

Detection of a Substantial Rate of Multidrug-Resistant Tuberculosis in an HIV-Infected Population in South Africa by Active Monitoring of Sputum Samples

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Background. Tuberculosis (TB) coinfection with human immunodeficiency virus (HIV) is a substantial problem in South Africa. There has been a presumption that drug-resistant strains of TB are common in South Africa, but few studies have documented this impression.

Methods. In Phidisa, a joint observational and randomized HIV treatment study for South African National Defence Force members and dependents, an initiative was launched to test subjects (by use of microbiologic TB test) who appeared to be at high risk. We report results for HIV-infected subjects.

Results. TB was identified by culture in 116 (19.9%) of 584 patients selected for sputum examination on the basis of suggestive symptoms. Smear was an insensitive technique for confirming the diagnosis: only 33% of culture-positive patients were identified by smear, with a 0.2% false-positive rate. Of the 107 culture-positive individuals with susceptibility testing, 22 (20.6%) were identified to be multidrug resistant (MDR), and 4 (3.7%) were identified to be extensively drug resistant. Culture-positive cases with a history of TB treatment had more than twice the rate of MDR than those without (27.1% vs 11.9%; $P = .05$).

Conclusions. TB is common in this cohort of HIV-infected patients. Smear was not a sensitive technique for identifying culture-positive cases in this health system. Drug susceptibility testing is essential to proper patient management because MDR was present in 20.6% of culture-positive patients. Better management strategies are needed to reduce the development of MDR TB, because so many of these patients had received prior antituberculous therapy that was presumably not curative.

Tuberculosis (TB) is well recognized to be a common disease in many parts of the world [1–3]. Clinicians often make empiric diagnoses without laboratory confirmation in countries with high prevalence rates, because they are highly familiar with the disease and have practiced for many years without extensive laboratory resources, and because investment in technology to perform smears, cultures, conventional susceptibility testing, or molecular diagnostics may not be feasible.

In South Africa, there is considerable discussion about multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB, but few reports document specific resistance rates [2, 4–13]. Current national guidelines in South Africa do not recommend routine TB drug susceptibility testing for new cases of TB [13–16]. This study reports how frequently TB isolates were resistant to standard antituberculous drugs in a South African cohort of patients with human immunodeficiency virus (HIV) infection who were frequently treated empirically for TB.

METHODS

Project Phidisa is a joint observational and randomized HIV treatment study for members of the South African National Defence Force and their dependents. The study was a collaboration between the National Institute of Allergy and Infectious Diseases and the South

Received 8 July 2009; accepted 13 November 2009; electronically published 2 March 2010.

^a “Phidisa” means “to heal” in Setswana, 1 of South Africa’s 11 official languages.

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Clinical Infectious Diseases 2010;50:1053–1059

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1058-4838/2010/5007-0016\$15.00

DOI: 10.1096/651119

Table 1. Phidisa Baseline Characteristics for 584 HIV-Positive Subjects in the Sputum Initiative Cohort

Characteristic	Value
Age, mean \pm SD, years	35.9 \pm 6.0
No. (%) of male subjects	398 (68.2)
CD4 ⁺ cell count, cells/ μ L	
Median	144
25th percentile	68
75th percentile	276
HIV RNA level, copies/mL	
Median	130,853
25th percentile	40,300
75th percentile	283,500

NOTE. "Phidisa" means "to heal" in Setswana, 1 of South Africa's 11 official languages. SD, standard deviation.

African National Defence Force during the period from January 2004 to March 2008. Participants in the observational study were screened for HIV infection and monitored longitudinally for disease progression. From January 2004 to December 2007, all patients in this protocol identified as positive for HIV were invited to enroll in a treatment study if they were treatment naive with a CD4⁺ T cell count of <200 cells/ μ L or if they had an AIDS-defining illness. The treatment study randomized patients to 1 of 4 active therapy arms at 1 of 3 urban and 3 rural military bases in South Africa [17, 18]. This aspect of Project Phidisa assessed the impact of 4 different antiretroviral regimens for treatment of drug-naive HIV-infected individuals. Currently, an observational-only study continues to collect data.

Sputum initiative. An initiative was undertaken during the fourth year of Phidisa to obtain sputum samples from subjects, in either the observational or randomized component of the study, who appeared to be at especially high risk for TB based on self-reporting or clinically observed weight loss, chronic cough, or fever. Staff were trained to identify patients who had cough for >2 weeks, substantial weight loss, or unexplained fever. Such patients were encouraged to provide at least 1 expectorated or induced sputum sample. Specimens were obtained using the available equipment, environmental control technology, and personnel needed for clinical care at each site, and varied by locality. Patients who were unable to produce expectorated sputum were induced with hypertonic saline if appropriate facilities were available.

Samples were transported to a commercial reference laboratory (the Bio Analytical Research Corporation [BARC] laboratory, which is affiliated with Lancet Laboratories [<http://www.lancet.co.za/>]) in Johannesburg, South Africa, within 24 h of being produced and evaluated by direct microscopy (using both Ziehl-Nielsen [ZN] and auramine O [AO] staining), conventional culture (BACTEC; Becton Dickinson), and polymerase chain reaction (PCR; Haines and Light Cyler 1.5). Certain stud-

ies could not be performed on some days when equipment was not functioning. Results of all tests were reported promptly to the ordering healthcare provider following standard clinical practice.

A patient was designated to have a true-positive culture result for TB if an organism grew from at least 1 specimen that was identified by biochemical testing as *Mycobacterium tuberculosis*. Susceptibility testing was reported separately for PCR and culture techniques.

Assessment of presence of *M. tuberculosis*. Sputum was concentrated by centrifugation at 3,000 rpm for 15 min. Duplicate smears were prepared for direct microscopy. For light microscopy, 1 slide was stained by use of the ZN technique. For fluorescence microscopy, the other slide was stained with AO. For both slides, 100 high-power fields were read before a smear was declared negative. For a smear to be considered positive, at least 2 organisms had to be seen.

All specimens were cultured by inoculating concentrated sputum in liquid medium in BBL Mycobacterial Growth Indicator Tubes (MGITs) using the Bactec MGIT 960 system (Becton Dickinson). Cultures were held for 6 weeks before reporting the sputum sample as negative. Colonies were identified as *M. tuberculosis* by use of PCR (Light Cyler 1.5) and confirmed by use of the Hain Lifescience genotype MTBDRplus assay. TB identification by PCR was also done on the sputum sample.

Assessment of resistance. Drug susceptibility testing with broth dilution was determined for 4 drugs per sample: ethambutol, isoniazid, rifampicin, and either streptomycin or pyrazinamide, according to availability in the Bactec system. Individuals with *M. tuberculosis* resistant to both isoniazid and rifampicin on culture were classified as having MDR TB and had further culture susceptibility testing for 3 second-line drugs: ethionamide, kanamycin, and ofloxacin. XDR TB was defined as MDR plus resistance to ofloxacin plus either streptomycin or kanamycin. Molecular testing (Lifescience genotype

Table 2. Sensitivity and Specificity of Results from Polymerase Chain Reaction (PCR), Auramine O (AO) Smear, and Ziehl-Nielsen (ZN) Smear, Compared with Results from Culture

Test, result	Culture-positive subjects	Culture-negative subjects
PCR		
Positive	52/103 (50.5)	47/466 (10.1)
Negative	51/103 (49.5)	419/466 (89.9)
AO smear		
Positive	38/115 (33.0)	1/512 (0.2)
Negative	77/115 (67.0)	511/512 (99.8)
ZN smear		
Positive	38/109 (34.9)	1/481 (0.2)
Negative	71/109 (65.1)	480/481 (99.8)

NOTE. Data are proportion (%) of subjects.

Table 3. Sensitivity and Specificity of Results from Polymerase Chain Reaction (PCR), for Auramine O Smear-Positive Subjects (n = 39)

Test, result	Culture-positive subjects	Culture-negative subjects
PCR		
Positive	34/37 (91.9)	0/1 (0)
Negative	3/37 (8.1)	1/1 (100)

NOTE. Data are proportion (%) of samples. There were a total of 39 samples, but PCR result was missing for 1 AO smear-positive sample.

MTBDRplus assay; Hain Lifescience) was performed to determine the isoniazid and rifampicin susceptibility of *M. tuberculosis*.

Statistical analysis. Patient characteristics, including age, sex, CD4⁺ cell count, and HIV RNA level at Phidisa baseline, were assessed. For the analysis of the TB identification and drug sensitivity, only those specimens that had complete culture results and that were collected from an individual known to be HIV infected at time of specimen collection were included. For patients found to be positive for TB on culture, their medical records were examined for evidence of prior history of TB and for prior history of TB treatment. Fisher's exact test is used to test for a difference in MDR rates based on known TB history.

Assessment of presence of *M. tuberculosis*. Using culture as the gold standard, the sensitivity and specificity of AO smear, ZN smear, and PCR were calculated. For this calculation, if a subject had multiple specimens, only the first culture-positive test and the first culture-negative test were included in the analysis. Sensitivity and specificity were also examined separately for a subject whose CD4⁺ cell count was ≤50, between 50 and 200, or >200 cells/μL; only subjects with a CD4⁺ cell count measured within 90 days before, and no later than 30 days after, the sputum collection date were considered for this analysis. The Cochran-Armitage trend test was used to see whether there was a significant trend for decreasing sensitivity with increasing CD4⁺ cell count. Test agreement between culture and PCR was considered separately for smear-negative and smear-positive samples.

Assessment of resistance. The rate of resistance for each drug tested, the rate of MDR TB, and the rate of XDR TB were reported. If a person had >1 set of susceptibility tests, then only results taken from the first culture-positive sample were included in the comparison of PCR and culture diagnostic susceptibility testing. The agreement between PCR and culture was assessed for isoniazid, rifampicin, and multidrug resistance. Additionally, for all culture-positive samples, a chart review was done to determine any prior history of TB or TB treatment within the past 3 years.

PCR drug sensitivity validation. After the sputum initiative had been ongoing for ~18 months, independent validation of a subset of drug sensitivity data was done. Susceptibility

results were assessed for accuracy by sending isolates to a second laboratory, the South African Medical Research Council (MRC) laboratory in Overport, South Africa, which did extensive work for research studies, and retesting the isolates in a blinded fashion. The MRC laboratory did the molecular diagnostic susceptibility testing (using MTBDRplus PCR; Hain) from cultured samples sent in MGITs (except for one in a Versatrek bottle from Trek Diagnostic Systems). The 30 samples were a convenience sample; that is, all frozen samples that were still in storage at the time of validation were reassessed by the MRC laboratory.

RESULTS

There were 832 sputum samples obtained from 613 HIV-infected patients during the period from May 2007 to December 2008. From these samples, 785 specimens (94.4%) from 584 individuals had complete culture results. There were 47 specimens missing culture results; 42 tests had indeterminate results, mostly due to overgrowth, and the remaining 5 culture tests were not completed due to administrative or laboratory errors. Of 584 individuals, 116 (19.9%) had at least 1 culture result positive for TB.

Table 1 presents the Phidisa baseline characteristics of patients in the sputum initiative cohort. For the calculation of PCR and smear test accuracy, data from 512 first culture-negative samples tested and 116 first culture-positive samples tested were analyzed. Tables 2 and 3 present the degree of agreement among smear, culture, and PCR. Using culture as the gold standard, the sensitivity (ie, the percentage of culture-positive samples that tested positive) and specificity (ie, the percentage of culture-negative samples that tested negative) are shown for PCR and smear testing. PCR testing had a low positive predictive value (52 [52.5%] of 99 samples), as a result of the moderate specificity (419 [90%] of 466 samples) and largely culture-negative population that was tested. The positive predictive value for AO and ZN smears was 97.4% (ie, 38 of 39 samples). ZN smear, AO smear, and PCR had decreasing trends for sensitivity with increasing CD4⁺ cell counts, but these trends were not significant (Table 4). There was no variation in specificity (Table 5).

Drug susceptibility. Figure 1 displays the resistance rates for first-line drugs. Of the 116 subjects who had at least 1 culture-positive test result, 107 (92.2%) had drug susceptibility test results for their first culture-positive sample. Of these 107 subjects, 20 (18.7%) had TB infection that was determined to be MDR on the basis of initial diagnostic susceptibility testing, 22 (20.6%) had TB infection that was determined to be MDR on the basis of initial or later diagnostic susceptibility testing, and 3 (2.8%) had TB infection that was determined to be XDR on the basis of initial diagnostic susceptibility testing. Another

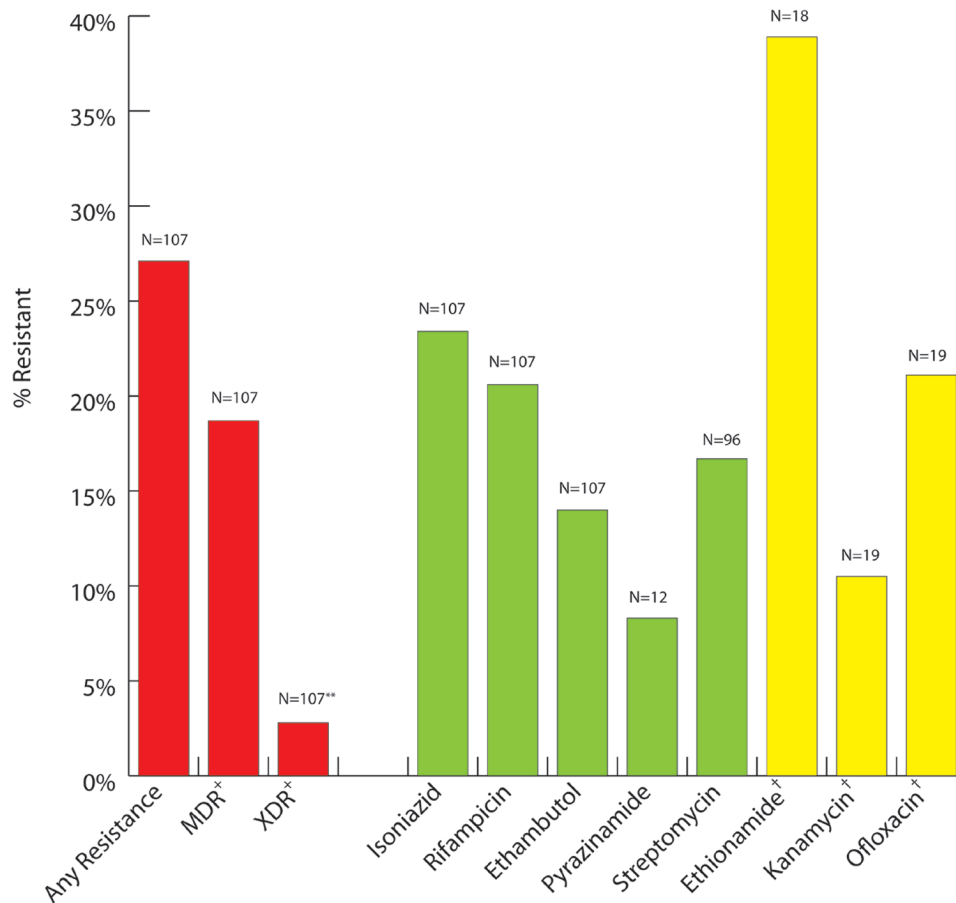


Figure 1. Drug sensitivity test results (for 107 isolates), performed by use of a culture-based method. A first-line susceptibility test result was available for 107 of 116 culture-positive individuals. If a subject had >1 sample, the first diagnostic susceptibility test result was presented. Not all isolates were tested for all drugs. [†]A second-line susceptibility test was performed for only 19 of 20 individuals who had MDR findings on their first culture-positive sample. [‡]Multidrug-resistant (MDR) tuberculosis (TB) is defined as resistant to both isoniazid and rifampicin. Extensively drug-resistant (XDR) TB is defined as multidrug resistance plus resistance to ofloxacin and streptomycin or kanamycin. ******Complete second-line test results were missing for 2 of 20 individuals whose samples were determined to be MDR from their first diagnostic susceptibility test result. One additional individual was found to have XDR TB from a subsequent diagnostic susceptibility test result.

subject was determined to have XDR TB, which was detected 2 months after an initial pan-sensitive test.

Figure 2 shows the rate of MDR TB by history of TB treatment, as determined by self-report and chart review. The rate of MDR TB (alone or along with XDR TB) was 11.9% for patients with no known history of TB treatment and 27.1% for patients with a history of TB treatment ($P = .05$).

Of the 107 individuals with drug susceptibility results on their initial culture-positive sample, 103 (96.3%) had concurrent PCR drug susceptibility results for isoniazid and rifampicin. PCR results agreed with the culture isoniazid and rifampicin sensitivity results for 94 (91.3%) of 103 subjects. Using only PCR sensitivity to isoniazid and rifampicin to define multidrug resistance, 13 (65%) of 20 individuals would have been correctly identified as have MDR TB, with no false MDR detections (data not shown).

Since patients received their antituberculous therapy in a

different healthcare system, information was not routinely available regarding the antituberculous drugs prescribed, the duration of therapy, adherence to therapy, or follow-up microbiologic results.

PCR validation study. There were 28 samples positive for TB on culture that were sent to the MRC laboratory, where they were retested by use of molecular techniques for isoniazid and rifampicin susceptibility (ie, diagnostic susceptibility testing). All 28 samples were classified as susceptible to isoniazid by use of PCR at the MRC laboratory, but 1 (3.6%) of these samples was designated as resistant to isoniazid by use of PCR at the BARC laboratory. The overall agreement rate between the study and MRC PCR results for rifampicin resistance was 85.7% (24 of 28 samples). Two samples were shown to be resistant to rifampicin, and 22 samples were shown to be sensitive to rifampicin, by both the MRC laboratory and the BARC laboratory; however, there were 4 (15.4%) of 26 rifampicin-

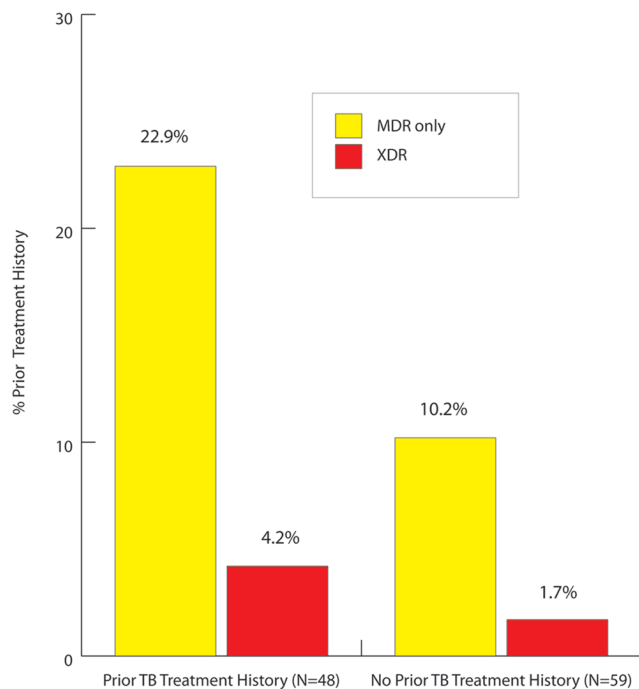


Figure 2. Rates of multidrug-resistant (MDR) tuberculosis (TB) alone and rates of MDR and extensively drug-resistant (XDR) TB, by history of prior TB treatment history ($n = 107$). MDR status was based on drug susceptibility testing on the first positive culture sample for individuals with multiple samples. Data on the percentage of patients with a prior history of TB treatment are based on patient self-reports and patient medical records for isolates that were susceptible to drugs, MDR, or XDR.

susceptible samples from the MRC laboratory that were classified as resistant by the BARC laboratory. None of the 28 samples were classified as MDR by the MRC laboratory, but 1 (3.6%) of the 28 samples was classified as MDR by the BARC laboratory.

DISCUSSION

South Africa has a particularly heavy burden of TB; in 2005, it was estimated that there were 285,000 incident cases of TB in South Africa [1, 18]. Thus, strategies to screen for TB, to treat TB effectively, and to prevent transmission are desperately needed.

This study demonstrated that clinicians could identify patients with a high likelihood of TB using general clinical parameters that focused on self-reported or clinically observed chronic cough, apparent weight loss, or fever. In these HIV clinics, 19.9% of patients who were asked to provide sputum samples did in fact have culture results positive for TB. Other HIV clinics in South Africa have reported high yields. Yields of 19%–26% have recently been reported in several South African HIV programs that obtained either expectorated or induced sputum samples on ≥ 1 occasion from all patients at-

tending HIV clinics, regardless of the presence of suggestive symptoms or signs [18–21]. Thus, in South Africa, active screening of symptomatic and perhaps asymptomatic HIV-infected patients, at regular intervals, seems likely to be highly effective.

Many programs in developing countries rely on smear alone to identify patients with TB. However, smear was an insensitive technique for confirming TB in this South African setting for all CD4⁺ T cell count groups (Table 4); only 33% of culture-positive patients were determined to be so by AO smear. This is consistent with the rates of 8%–29% reported in recent investigations from South Africa and other developing countries involving HIV-infected patients [19, 20, 22–25], marginally below the rates of 45%–80% reported by the Centers for Disease Control and Prevention [23], but well below the 93% rate that some reference laboratories can attain [26]. The performance characteristics of smear might be enhanced if laboratory technicians had better training or if they had the opportunity to spend more time on each specimen. However, other techniques are needed to rapidly identify patients with TB, especially if the number of organisms is small [3, 22, 25, 26].

A nucleic acid amplification test would be a logical technique to identify more culture-positive patients at the time of their initial visit, and to identify those organisms that were resistant to isoniazid or rifampin [3, 23, 27–32]. In the United States, the Centers for Disease Control and Prevention recently issued updated guidelines supporting such an approach [23].

In the current study, PCR had a low positive predictive value (52.5%), because of the moderate specificity (90%) and largely culture-negative population that was tested (TB prevalence, 19.9%). Thus, as performed in this study, PCR alone was not sensitive or specific enough to be reliably and definitively diagnose TB. On the other hand, in this setting, PCR had a high negative predictive value (89.1%). These PCR results were obtained from a large, widely used commercial laboratory. A research laboratory might have achieved different results.

Most TB programs in South Africa do not use culture and susceptibility testing as part of their TB program, because of cost constraints and the absence of appropriate technical and personnel resources [1–3, 13–15]. It is well known that MDR TB and XDR TB are present in South Africa, yet there are few published data documenting their prevalence.

The World Health Organization's global report on antituberculosis drug resistance in the world has recently reported that Africa recorded 1 of the lowest median levels of drug resistance worldwide; the mean rate of MDR TB in Africa was 2.2% (range, 0.0%–5.8%) [1, 5, 8, 11, 13]. South Africa, in particular, was reported to have a prevalence of MDR TB of 3.1% [1, 5, 8, 13]. There has been much discussion about why these rates were so low, but underreporting has been suspected [11, 33]. In many reports, it is not clear whether the data

Table 4. Sensitivity of Direct Sputum Tests According to CD4⁺ Cell Count

CD4 ⁺ cell count	No. of culture-positive samples (n = 105)	Sensitivity, % (ie, proportion of positive samples)		
		ZN smear	AO smear	PCR
≤50 cells/μL	22	45 (9/20)	42.9 (9/21)	66.7 (12/16)
51–200 cells/μL	55	33.3 (18/54)	32.7 (18/55)	47.1 (24/51)
>200 cells/μL	28	25.0 (6/24)	21.4 (6/28)	45.8 (11/24)

NOTE. Differences between CD4 cell count groups did not reach statistical significance. The samples in this analysis were limited to 105 of 116 culture-positive samples obtained from subjects with a CD4⁺ cell count measured within 90 days before, and no later than 30 days after, the sputum collection date. AO, auramine O; PCR, polymerase chain reaction; ZN, Ziehl-Nielsen.

included both treatment-naïve and treatment-experienced patients.

Other studies reporting national data and regional surveys suggested that rates of MDR TB began to increase in the late 1990s in South Africa [1–12, 19, 34, 35]. A systematic survey in 2001–2002 of 9 South African provinces, conducted by the Tuberculosis Lead Programme of the MRC, reported a rate of multidrug resistance in isolates from patients with newly diagnosed TB of 1.6% (range, 0.9%–2.6% in the provinces), and a rate of multidrug resistance in isolates from patients with previously treated TB of 6.6% (range, 3.9%–13.7% in the provinces) [10, 11]. The Tuberculosis Strategic Plan for South Africa, 2007–2011, reported that, from 2004 to 2007 there, were >11,000 laboratory-confirmed cases of MDR TB in South Africa, many of which came from the Western Cape [13]. For 2006, the reported rates of MDR TB were 0.4%–2.67% by province. During the same period, there were >800 cases of XDR TB reported.

In this current HIV-positive patient population of geographically mobile, actively employed individuals and their dependents, multidrug resistance was present in 20.6% of TB cases. PCR testing of isolates for isoniazid and rifampin resistance showed a high correlation with culture-based results, providing additional confirmation of the accuracy of the results. The percentage of patients with MDR TB who reported no prior TB treatment was 11.9%, which is consistent with several recent

reports from South Africa [6–12, 19]. The percentage of patients with MDR TB who reported prior TB treatment was considerably higher than previously reported, 27.1%.

HIV-infected patients with TB have a poorer 1-year survival rate than do HIV-infected patients without TB. MDR or XDR TB adds to mortality incrementally. A recent study in South Africa reported 1-year survival rates of 69% for patients coinfecting with HIV and MDR TB and 82% for patients coinfecting with HIV and XDR TB [12, 15].

In the HIV-infected patient population studied in South Africa, where unrecognized TB was common, more consistent and uniform interventions are needed to screen high-risk patients for TB. It seems likely that, given the national prevalence of TB, all HIV-infected patients in South Africa should be considered high risk, regardless of CD4⁺ T cell count or symptomatic status. More rapid and accurate diagnosis and more effective management strategies are needed if the cycle of transmission and poor outcome in the community, in the workplace, and in healthcare settings is to be broken.

Acknowledgments

We thank Natalie Bracher, Nicolaas Poole, and Jessica Trusler at the BARC laboratory as well as Paul Khabo and other members of the Phidisa study team (ie, the Executive Committee, Data Safety Monitoring Committee, and site personnel) for their efforts in providing the information necessary for this report. We also thank the patient volunteers who par-

Table 5. Specificity of Direct Sputum Tests According to CD4⁺ Cell Count

CD4 ⁺ cell count	No. of culture-negative samples (n = 477)	Specificity, % (ie, proportion of positive samples)		
		ZN smear	AO smear	PCR
≤50 cells/μL	52	100 (48/48)	100 (52/52)	87.5 (42/48)
51–200 cells/μL	193	99.5 (181/182)	99.5 (192/193)	88.2 (150/170)
>200 cells/μL	232	100 (216/216)	100 (232/232)	92.0 (196/213)

NOTE. Differences between CD4 cell count groups did not reach statistical significance. The samples in this analysis were limited to 477 of 512 culture-negative samples obtained from subjects with a CD4⁺ cell count measured within 90 days before, and no later than 30 days after, the sputum collection date. AO, auramine O; PCR, polymerase chain reaction; ZN, Ziehl-Nielsen.

ticipated in the study and the healthcare providers who contributed to this effort.

Financial support. US Department of Health, National Institute of Allergy and Infectious Diseases, and South African National Defence Force, under a collaborative agreement.

Potential conflicts of interest. All authors: no conflicts.

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