cases per 100 000 population in 2004 to 293 cases per 100 000 population in 2008 ($p<0.001$). This was followed by a slight decrease in incidence to 288 cases per 100 000 population in 2009. Resistant *tuberculosis* incidence significantly increased from 5 cases per 100 000 population in 2004 to 15 cases per 100 000 population in 2007, 2008 and 2009 ($p<0.001$).

Figure 3.3: Incidence of MDR and XDR *tuberculosis*: Eastern Cape, 2004-2009

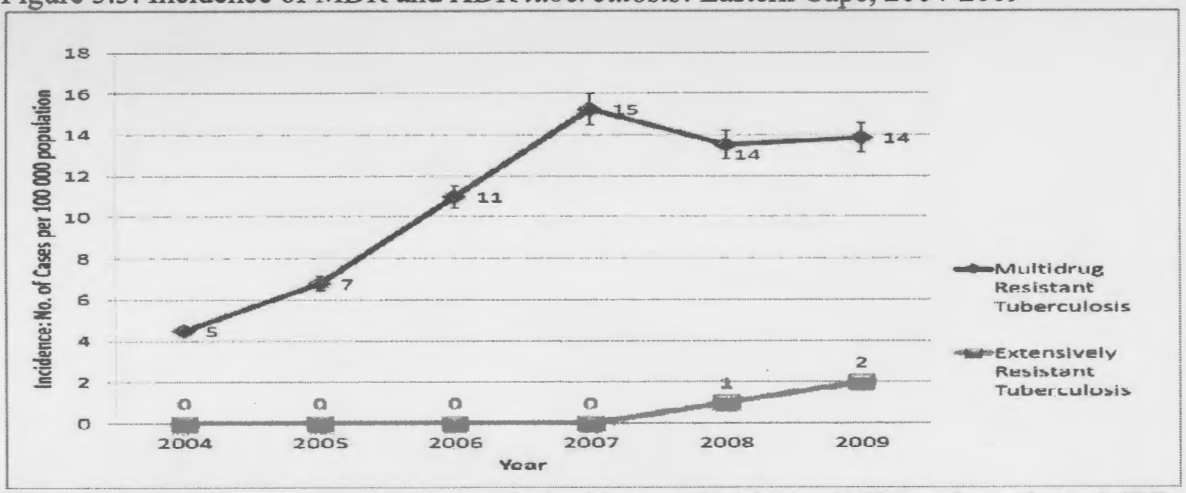


Figure 3.3 presents the annual changes in incidence of MDR and XDR *tuberculosis*. MDR *tuberculosis* incidence rose from 5 cases per 100 000 population in 2004 to a peak of 15 cases per 100 000 population in 2007, which was followed by a slight decrease to 14 cases per 100 000 population in 2008 and 2009. The incidence of XDR *tuberculosis* increased from 1 case per 100 000 population in 2008 to 2 cases per 100 000 population in 2009. Both these tests were found to be highly statistically significant ($p<0.001$).

3.1.2.2 Distribution of tuberculosis in Eastern Cape districts

This section describes the differences in *tuberculosis* distribution by district in the Eastern Cape from 2004 to 2009.The overall geographic distribution by district is shown first followed by a further disaggregation by place and time.

Figure 3.4 Incidence of *tuberculosis* by district: Eastern Cape, 2004-2009

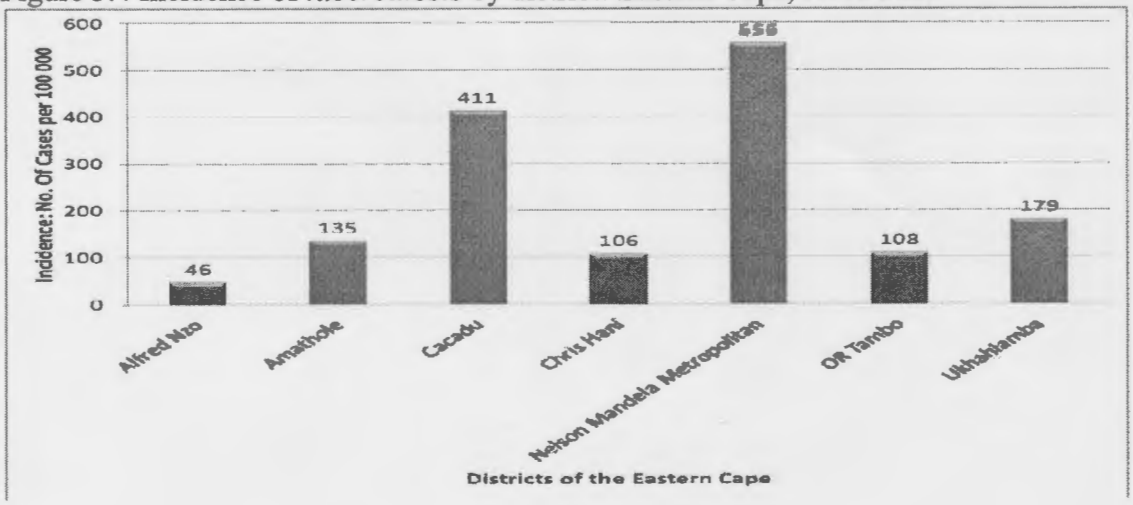
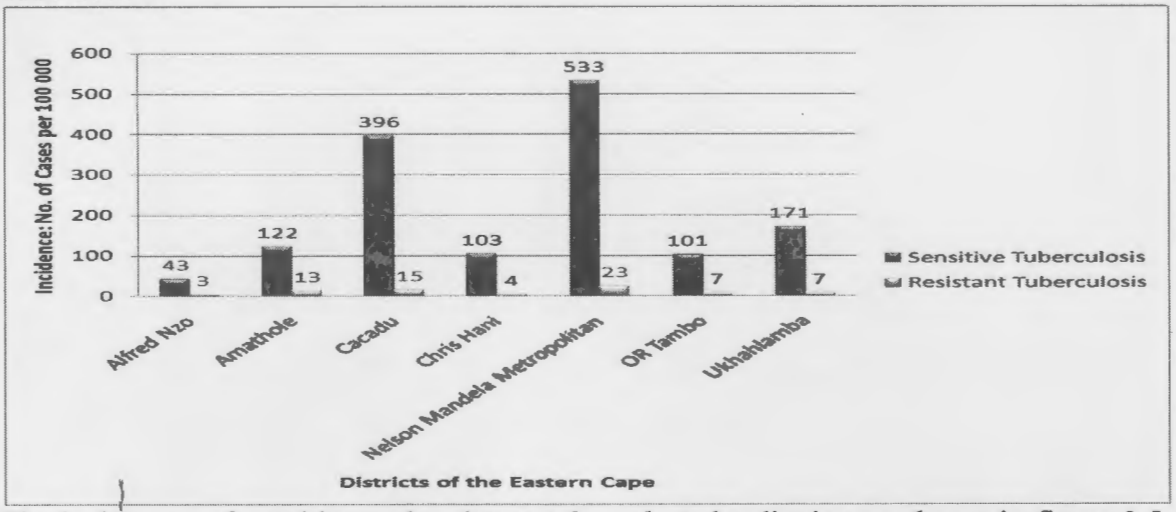


Figure 3.4 shows the distribution of *tuberculosis* in districts of the Eastern Cape for the overall study period. The Nelson Mandela Metropolitan had the highest incidence at 556 cases per 100 000 population followed by Cacadu with 411 cases per 100 000 population.

Figure 3.5: Incidence of sensitive and resistant *tuberculosis* by district: Eastern Cape, 2004-2009



The incidences of sensitive and resistant *tuberculosis* by district are shown in figure 3.5. The Nelson Mandela Metropolitan had the highest incidence of both sensitive and resistant *tuberculosis* at 533 and 23 cases per 100 000 population respectively followed by Cacadu with 396 cases per 100 000 population of sensitive tuberculosis and 15 cases per 100 000 population of resistant *tuberculosis*.

Figure 3.6: Incidence of MDR and XDR *tuberculosis* by district: Eastern Cape, 2004-2009

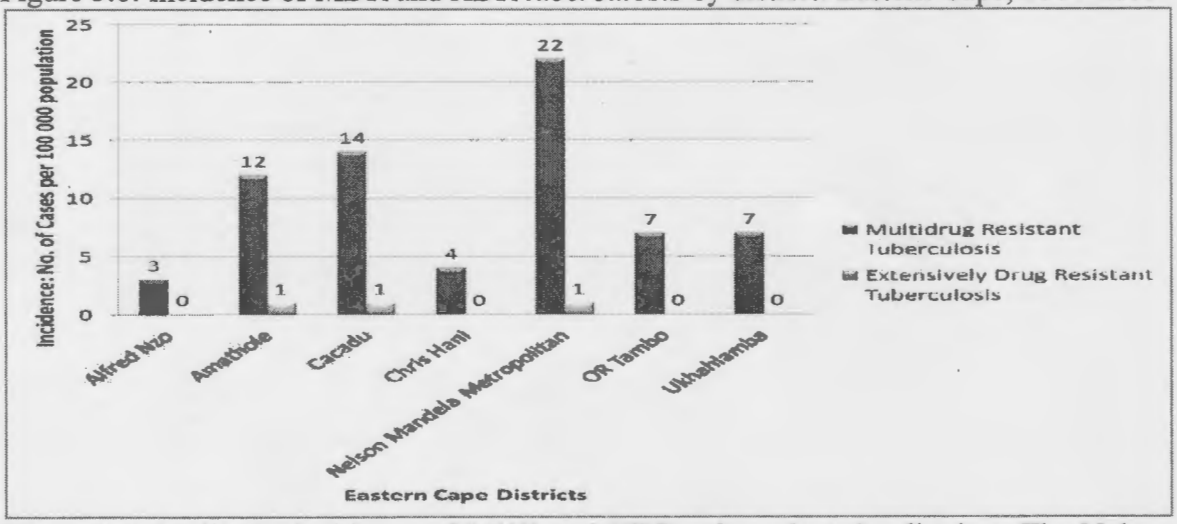
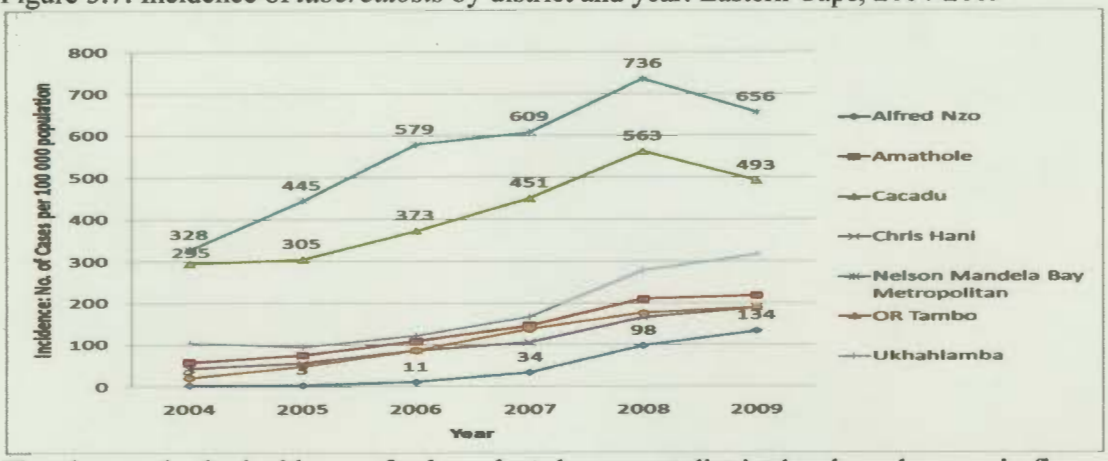


Figure 3.6 details the incidence of MDR and XDR *tuberculosis* by districts. The Nelson Mandela Metropolitan had the highest incidence of MDR *tuberculosis* with 22 cases per

100 000 population followed by Cacadu with 14 cases per 100 000 population. The incidence of XDR *tuberculosis* was 1 case per 100 000 population in the Nelson Mandela Metropolitan, Cacadu and Amathole districts. XDR *tuberculosis* was however not observed in other districts over the study period.

Figure 3.7: Incidence of *tuberculosis* by district and year: Eastern Cape, 2004-2009



The changes in the incidence of *tuberculosis* by year at district level can be seen in figure 3.7. Despite there being considerable differences between the incidences of various districts, a general upward trend in *tuberculosis* incidence occurred in all districts. In figure 3.7 it is clear that the Nelson Mandela Metropolitan had the highest incidence of *tuberculosis* consistently through 2004 to 2009 followed by the Cacadu district. These two districts' *tuberculosis* incidences were significantly higher than the other districts' incidences throughout the study period. Alfred Nzo district consistently had the lowest *tuberculosis* incidence from 2004 to 2009.

Figure 3.8: Incidence of resistant tuberculosis by year at the district level: Eastern Cape, 2004-2009

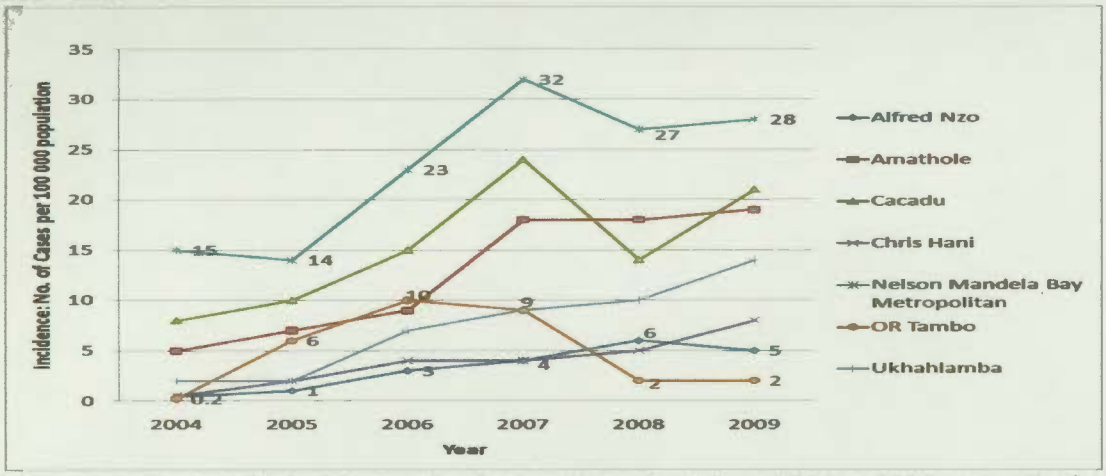


Figure 3.8 presents the incidence of resistant *tuberculosis* from 2004 to 2009 at the district level. The incidence of resistant *tuberculosis* generally increased for all districts over the study period except for the OR Tambo district which initially increased from 0 to 10 cases per 100 000 population in 2006 then decreased to 2 cases per 100 000 population in 2008 and 2009. The Nelson Mandela Metropolitan had the highest incidence throughout the time period, rising from 15 cases per 100 000 population in 2004 to 28 cases per 100 000 population in 2009. This was followed by Cacadu and Amathole districts respectively.

3.1.3 Laboratory tuberculosis testing trends

This section details the Eastern Cape laboratories' performance and diagnosing test patterns, regardless of whether the tests had a positive or negative *tuberculosis* result, from 2004 to 2009. The laboratory load and performance are described first for the entire province according to the total numbers of tests done over the years and the average times taken for each test to be conducted. Secondly these same parameters are further disaggregated to the NHLS branch level to characterise performance at a lower level.

Figure 3.9: Overall laboratory microscopy loads over time: Eastern Cape, 2004-2009

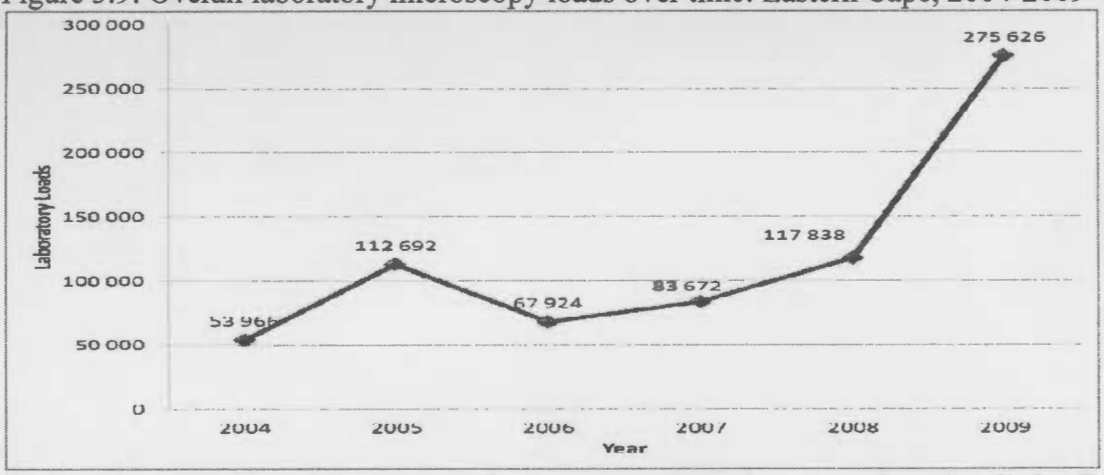
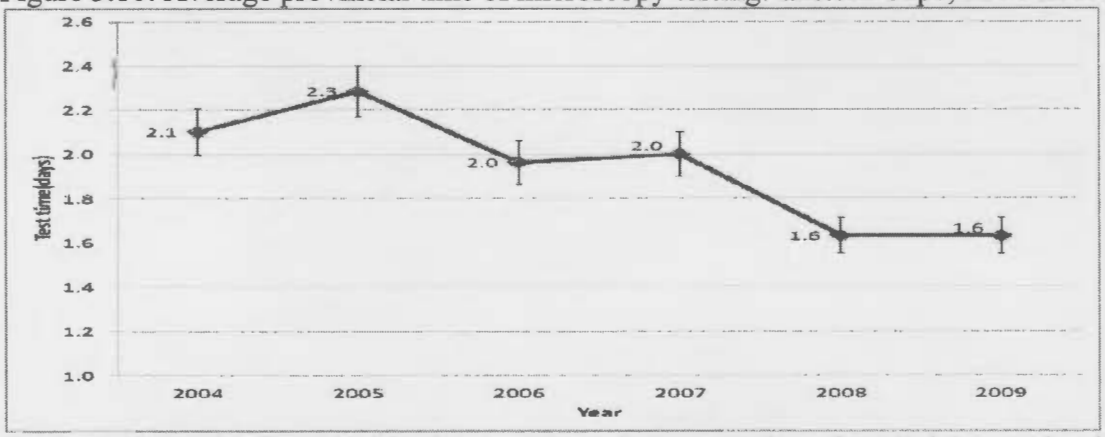


Figure 3.9 shows the total number of microscopy tests that were done in all the Eastern Cape laboratories from 2004 to 2009. The microscopy test load increased from 53 966 tests in 2004 to 275 626 tests in 2009, a five-fold increase in the space of six years.

Figure 3.10: Average provincial time of microscopy testing: Eastern Cape, 2004-2009



As seen above in figure 3.10, the average microscopy time for all laboratories in the Eastern Cape was first just above or at 2 days in 2004 and 2007, then significantly below this in 2008 and 2009 versus earlier years.

Figure 3.11: Overall laboratory culture loads over time: Eastern Cape, 2004-2009

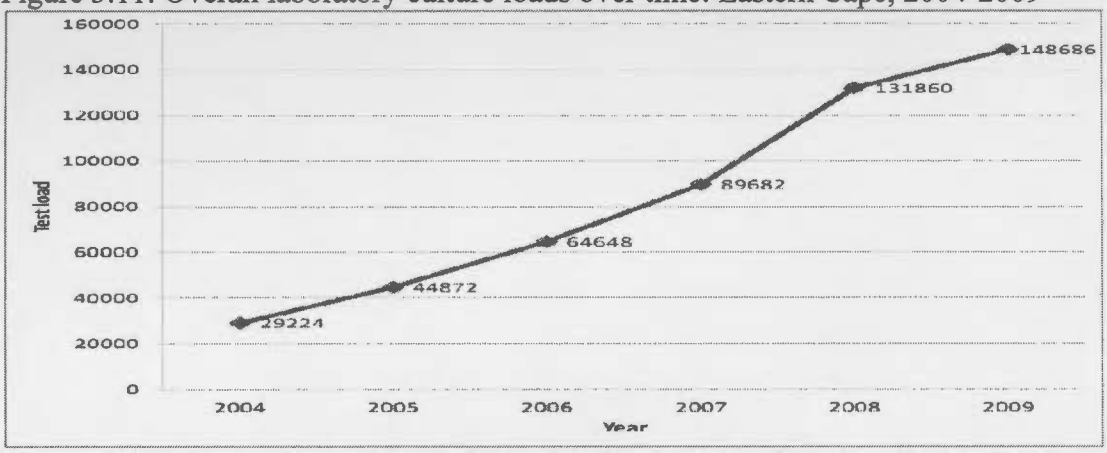
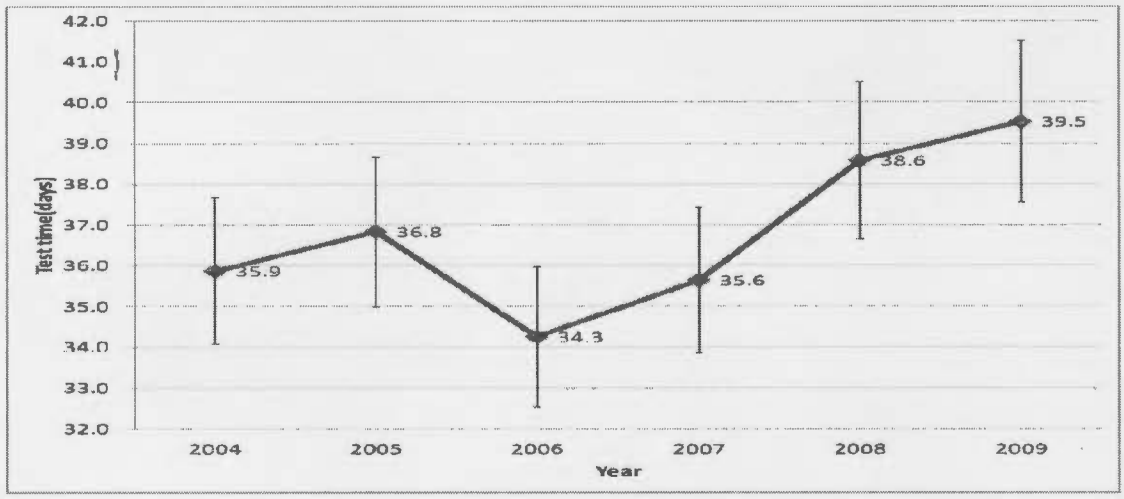


Figure 3.11 shows the total number of culture tests done in all the Eastern Cape laboratories from 2004 to 2009. From the graph above it can be seen that the culture test load increased five-fold, from 29 224 cultures in 2004 to 148 686 cultures in 2009.

Figure 3.12: Average provincial time taken to complete culture testing: Eastern Cape, 2004-2009



As seen above in figure 3.12, there was a general upward trend or increase in the average time taken to complete culture testing for all laboratories in the Eastern Cape. The time taken first increased slightly from 35.9 days to 36.8 days in 2005 then decreased to 34.3 days in 2006 and finally increased continuously to 39.5 days in 2009.

Figure 3.13: Overall laboratory polymerase chain reaction (PCR) loads over time: Eastern Cape, 2004-2009

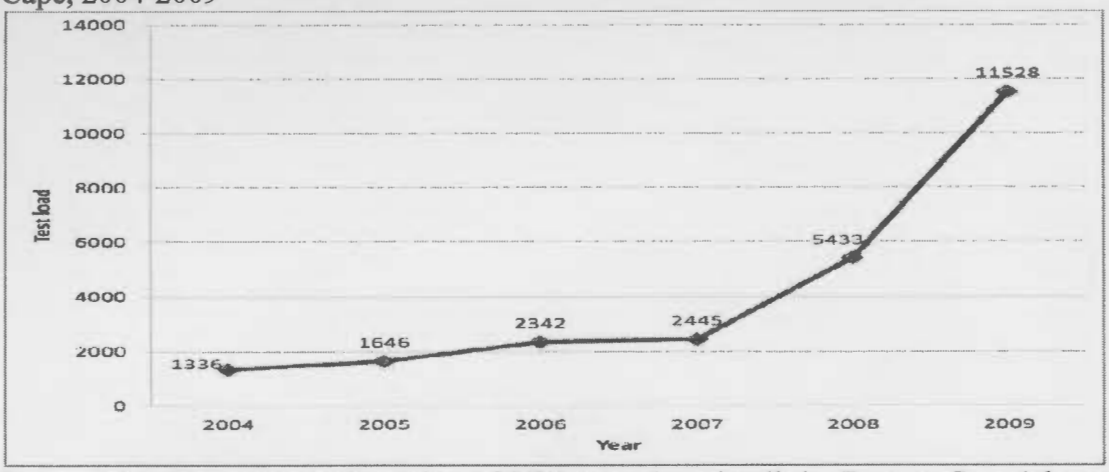
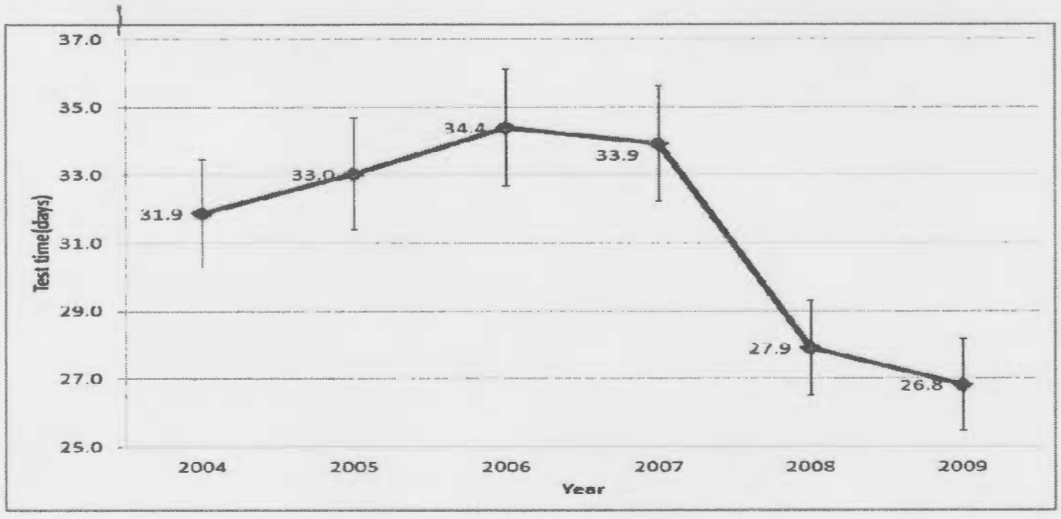


Figure 3.13 shows the total number of PCR tests done in all the Eastern Cape laboratories from 2004 to 2009. From the graph above it can be seen that the PCR test load increased almost nine-fold, from 1 336 tests in 2004 to 11 528 in 2009.

Figure 3.14: Average provincial time taken for PCR testing: Eastern Cape, 2004-2009



As seen in figure 3.14 above there was a downward trend or decrease in the time taken for PCR testing from 2004 to 2009. The PCR testing time decreased from 31.9 days in 2004 to 26.8 in 2009.

Tuberculosis diagnosis is currently confirmed in South Africa through culture and PCR, therefore from the above plots of average provincial times for these two tests, diagnosis

confirmation by 2009 occurred at approximately 27 to 40 days on average after sputum collection.

Figure 3.15: Average susceptibility testing time for selected laboratories: Eastern Cape, 2004-2009

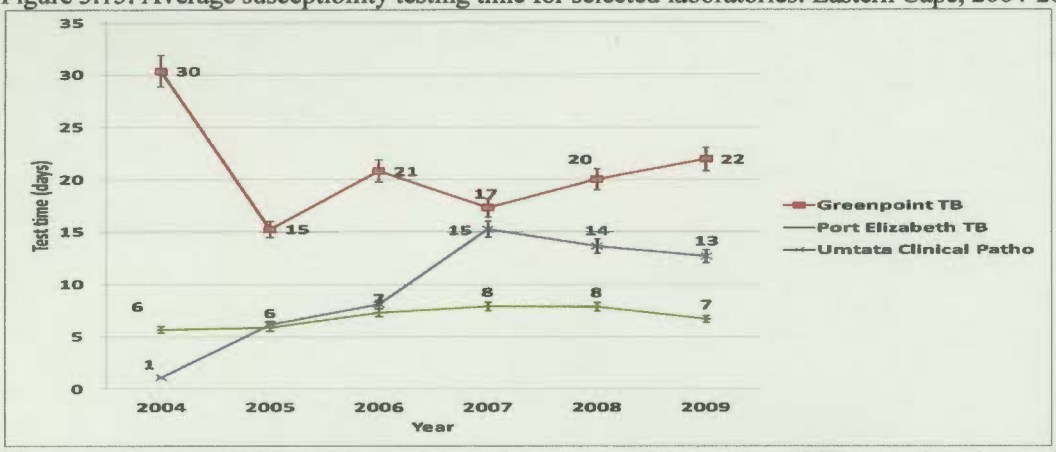


Figure 3.15 shows the average time taken to conduct susceptibility testing in various laboratories of the Eastern Cape. This analysis was conducted at the laboratory and not provincial level because very few laboratories in the province had the facilities to carry out this testing. In addition to this, the three laboratories were the only ones that had data for more than half of the study period. The average time taken for susceptibility testing to be conducted varied between the laboratories. For some laboratories like Umtata clinical pathology the average time increased over the study period, but for Greenpoint laboratory the average time decreased. Port Elizabeth TB laboratory maintained a fairly constant average time throughout the study period. It is important to note however that later in the study period the susceptibility testing process comprised more tests. In 2004 the process comprised seven tests, decreased to four tests in 2005, increased to eight tests in 2006 and then finally involved 11 tests from 2007 onwards. This variation in the process would explain the dip in the average time from 2004 to 2005 for Greenpoint TB laboratory and would also explain the stable average times seen from 2007 to 2009 in all three laboratories.

Figure 3.16: Microscopy load by laboratory branches: Eastern Cape, 2004-2009

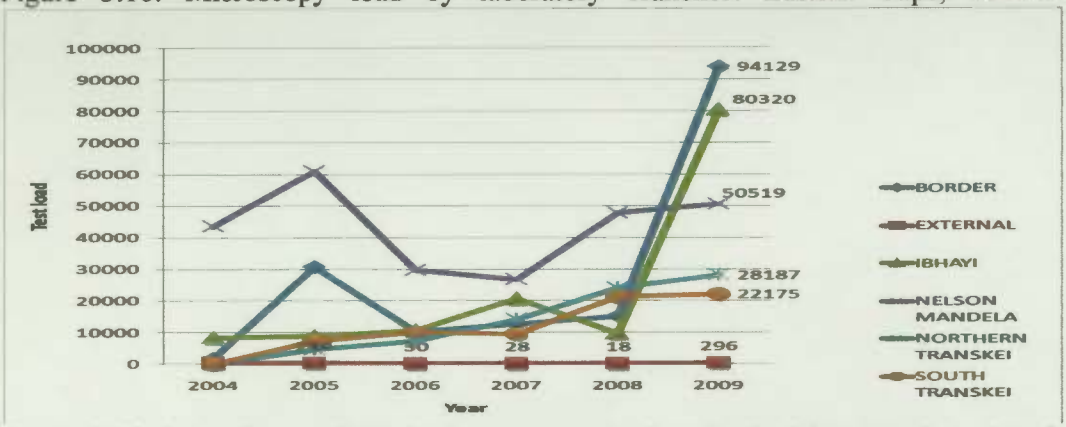
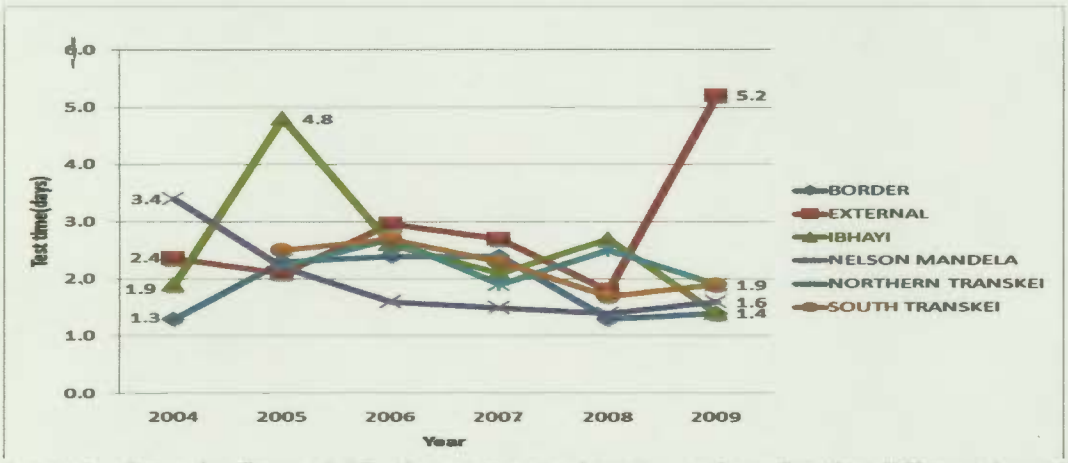


Figure 3.16 shows the number of microscopy tests done in the laboratory branches from 2004 to 2009. From the graph above it can be seen that the microscopy load generally increased throughout the years for all the branches, but in 2009 the Border branch processed the most specimens followed by Ibhayi and the Nelson Mandela branch.

Figure 3.17: Average time of microscopy testing by laboratory branch: Eastern Cape, 2004-2009



As seen above in figure 3.17, the average microscopy time for the different branches fluctuated over time. All the branches generally decreased their average microscopy time from 2004 to 2009 except for the External branch which more than doubled its average microscopy time from 2.4 days in 2004 to 5.2 days in 2009.

3.2 INFERENTIAL ANALYSIS RESULTS

3.2.1 Tuberculosis spatial analysis

This section details the spatial statistical analyses conducted to establish where the significant clusters of excess *tuberculosis* incidence occurred in the Eastern Cape. Purely spatial analysis for the entire study period is first described followed by space-time analysis where smaller annual time frames were analyzed. Results of clustering analysis are given at sub-district level.

It is important to note that all cluster results consist of four components- the observed number of cases, the expected number of cases if cases were evenly distributed in all sub-districts throughout the province, a relative risk (RR) that ratios the observed and expected number of cases and the significance (p-value) of the clustering. In all instances the null hypothesis is that all sub-districts in the province have an even distribution of cases (i.e. the expected number of cases equals the observed number of cases) and therefore no difference in underlying risk between sub-districts. Significant primary and secondary clusters (ordered by descending likelihood) are reported.

3.2.1.1Purely spatial analysis

This section describes the purely spatial clustering analysis results identifying sub-districts with significant *tuberculosis* excess for the aggregated study period of 2004 to 2009.

Table 3.5: Sub-districts with *tuberculosis* (any, resistant, XDR) excess in the Eastern Cape Province for 2004 to 2009 using a purely spatial clustering analysis only

Tuberculosis				
Sub-district	Observed	Expected	Relative Risk	P-value
Primary Clusters				
Cambedoo,Blue Crane, Nxuba, Nelson Mandela,Inxuba Yethemba,Sunday's River Valley, Ikwezi, Tsolwana, Makana, Kouga	47828	18036.16	4.87	<0.001
Secondary Clusters				
Qaukeni	4659	3565	1.33	<0.001
Maletswai	816	544	1.5	<0.001
Sakhisizwe	831	696	1.2	<0.001
Resistant Tuberculosis				
Sub-district	Observed	Expected	Relative Risk	P-value
Primary Clusters				
Blue Crane, Makana, Ndlambe, Sunday's River Valley, Kouga,Buffalo City, Ngqushwa, Nkonkobe, Inxuba Yethemba, Nelson Mandela	2865	1564	3.33	<0.001
Secondary Clusters				
Qaukeni	280	190	1.5	<0.001
XDR Tuberculosis				
Primary Clusters				
Sub-district	Observed	Expected	Relative Risk	P-value
Blue Crane, Makana, Ndlambe, Sunday's River Valley, Kouga,Buffalo City, Ngqushwa, Nkonkobe, Inxuba Yethemba, Nelson Mandela	166	60	61.28	<0.001

Table 3.5 shows that there was non-random clustering (excess) of *tuberculosis* in various sub-districts of the Eastern Cape based on purely spatial analysis. The most likely significant cluster (P-value< 0.001) of high *tuberculosis* comprised the sub-districts of Cambedoo, Blue Crane, Nxuba, Nelson Mandela, Inxuba Yethemba, Sunday's River Valley, Ikwezi, Tsolwana, Makana and Kouga. This primary cluster had 47 828 *tuberculosis* observed cases which was 2.7 times more than the expected 18 036 cases and a relative risk of 4.87. All these ten sub-districts are located in the western part of the

province relatively close to each other (Figure 3.18). Three significant secondary clusters (P-value<0.001) of *tuberculosis* excess were identified in the northern parts of the province, namely Qaukeni (4 659 cases observed, 3 565 expected, RR=1.33), Maletswai (816 cases observed, 544 expected, RR=1.5) and Sakhisizwe (831 cases observed, 696 expected, RR=1.2).

The statistically significant most likely cluster (P-value<0.001) of resistant *tuberculosis* was in the south-western part of the province comprising Blue Crane, Makana, Ndlambe, Sunday's River Valley, Kouga, Buffalo City, Ngqushwa, Nkonkobe, Inxuba Yethemba sub-districts and the Nelson Mandela Metropolitan. The cluster had 2 865 resistant *tuberculosis* cases (1 564 cases expected) and a high relative risk of 3.33, indicating that the underlying risk of resistant *tuberculosis* in this cluster was 3.33 times greater than that of the baseline rate. Qaukeni district was the only significant secondary cluster (P-value<0.001) of excess drug resistant *tuberculosis* (280 cases observed, 190 expected, RR=1.5).

Finally the most likely significant cluster (P-value<0.001) of XDR-*tuberculosis* excess comprised the south-western sub-districts of Blue Crane, Makana, Ndlambe, Sunday's River Valley, Kouga, Buffalo City, Ngqushwa, Nkonkobe, Inxuba Yethemba and the Nelson Mandela Metropolitan. The cluster had 166 resistant *tuberculosis* observed cases (60 expected cases) and a very high relative risk of 61.28.

To graphically represent the purely spatial clustering results, three maps follow on depicting the primary and secondary clusters of *tuberculosis* (figure3.18), resistant *tuberculosis* (figure 3.19) and XDR-*tuberculosis* (figure3.20) discussed above.

Figure 3.18: Clusters of *tuberculosis* cases using a purely spatial clustering analysis scanning for high *tuberculosis* incidence, Eastern Cape, 2004-2009

Figure 3.19: Clusters of resistant *tuberculosis* cases using a purely spatial clustering analysis scanning for high resistant *tuberculosis* incidence, Eastern Cape, 2004-2009

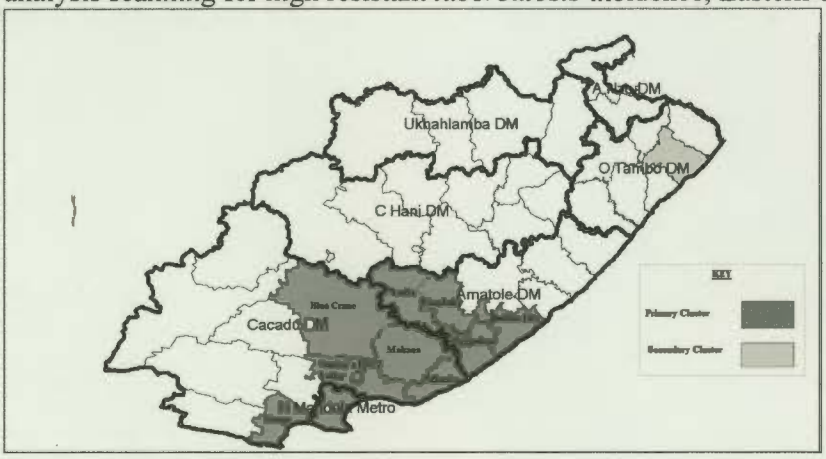
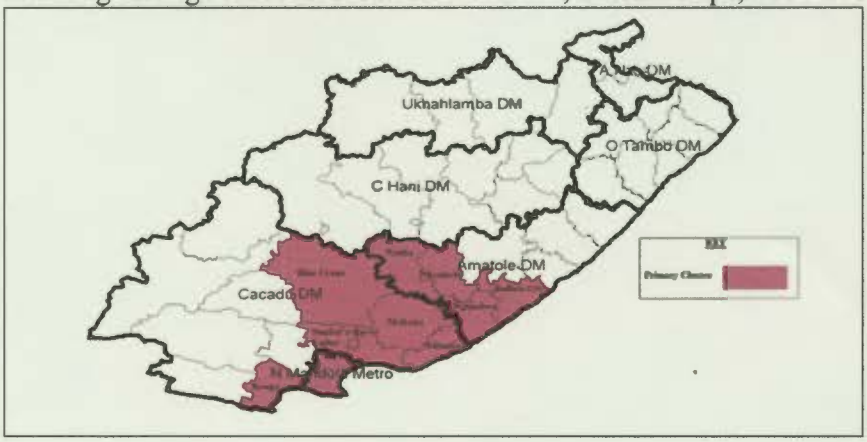


Figure 3.20: Cluster of XDR *tuberculosis* cases using a purely spatial clustering analysis scanning for high XDR *tuberculosis* incidence, Eastern Cape, 2004-2009



3.2.1.2 Spatio-temporal analysis

This section describes the Spatio-temporal clustering analysis results where the sub-districts with non-random clustering were established incorporating a temporal (yearly) element.

Table 3.6: Sub-districts with *tuberculosis* (any, resistant, XDR) excess in the Eastern Cape Province using space-time spatial analysis for high incidence of period 2004-2009

Tuberculosis					
Primary Cluster					
Year	Sub-district	Observed	Expected	Relative Risk	P-value
2007-2009	Cambedoo, Blue Crane, Ikwezi,Makana, Sunday's River Valley, Kouga, Inxuba Yethemba, Nxuba, Tsolwana, Nelson Mandela	28134	9028	4.19	<0.001
Secondary Clusters					
2007-2009	Qaukeni	3761	1822	2.11	<0.001
2007-2009	Sakhisizwe	478	334	1.44	<0.001
2007-2009	Elundini	976	779	1.26	<0.001
2008-2009	Maletswai	646	189	3.43	<0.001
2008-2009	Buffalo City	4083	3123	1.32	<0.001
2009	Mbashe	820	565	1.46	<0.001
Resistant Tuberculosis					
Year	Sub-district	Observed	Expected	Relative Risk	P-value
Primary Cluster					
2007-2009	Blue Crane, Makana, Ndlambe, Sunday's River Valley, Kouga, Buffalo City, Ngqushwa, Nkonkobe, Inxuba Yethemba, Nelson Mandela	1893	785	3.46	<0.001
Secondary Clusters					
2006-2007	Mbashe, King Sabata Dalindyebo	224	161	1.42	0.0023
2006-2008	Qaukeni	186	96	1.98	<0.001
XDR Tuberculosis					
Year	Sub-district	Observed	Expected	Relative Risk	P-value
Primary Cluster					
2008-2009	Blue Crane, Makana, Ndlambe, Sunday's River Valley, Kouga, Buffalo City, Ngqushwa, Nkonkobe, Inxuba Yethemba, Nelson Mandela	166	20	248.92	<0.001

The space-time spatial clustering analysis is summarised in tabular form in table 3.6. The space-time spatial analysis identified areas and time spans of excess *tuberculosis*. The primary significant cluster (P-value<0.001) of tuberculosis excess cases occurred later in the study (viz. 2007 to 2009) and consisted of the Cambedoo, Blue Crane, Ikwezi, Makana, Sunday's River Valley, Kouga, Inxuba Yethemba, Nxuba, Tsolwana sub-districts and the Nelson Mandela Metropolitan. This cluster had 28 314 observed *tuberculosis* cases

(expected-9 028) and a high relative risk of 4.19. All eleven sub-districts are in close proximity to each other and are situated in the west-central area of the province. Three statistically significant secondary clusters (P-value<0.001) were identified for the period of 2007 to 2009, namely Qaukeni (3 761 *tuberculosis* cases observed, 1 822 expected, RR=2.11), Sakhisizwe (478 *tuberculosis* cases observed, 334 expected, RR=1.44) and Elundini (976 *tuberculosis* cases observed, 779 expected, RR=1.26). For the period of 2008 to 2009, two statistically significant secondary clusters (P-value<0.001) were identified: Maletswai (646 *tuberculosis* cases observed, 189 expected and a high relative risk of 3.43) and Buffalo City (4 083 cases observed, 3 123 expected, RR=1.32). 2009 one significant secondary cluster (P-value<0.001) was identified in Mbashe sub-district (820 cases observed, 565 expected, RR=1.46).

The statistically significant primary cluster (P-value<0.001) for resistant *tuberculosis* occurred from 2007 to 2009 and comprised the Blue Crane, Makana, Ndlambe, Sunday's River Valley, Kouga, Nelson Mandela, Buffalo City, Ngqushwa, Nkonkobe and Inxuba Yethemba sub-districts, situated in the south-western part of the province. This significant cluster had 1 893 resistant *tuberculosis* cases while only 785 cases were expected and had a high relative risk of 3.46. A statistically significant secondary cluster (P-value=0.002) occurred during 2006 and 2007, encompassed Mbashe and King Sabata Dalinyebo sub-districts with 224 observed resistant *tuberculosis* cases, 161 expected cases and a relative risk of 1.42. The other secondary significant cluster occurred from 2008 to 2009 in Qaukeni sub-district and had 182 observed resistant *tuberculosis* cases, 96 expected cases and a relative risk of 1.98.

For XDR *tuberculosis* only one significant primary cluster (P-value<0.001) occurred from 2008 to 2009 in the south-western part of the province. This cluster consisted of the sub-districts of Blue Crane, Makana, Ndlambe, Sunday's River Valley, Kouga, Nelson Mandela, Buffalo City, Ngqushwa, Nkonkobe and Inxuba Yethemba. This cluster comprised of 166 observed XDR *tuberculosis* cases, 20 expected cases and a very large relative risk of 248.92.

To graphically represent the space-time clustering analysis findings, three maps follow below depicting the results just discussed.

Figure 3.21: Sub-district clusters of *tuberculosis* cases using a space-time clustering analysis scanning for high incidence only, Eastern Cape, 2004-2009

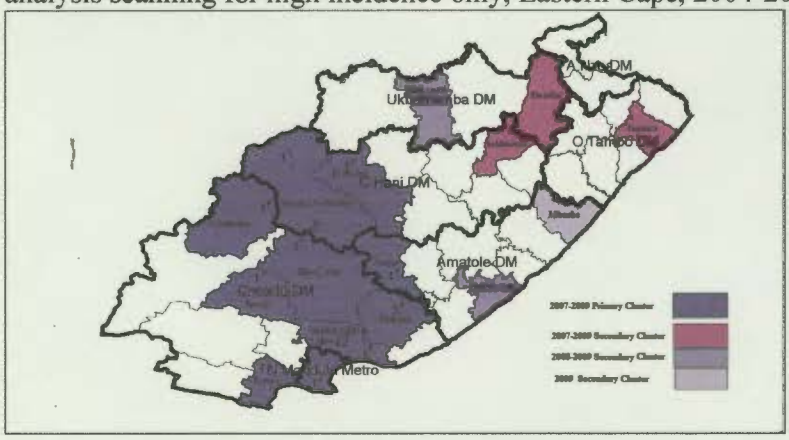


Figure 3.22: Sub-district clusters of resistant *tuberculosis* cases using a space-time clustering analysis scanning for high incidence only, Eastern Cape, 2004-2009

Figure 3.23: Sub-district clusters of XDR *tuberculosis* cases using a space-time clustering analysis scanning for high incidence only, Eastern Cape, 2004-2009

3.2.2 Regression Analysis

This section describes the logistic regression analyses conducted to establish the factors independently associated with *tuberculosis*. Three sets of analyses are presented, for the three outcomes namely pulmonary *tuberculosis*, resistant *tuberculosis* and XDR *tuberculosis*. For each outcome, univariate logistic regressions were first applied to see which factors were associated with the outcome and then all factors found to be significantly associated ($p<0.1$) at univariate level were placed in the multivariable logistic regression analysis to determine which factors were independent predictors of the outcome when adjusted for the influence of one another.

3.2.2.1 Factors associated with pulmonary tuberculosis

The first model looked at the factors that were associated with any *tuberculosis* as the outcome.

Table 3.7: Univariate and multivariable logistic regression analysis showing factors associated with any *tuberculosis*, Eastern Cape, 2004-2009

VARIABLE	UNIVARIATE			MULTIVARIATE		
	OR	(95% CI)	P-Value	OR	(95% CI)	P-Value
Gender						
Male	1			1		
Female	0.692	0.683-0.702	<0.001	0.668	0.658-0.678	<0.001
Age Categorised						
20-34 yr olds	1			1		
0-4 yr olds(2)	0.719	0.676-0.765	<0.001	0.727	0.681-0.778	<0.001
5-9 yr olds(3)	0.182	0.166-0.199	<0.001	0.166	0.150-0.183	<0.001
10-14 yr olds(4)	0.148	0.139-0.159	<0.001	0.140	0.130-0.151	<0.001
15-19 yr olds(5)	0.483	0.467-0.501	<0.001	0.464	0.446-0.483	<0.001
35-44 yr olds(6)	1.014	0.997-1.032	0.104	0.980	0.962-0.999	0.041
45-54 yr olds(7)	0.730	0.715-0.745	<0.001	0.696	0.680-0.712	<0.001
55+ yr olds(8)	0.375	0.366-0.383	<0.001	0.374	0.365-0.383	<0.001
Laboratory Inefficiency	1.213	1.197-1.230	<0.001	1.221	1.205-1.237	<0.001

As seen in table 3.7, univariate logistic regression found that the gender ($p<0.001$), age category ($p<0.001$) and laboratory inefficiency ($p<0.001$) were significantly associated with any *tuberculosis*. All these variables were therefore included in the multivariable logistic model. All three variables remained significantly associated with the outcome following multivariable adjustment. The odds of an individual having any *tuberculosis*

decreased by 34% for female individuals compared to male individuals. The highest risk age group was the 20-34 year olds followed by the 35-44 years age group. The odds of an individual having any *tuberculosis* was decreased in all age groups especially 5-9 years old and 10-14 years old compared to the 20 to 34 year old age group.

The evaluation of laboratory efficiency was analysed through using microscopy efficiency as a proxy measure which was established through calculating the province's average microscopy test time for each year and then dividing each laboratory's annual average microscopy test time by the province's annual average test time. Laboratory efficiency therefore had the value of one if it was on the provincial average. Laboratories that had a value less than one were more efficient (faster) than the province's annual average microscopy test time and laboratories that had a value greater than one were less efficient (slower) than the province's annual average microscopy test time. The odds of an individual having any *tuberculosis* was increased by 22% for a day's increase in laboratory inefficiency (or delay) following multivariable adjustment.

3.2.2.2 Factors associated with resistant tuberculosis

The next model sought to establish the factors that were associated with resistant *tuberculosis* i.e. both MDR and XDR-TB.

Table 3.8: Univariate and multivariable logistic regression analysis showing factors associated with resistant *tuberculosis*, Eastern Cape, 2004-2009

VARIABLE	UNIVARIATE			MULTIVARIATE		
	OR	(95% CI)	P-Value	OR	(95% CI)	P-Value
Gender						
Male	1			1		
Female	0.781	0.736-0.828	<0.001	0.772	0.722-0.824	<0.001
Age Categorised						
20-34 yr olds	1			1		
0-4 yr olds(2)	0.433	0.309-0.609	<0.001	0.456	0.317-0.654	<0.001
5-9 yr olds(3)	0.235	0.166-0.334	<0.001	0.202	0.135-0.303	<0.001
10-14 yr olds(4)	0.132	0.096-0.182	<0.001	0.140	0.100-0.195	<0.001
15-19 yr olds(5)	0.495	0.425-0.576	<0.001	0.508	0.431-0.599	<0.001
35-44 yr olds(6)	1.034	0.961-1.112	0.368	1.000	0.922-1.085	0.987
45-54 yr olds(7)	0.742	0.680-0.810	<0.001	0.712	0.646-0.784	<0.001
55+ yr olds(8)	0.311	0.280-0.346	<0.001	0.311	0.278-0.349	<0.001
Laboratory Inefficiency	1.517	1.448-1.590	<0.001	1.527	1.457-1.600	<0.001

Table 3.8 summarises the results from the univariate and multivariable logistic regression analyses for resistant *tuberculosis*. Based on the univariate logistic regressions gender ($p<0.001$), age category ($p<0.001$) and laboratory inefficiency ($p<0.001$) were significantly associated with resistant *tuberculosis*. After multivariable logistic regression gender ($p<0.001$), age category ($p<0.001$) and the laboratory's inefficiency ($p<0.001$) remained significant and were therefore independently associated with resistant *tuberculosis*.

The odds of an individual having resistant *tuberculosis* decreased by 23% for female individuals compared to male individuals. The 20-34 and 35-44 year olds were the highest risk age groups for resistant *tuberculosis*. The odds of an individual having *tuberculosis* was decreased in all other age groups especially 5-9 years old and 10-14 years old compared to the 20 to 34 year old age group. The odds of an individual having resistant *tuberculosis* was increased by 52% for a day's increase in laboratory inefficiency (or delay).

3.2.2.3 Factors associated with XDR tuberculosis

The final model looked at the factors that were associated with XDR-TB.

Table 3.9: Univariate and multivariable logistic regression analysis showing factors associated with XDR *tuberculosis*, Eastern Cape, 2004-2009

VARIABLE	UNIVARIATE			MULTIVARIATE		
	OR	(95% CI)	P-Value	OR	(95% CI)	P-Value
Gender						
Male	1			1		
Female	2.020	1.488-2.743	<0.001	2.026	1.469-2.795	<0.001
Age Categorised						
20-34 yr olds	1			1		
0-4 yr olds(2)	1.438	0.518-3.994	0.486	1.462	0.517-4.131	0.474
5-9 yr olds(3)	omitted			omitted		
10-14 yr olds(4)	0.631	0.087-4.583	0.649	0.499	0.068-3.679	0.495
15-19 yr olds(5)	1.816	0.946-3.488	0.073	1.815	0.933-3.531	0.079
35-44 yr olds(6)	1.817	1.065-2.194	0.021	1.757	1.213-2.546	0.003
45-54 yr olds(7)	0.936	0.565-1.551	0.798	1.119	0.668-1.876	0.669
55+ yr olds(8)	0.424	0.209-0.858	0.017	0.317	0.155-0.651	0.002
Laboratory Inefficiency	12.615	8.926-17.830	<0.001	15.548	10.887-22.204	<0.001

As seen in table 3.9, following univariate logistic regression gender ($p<0.001$), age groups 35-44 and 55+ ($p=0.021$ and 0.017 respectively) as well as laboratory inefficiency ($p<0.001$) were significantly associated with XDR *tuberculosis*. After multivariable logistic regression gender ($p<0.001$), age groups 35-44 and 55+ ($p=0.003$ and 0.002 respectively) as well as laboratory's inefficiency ($p<0.001$) remained significant and were therefore independently associated with XDR *tuberculosis*.

The odds of an individual having XDR *tuberculosis* remained double for female individuals compared to male individuals following multivariable adjustment. The 35-44 and 15-19 year olds were the highest risk age groups for XDR *tuberculosis*. The odds of an individual having XDR *tuberculosis* was significantly increased by 81% for 15-19 year olds, 75% for 35-44 year olds and significantly decreased by 69% for individuals older than 55 years compared to 20 - 34 year old individuals. The odds of an individual having XDR *tuberculosis* was increased 15.55 fold for each day's increase in laboratory inefficiency (or delay).

CHAPTER FOUR

In this chapter the findings of the study are discussed with reference to relevant and available literature. Limitations of the study are then also discussed.

4.1 DISCUSSION

The first objective of the study was to determine temporal patterns and changes in the incidence proportion of sensitive and resistant *tuberculosis* in the Eastern Cape from 2004 to 2009. Findings show any *tuberculosis*, sensitive *tuberculosis* and resistant *tuberculosis* increased and had statistically significant increasing linear trends across the years. *Tuberculosis* was initially predicted to increase globally by Dolin, Raviglione and Kochi from 7.5 million cases in 1990 to 8.8 million cases in 1995 and 10.2 million cases in 2000 (45). The findings of this study correlate with two studies: The first was conducted in a peri-urban township near Cape Town in 2005 and found that the *tuberculosis* notification rate increased by 2.5 fold from 1996 to 2004 (46). The second study done by Davies found that *tuberculosis* was increasing globally and was expected to continue increasing for longer than ten years further (47). The main reasons for this continued increase were cited as being the spread of HIV (immunocompromised and thus more susceptible), demographic changes and increasing poverty levels that promote the spread of *tuberculosis* through crowding and malnutrition (47). A plateau was identified in any, sensitive and resistant *tuberculosis* from 2008 to 2009. Such a phenomenon has been explained in previous literature to be due to mass vaccination, drug regimens and new diagnostics resulting in reductions in incidence (48). The explanation is the most likely for this study due to the plateau following the roll out of the complete 'Stop TB' campaign in the Eastern Cape which included case finding, vaccinations and *tuberculosis* treatment by government.

The second objective to determine changes in laboratory load and laboratory efficiency in the Eastern Cape districts revealed laboratories that were experiencing high workloads and changing inefficiency over time. The laboratory load of the Eastern Cape from 2004 to 2009 for microscopy, culture and PCR tests increased reflecting the increasing number of *tuberculosis* 'suspects' over the study period. Similar findings were concluded in a Malawian study carried out in 2001, where the main cause of increasing numbers of suspects was deemed to be the concurrent HIV epidemic (49). During the same interval, the Eastern Cape's laboratories' efficiency of microscopy and PCR testing efficiency increased by five days, i.e. the time taken to obtain results from these diagnostic tests decreased. The microscopy efficiency progress is due to South African laboratories changing from conventional direct microscopy to fluorescence microscopy and more laboratories being equipped to perform successful microscopy with a resultant improvement in speed (50, 51). PCR testing time decreased due to a new PCR facility being established in Port Elizabeth (51). The efficiency of culture testing decreased over the study period. This occurred because the increase in the load of culture tests was too excessive to be serviced by the few laboratories able to do culture testing in the area. In addition to this of the laboratories that were able to do culture testing only three in the entire region use the rapid Mycobacteria Growth Indicator Tube (MGIT) liquid media for culture (52). This therefore deems the process to be extremely slow in laboratories without this facility. Susceptibility testing was analysed at laboratory level. Umtata Clinical Pathology laboratory's test time increased presumably due to an inability to manage the increased load. Port Elizabeth TB's laboratory test time remained fairly constant and Greenpoint TB's test time decreased despite increased loads. The three laboratories however have varying resources and infrastructure with Greenpoint TB laboratory having the most resources and best infrastructure followed by Port Elizabeth TB and finally

Umtata Clinical Pathology laboratory (52). The discrepancies in infrastructure could be the rationale behind differences in the test times.

The third objective was to determine the distribution and changes in the distribution of sensitive and resistant *tuberculosis* in districts of the Eastern Cape overall and by year. Variations in the increase of incidence rates were seen as districts containing or near large cities (Nelson Mandela Metropolitan, Cacadu, UKhahlamba and Amathole) had much higher incidence rates than those further away from cities and consisting more of rural areas. These findings are consistent with the world health organisation's bulletin of 2000, focusing on the environment and health, which suggested that characteristics of urban poverty were important facilitators of infectious disease transmission (53).

Characteristics of urban poverty are caused by rapid urbanization as people seek better employment, lifestyle and education (54). Urban health hazards associated with health risk include poverty, over-crowding, poor housing, air pollution, contaminated drinking water, lack of sunshine, dampness, poor sanitation and waste disposal, malnutrition, alcohol abuse, tobacco smoking (55). These hazards collectively lower the defence mechanisms of the body making it unable to conquer invading micro-organisms and become infected (56). The lower classes of society are found in poor settings. Low socio-economic status has been linked to a low understanding of *tuberculosis*, high possibility of infection and spread with resultant poor and/or deferred seeking out of health care (57). In addition to this, once patients are on treatment, poor adherence arises due to competing needs, the necessity to work in order to earn a salary and family responsibilities (58).

Over-crowding, as is found in urban townships and squatter camps, facilitates the spread of infection as the air space shared is decreased; the risks of transmission are increased, with amplified contact to M. *Tuberculosis* (57). The tin shacks that communities of squatter camps live in are the epitome of poor housing. This type of housing has poor ventilation which prolongs exposure with infectious droplet nuclei and gets wet through flooding, easily causing dampness that supports viability of the *tuberculosis* organism. Lack of sunshine results in the same effects on the bacillus as sunshine is known to dry out the organism rendering it too weak to initiate infection (57). City air is polluted with dust containing a large volume of soot from factories. This dust and smoke is deposited in the lungs and eventually lowers the capacity of the lung tissue thereby predisposing an individual to *tuberculosis* (56).

Malnutrition and poor eating habits of urban life cause the immune system to be more susceptible to pathogenic organisms. This is further exacerbated by the consumption of contaminated drinking water which introduces pathogens into the body (57). Substance (alcohol and tobacco) misuse is engaged in as a coping mechanism for impoverishment. This however lowers the body's defence mechanisms and increases the susceptibility to infection (58). It is a culmination of all these factors that have led to the higher prevalence of *tuberculosis* in districts containing or near large cities in the Eastern Cape.

Spatial clustering analysis and particularly space-time clustering analysis identified sub-districts with excess *tuberculosis* both in space and space-time. These are the areas of most concern where *tuberculosis* continues to spread at uncontrolled levels. Primary clusters were seen to involve sub-districts in proximity or within large cities which correlates with the findings that people living under poverty in urban areas are more susceptible to

acquiring *tuberculosis* (47, 53). Studies conducted in Canada and Asia cited urban areas to have higher risk due to urban poverty conditions causing higher rates of *tuberculosis* transmission and challenges to the contemporary *tuberculosis* control programmes (55, 59) . The effects of these conditions have been discussed above in detail and all play a vital role in the continued spread of *tuberculosis* in the Eastern Cape.

The fourth objective of establishing the association between available socio-demographic factors, laboratory efficiency and pulmonary *tuberculosis* incidence in the Eastern Cape showed varying strength of association across the *tuberculosis* type dependent variables. Multivariable regression confirms that *tuberculosis* is independently associated with gender, age and laboratory inefficiency. In particular the findings reveal that male sex, ages between 20 and 35 and laboratory inefficiency or delay are all significantly associated with *tuberculosis* and resistant *tuberculosis*. The association with male gender is in line with findings of a study done in 2007 by Kolappan et al (60). However the same study found that *tuberculosis* was associated with increasing age which is contrary to our findings where age groups above and below the 20-34 year old age group were at significantly lower risk of acquiring *tuberculosis*. The pattern was similar for resistant *tuberculosis*, except for the 35-44 year old age group which was also an independent predictor of resistant *tuberculosis* (60).

Our results for XDR *tuberculosis* regression reveal that female sex, 0-4 year, 15-19 year and 35-44 year age groups as well as laboratory inefficiency are all significantly associated with XDR *tuberculosis* incidence. The association with female gender is surprising as most literature has documented *tuberculosis*, resistant or not, being more common among males due to their lower treatment adherence levels (61). Various studies have however

established an association between female gender and MDR *tuberculosis* i. e. female gender being a risk factor for MDR *tuberculosis* (61-63). Reasons for the association between female sex and XDR *tuberculosis* are explained in the study by Mdivani et. al which concluded that women were the primary caregivers in homes and the healthcare system thus elevating their exposure to infectious *tuberculosis* cases (61). This is indeed true in the Eastern Cape where people often come home to die in the late stages of HIV and *tuberculosis* disease. A study performed in the Eastern Cape town of Grahamstown looking at the perceptions and knowledge towards tuberculosis treatment initiation and adherence found that though knowledge of *tuberculosis* was good, stigma within the community influenced *tuberculosis* patients not to seek healthcare and prevented adherence to treatment due to *tuberculosis* being associated with irresponsible behaviour and HIV (35). Stigma and common perceptions in the area would likely cause patients to refute their diagnosis due to fearing of stigma and this then further exacerbates the spread of infection and drug resistance (35). Eastern Cape women caring for relatives with late stages of illness may also acquire XDR *tuberculosis* through care giving rather than due to treatment default. Such female sufferers would however delay or reject diagnosis and treatment due to community perceptions and stigma.

Findings show that 0-4 year, 15-19 year and 35-44 year age groups are associated with XDR *tuberculosis*. This correlates with a few studies the first being one conducted in New York which found drug resistance associated with ages younger than 45 years old (63). Other studies have shown that persons older than 65 years were less likely to have drug resistance and that drug resistance occurrence decreased with increasing age (64, 65).

Tuberculosis, resistant *tuberculosis* and XDR *tuberculosis* are all independently associated with laboratory inefficiency. Laboratory delay in this study had an increasingly detrimental effect (higher risk) as we move from any *tuberculosis* to resistant *tuberculosis* to XDR *tuberculosis*. Laboratory diagnosis delay has been long documented to be associated with the transmission of *tuberculosis*. A review conducted by Storla et. al on delay in the diagnosis and treatment of *tuberculosis* concluded that delayed diagnosis and treatment initiation disseminates *tuberculosis* into communities (32). Demissie, Lindtjorn and Berhane in their 2002 study stated that diagnosis delay caused transmission of the disease, boosted the threat of mortality and exacerbated the disease (66). No previous studies have shown the dynamic influence of laboratory delay on various levels of *tuberculosis* resistance as found in this study. From these findings it is seen that laboratory efficiency is imperative not only to decrease *tuberculosis*, but more especially to decrease the spread of resistant *tuberculosis*.

4.2 LIMITATIONS OF THE STUDY

1. The data were from the NHLS central repository database -being routine laboratory based services data. This posed three limitations to our study namely:
    a. The data had many errors requiring extensive cleaning and reviewing.
    b. The data had no clinical information
    c. Finally the dataset was limiting in terms of the variables available for analysis because only the variables already recorded by the laboratory services could be used. Very few variables were therefore used in the multivariable model construction as those used were the only available demographic variables.
2. Selection bias: the dataset consisted of suspected *tuberculosis* cases, the majority of which presented to a health facility in the Eastern Cape. These patients may have

been quite different from people who did not go to health facilities when they were sick due to traditional beliefs, lack of funds or transport or lack of awareness.

3. Due to this being an analytical cross-sectional study the issue of temporality arises and therefore it was not possible to infer cause and effect in certain instances such as when looking at the relationship between demographic factors, laboratory efficiency and *tuberculosis* incidence proportion. As a result it was difficult to add to a causality argument due to not knowing what happened first.
4. Limitation of denominators: the population data being collected in 2001 then 2007 posed a high possibility of the population denominator data being incorrect for the other years when the census or community survey had not been conducted.

CHAPTER FIVE

This chapter contains conclusions obtained from the findings of the study. Some recommendations are then also made based on the study findings.

5.1 CONCLUSION AND RECOMMENDATIONS

The incidence of *tuberculosis* in all its forms has increased significantly in the Eastern Cape between 2004 and 2009. This shows that control measures have not been adequate to treat the infected and to curb further spread of the infection. Efforts of actively searching for new cases and treatment defaulters therefore need to be scaled up.

Further studies looking at the numbers of patients that started treatment after sputum was tested for *tuberculosis* are necessary to establish the level of primary defaulters as well as studies that follow up patients who did start treatment to establish secondary defaulters. This will assist in establishing whether control measures need to centre on new case finding or ensuring patient compliance to the treatment regimen.

Future studies need to incorporate clinical and serological information including HIV status of suspected *tuberculosis* cases to establish whether any other underlying conditions could be risk factors and responsible for the rapid propagation of the disease.

The laboratory load and corresponding *tuberculosis* suspects of the Eastern Cape increased considerably from 2004 to 2009. This has led to decreased laboratory functioning with specific impact on culture and sensitivity testing. It is recommended that all laboratories that do culture testing in the province, have their facilities upgraded to include the rapid MGIT liquid media approach for culture testing. In addition to this it is recommended that the infrastructure for the two main laboratories used for susceptibility testing in the

province viz. Port Elizabeth TB and Umtata Clinical Pathology laboratory be standardised to the Greenpoint TB laboratory's standards to ensure that they are equipped to work as efficiently and effectively as the Western Cape laboratory.

It is recommended that a similar study be conducted to establish laboratory load and efficiency for 2010 and 2011 in order to obtain the most up-to-date information and confirm ongoing trends. It would be of great value to incorporate such studies as part of the annual laboratory system assessment. The detrimental effect of laboratory inefficiency/delay to the potential spread of resistant *tuberculosis* shown in these analyses suggests that optimal functioning laboratory services are crucial in reducing the spread of *tuberculosis* and *tuberculosis* resistance. The prioritization of *tuberculosis* laboratory services has been implemented in South Africa. Initially little attention was given to these services with resultant overworked staff and outdated or non-functional laboratory apparatus. In recent times however, this has changed with laboratories being upgraded and better equipped and staffed throughout the country (51, 67). It is hoped that consistent progress in this direction will further decrease test times and eventual test loads per laboratory in the future.

Tuberculosis in all its forms has proliferated and concentrated in districts with or near large cities viz. Nelson Mandela Metropolitan, Cacadu, UKhahlamba and Amathole rather than in the rural areas. This finding can be used as a motivation for government to focus on rural development in the Eastern Cape in order to prevent current trends of urban migration. Rural communities need to be sensitised on the effects of rural urban migration and on methods and benefits of developing themselves in villages.

Sub-districts with excess *tuberculosis* in time periods extending to 2009 should be target areas for urgent mass treatment as well as *tuberculosis* control and urgent prevention campaigns implemented by provincial health authorities. Multisectoral collaboration of the departments of health, environment, housing, social welfare, urban planning etc. needs to be established in the districts containing or near large cities to decrease levels of urbanization's detrimental effects in order to improve living conditions and decrease the high *tuberculosis* incidence rates observed in these areas. Particular emphasis needs to be placed on alleviating overcrowding in such areas. In addition to this, establishment of interventions specifically aimed at poverty alleviation need to be prioritized as a strong link was confirmed between *tuberculosis* and poverty in this study and this group represents a vulnerable subpopulation.

Regression analysis established that *tuberculosis* in its various forms is significantly associated with gender, age and laboratory inefficiency. In light of this socio-demographic factors found to be associated with various *tuberculosis* types need to be adopted as target populations for educational prevention and case tracing campaigns. These campaigns can be integrated into health programmes already in place for certain population groups. Women can be tested for *tuberculosis* at antenatal clinics and contraception points whereas children can be tested during vaccination as well as through school health programmes. Men can be targeted during specific health campaigns that encompass screening and education on male related diseases. Targeting age groups out of school will however need wider reaching strategies such as the use of mass media, television, radio and newspaper educational notices.

Finally the following policy recommendations are made in order to enhance the guidelines of the National *Tuberculosis* Control Programme of 2004:

-Quarterly monitoring of proposed external quality assurance systems at laboratories needs to be implemented to ensure that laboratories that perform *tuberculosis* drug susceptibility tests adhere to this policy guideline. This will ensure that laboratories with susceptibility testing are functioning at optimum levels more often than not.

-Over and above frequent surveys of drug resistant *tuberculosis* incidence and prevalence being conducted, it is recommended that provincial periodic surveys of sensitive *tuberculosis* be made mandatory as well to guarantee current data of *tuberculosis* levels nationally that can be compared to the records of the tuberculosis registry. This will then allow mistakes and shortcomings in the registry to be picked up and addressed.

-It is recommended that patients that have given in sputum samples, but have not gone back to clinics to collect results and yet have *tuberculosis* be followed up. This would involve going to the address that the patient supplied on their clinic file in order to inform them of their positive status and need to begin treatment.

-In closing it is imperative that education, influence and persuasion mechanisms be employed for the general public to be alerted of the importance of having timeous *tuberculosis* tests when any relevant symptoms are present. This would have to include campaigns geared at eliminating the stigma associated with *tuberculosis* in order for the fear of testing to be eradicated.

6.0 REFERENCES:

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Sibusiso Mkwananzi

CLEARANCE CERTIFICATE	**M10M101107**
PROJECT	Temporal and spatial Changes in the Tuberculosis Burden and Laboratory Services of the Eastern Cape
INVESTIGATORS	Dr Sibusiso Mkwananzi.
DEPARTMENT	School of Public Health
DATE CONSIDERED	26/11/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 26/11/2010 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Mr B Sartorius

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...