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Prevalence of Tuberculosis in Selected Prisons in Ghana: A Baseline Study Using the Capilia^{tm} TB-Neo Assay

Oti K. Gyamfi[1], Isaac K. A. Galyuon[2], Foster K. Kyei[3], Emmanuel P. Otwe[4], Daniel Asante[5], Kwasi K. Addo[6], Kofi D. Bedzra[7], Samuel Kudzawu[8], Sjoukje Kuijper[9], Arend J. H. Kolk[10]

[1]Adjunct, Public Health Programme, School of Governance and Public Service (SGPS), Ghana Institute of Management and Public Administration (GIMPA), Greenhill, Achimota, Accra, Ghana.
[2,3,4,5]Dept. of Molecular Biology & Biotechnology, School of Biological Sciences, College of Agriculture & Natural Sciences, University of Cape Coast, Cape Coast, Ghana.
[6]Dept. of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana.
[7]Cellular and Clinical Research Centre, Radiological and Medical Sciences Research Institute, Ghana Atomic Energy Commission, Kwabenya, Accra, Ghana.
[8]Chest Clinic/Tuberculosis Reference Laboratory, Dept. of Chest Diseases, Korle Bu Teaching Hospital, Korle Bu, Accra, Ghana.
[9,10]Analytical Chemistry Group, Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Amsterdam, The Netherlands.

Abstract

Introduction: There is very little information on the nature and extent of Tuberculosis in institutionalised sections of the Ghanaian population such as the prisons is disturbing, and therefore any attempt in rapidly and accurately diagnosing the disease should serve as a very welcome control and management intervention.

Methodology: A total of 214 coughing prison inmates from 9 selected prisons were screened for tuberculosis. Isolates recovered from the cultured sputa elicited from these coughing inmates were analysed using the Capilia^{TM} TB-Neo assay, together with primary tests such as ante- and post-culture microscopy, Conventional biochemical tests and the 16S rDNA PCR for the insertion sequence *IS*6110.

Results: A total of 43 out of the 54 isolates harvested from culture were acid-fast by microscopy, out of which 30 were confirmed as *Mycobacterium tuberculosis*-complex by both the Capilia^{TM} TB-Neo assay, 16S rDNA PCR and conventional biochemical tests. The Capilia^{TM} TB-Neo assay detected 13 isolates as not harbouring the MPB64 gene and therefore categorised as non-tuberculous mycobacteria. Nine (9) out of these 13 were of sufficient purity to be sequenced and 8 confirmed as non-tuberculous mycobacteria and one as *Nocardia nova*.

Conclusions: The Capilia^{TM} TB-Neo assay can be a useful additional test to include as part of the traditional first-line screening tools in a resource-stretched setting. The detection of a member of *Nocardiaceae,* preliminarily mimicking members of the *Mycobacteriaceae,* indicates the inclusion of a

more specific genetic test in a primary screening methodology, if control and management measures are to be efficient.

Keywords: Tuberculosis; Prisons; Capilia^{TM} TB-Neo assay; MPB64 gene

Introduction

Tuberculosis (TB) is one of the most serious public health problems confronting the world today. The World Health Organisation (WHO) estimates that globally 10.8 million people became ill with tuberculosis in 2023 rising from a figure of 10.7 million in 2022 and 10.4 million in 2021 [**1**]. Since 2021, more than 10 million people continue to be afflicted by tuberculosis globally every year [1]. According to the WHO, TB re-emerged as the leading cause of death from a single aetiologic agent globally in 2023, after a three-year interregnum in which the coronavirus disease (Covid-19) held sway and caused twice as many human deaths as HIV/AIDS. The flagship aetiologic agent of TB disease, *Mycobacterium tuberculosis* (MTB), is a member of the *Mycobacterium tuberculosis*-complex (MTBC), a group of closely-related pathogens belonging to *Mycobacteriaceae*, which also includes *Mycobacterium bovis, Mycobacterium bovis* Bacillus Calmette–Guérin (BCG)*, Mycobacterium caprae, Mycobacterium africanum, Mycobacterium microti, Mycobacterium canettii, Mycobacterium pinnipedii, Mycobacterium orygis, Mycobacterium mungi* and *Mycobacterium surricatae* [2]. Tuberculosis is spread mainly through coughing by infected individuals where the bacilli are carried air-borne in aerosolised sputum droplets. When breathed in, these droplets may lead to TB disease. According to the World WHO, TB re-emerged as the leading cause of death from a single aetiologic agent globally in 2023, after a three-year interregnum in which SARS-Cov-2 (or COVID-19) held sway and caused twice as many human deaths as HIV/AIDS [1]. In resource-stretched settings such as in parts of rural Ghana, the availability of rapid diagnostic tests for application to notified cases at diagnosis time has always been problematic [3]. For example in 2017, only a paltry 6% of notified cases in Ghana had the benefit of being tested with a rapid diagnostics test at diagnosis time [3]. Current available data for TB infections in 2023 indicate that in terms of notifications, new and relapse cases were 19,000 per 100,000 population and 56 per 100,000 population per year respectively. Fortunately, 99% of these notified cases were tested with a rapid diagnostics test at diagnosis. This indicates a burgeoning work load saddling health centres in inaccessible rural locales. In these resource-stretched settings, the need for the inclusion of an accurate, efficient and relatively inexpensive diagnostics test at diagnosis cannot be further emphasised. Estimated data describing Ghana's tuberculosis burden gives a total tuberculosis incidence and incidence rate of 44,000 per 100,000 population (69,000 – 78,000) and 129 (57 – 236) per 100,000 population per year respectively [1]. The same data set gives mortality from TB amongst HIV-positive subjects of 2,300 (1,100 – 4,100) per 100,000 population from a mortality rate of 6.9 (3.1 – 12.0) per 100,000 per year [1]. A silver lining is the welcome drop in TB incidence of -19% [1].

The state of TB infections in penal populations is clearly worse than in the general population since tuberculosis rates in prisons are often 5 to 10-fold higher [4]. The stifling and over-crowded conditions in most prisons are conducive for the spread of TB [5,6] resulting in TB infections in both inmates and prison staff [5–**9**]. Also, in the penal environment, the fate of asymptomatic inmates is unknown and this could lead to infections within the general prison population including prison warders and visitors. Information on the state TB infections in prisons would be invaluable in the formulation of the appropriate management and control strategies for TB in the penal environment. Thus, the primary

objective of this study was to determine the prevalence of culture-active tuberculosis in coughing inmates in selected Ghanaian prisons using the Capilia™ TB-Neo assay in tandem with routine screening methods like microscopy and the 16S rDNA PCR assay. Whilst the former method exploits the presence or absence of the immunogenic protein MPB64 in the categorisation of MTB isolates, the latter exploits the existence of multiple copies of the insertion sequence IS*6110* in the genome of members of the MTBC.

Liquid culture broths of the flag-ship aetiologic agent of TB disease, *Mycobacterium tuberculosis,* have been known to exude more than 30 different proteins, the most important of which is the 24 kDA immunogenic protein, MPB64 [10–15]. Also, *Mycobacterium bovis* and some strains of its attenuated variant, *Mycobacterium bovis* BCG, also express the protein MPB64 in liquid media. The current view is that the MPB64 protein is highly specific to members of the MTBC [15-18]. Atypical or non-tuberculous mycobacteria (NTM) do not express this protein and therefore this property can be used to categorise Mycobacteria into MTBC and NTM. On the other hand, the 16S rDNA PCR assay exploits the existence of multiple copies of the insertion sequence IS*6110* on the genome of members of the MTBC [19–21]. However, some *M. bovis* strains have been known to harbour only one copy or none at all [19–22].

The Capilia™ TB-Neo assay (TAUNS Laboratories, Inc., Shizuoka, Japan) is one of the immunochromatographic assays (ICA) based on the MPB64 protein currently available. Another notable MPB64-based test is the BD MGIT™ TBc Identification Test (Becton, Dickinson and Company, Franklin Lakes, NJ, U.S.A.). A similar test, but based on the MPT64 protein antigen, is the SD TB Ag MPT 64 Rapid Test (Standard Diagnostics, Seoul, South Korea) [23]. The Capilia™ TB-Neo is a second generation version superseding the earlier Capilia™ test. Continuing validation work has uncovered the possibility of false-negatives because of mutations in the MPB64 gene [15].

Materials and Methods

In order to determine the expected frequency and attendant error in a positive diagnosis of TB in coughing prison inmates, TB Registers in prison infirmaries were cross-referenced with those in hospitals (in closest proximity to the prisons) for a positive diagnosis in the last 6 months prior to the commencement of the study. The information garnered was used to calculate the sample size for the study.

Inmate Population Description and Inclusion Criteria

The only criterion for inclusion was an active cough for at least one (1) week in an inmate, narrowed down from the usual Ghana National Tuberculosis Programme (NTP) criteria of: an active cough for at least 2 weeks, night sweats, weight loss, and fever. All sputum samples obtained were from eligible inmates found in selected prisons after the initial assessment survey. The prison inmates were all male and comprised convicted and remanded inmates. Convicted inmates refer to inmates duly convicted by a competent Ghanaian Court of Law. Remanded inmates refer to inmates whose cases have not been determined to completion by any competent Ghanaian Court of Law. Remanded inmates could also have their cases in the process of being determined. Usually, remanded inmates were characterised by considerable mobility, in and out of designated prisons, in order to satisfy the demands of the preliminary investigative process and court attendance. For remanded inmates, this resulted, in ad hoc and unplanned short-term detentions in various police stations because of logistical constraints (mainly

the means of transportation and feeding) resulting from a lack of adequate funding. Both convicted and remanded inmates had been gaoled on charges ranging from theft (mostly petty larceny and armed robbery), motor traffic offences, assault, internet fraud and in a few instances, murder. Permission was sought from, and granted by the Ghana Health Service Ethical Review Committee on Research Involving Human Subjects (ERCRIHS) before subsequent consent was obtained from inmates to be included in the study.

Sample size determination

As a baseline study, the sample size was computed using Fisher's Exact Test. This statistical test method was chosen because the study yields one of two possible outcomes, that is, TB present or TB absent and, can thus be assigned two possible categorical values. The possibility therefore exists for the definition of the relationship between these categorical values by the use of 2X2 contingency tables. The incidence or expected frequency (p) was determined as 83.3% (19 positives out of 23 suspected cases – using coughing as the sole criterion) whilst the margin of error (d) was determined as 5% (1 out of 19 positives failing to grow on culture). These indices gave a required sample size (n) of 214 coughing inmates. This population of coughing inmates was drawn from nine (9) selected prisons with a combined total population of 6,132 inmates, comprising of 106 remanded inmates and 108 convicted inmates.

Sputum sample collection

Two sputum samples each, comprising a mid-night sample (sample 'A') and an 'on-the-spot' sample (sample 'B'; given in the presence of a prison health official) were obtained from the inmates. Basic inmate data (age, prison identification code) and cell numbers were recorded. Samples were collected into appropriately labelled 50 ml screw-capped polypropylene tubes and an equal volume of 1% Cetyl-pyridinium chloride (1% CPC), was added before shipment to the laboratory for further analyses. The 1% CPC served both as a decontaminant and a transport medium [24,25].

Sputum Smear Microscopy and Culture

To each tube containing sputum in 1% CPC, sterile double-distilled pyrogen-free water was added to the 50-ml mark. The tubes were then centrifuged at 3000 x G for 15 minutes and then left for 15 minutes to reduce aerosols, before decanting the supernatant. Formed pellets then re-suspended in 1–2ml sterile distilled water, and subjected to ante-culture Ziehl-Neelsen (ZN) microscopy for acid-fast bacilli, and then finally inoculated (100µl) onto Löwenstein-Jensen (LJ) media slants in replicates – one glycerol-impregnated slant and the other pyruvate-impregnated slant. All 214 sputum samples were cultured whether they were acid-fast or not. Tubes were then loosely screwed and then incubated at 37°C by laying them onto a slanting bed for 4 days before standing them upright on test-tube racks, and observed for growths for periods up to 8 weeks. Tubes were monitored daily for the first 7 days (for fast or rapid growers) and then, thereafter, weekly for eight weeks. The general morphologies of the growths were observed and recorded as rough or smooth, eugenic or dysgonic, buff, white, yellow or orange. All growths were then each harvested into approximately 0.5ml sterile double distilled water and Middlebrook 7H9 broth (enriched with Oleic acid-albumin-dextrose-catalase, OADC) and then subjected to another round of post-culture ZN microscopy for the confirmation of acid-fastness. They were then stored at -20°C as isolates.

Conventional Biochemical Tests

Isolates were then sub-cultured on Coletsos® medium (bioMerieux Clinical Diagnostics, Marcy l'Etoile, France) at 37ºC in an atmosphere of 5% Carbon dioxide to yield purer harvests for classical biochemical identification tests.

Para-Nitrobenzoic acid (PNB) Susceptibility Test: This test [26] is used to differentiate members of *M. tuberculosis*-complex from other mycobacterial strains. Briefly explained, 0.1 ml of isolate suspension prepared from 3-week old sub-cultures was inoculated into 2 tubes of 0.5mg/ml PNB-containing and PNB-free LJ medium and incubated at 37ºC for 4 to 28 days. The tubes were observed on the 4th, 7th, 14th, 21st, and 28th days for visible growth of colonies or otherwise. The presence of members of the *M. tuberculosis*-complex was indicated by a lack of growth observed in any of the PNB-containing tubes after the 28 days of incubation. Non-tuberculous mycobacteria are resistant to PNB [27,28] whiles usually PNB included in media inhibits the growth of classical MTB, *M. africanum, M. bovis* and *M. microti* [29].

Niacin (Nicotinic acid) Accumulation Test [29,30]**:** Niacin is produced by all mycobacteria through a redox mechanism in the energy metabolism process. However, the MTBC accumulates its niacin because of the activity of nicotianimide adenine dinucleotide (NAD) and its inability to process the resulting niacin [29,31]. Thus niacin accumulation is used in differentiating members of the MTBC from other mycobacteria. Niacin Test Strips, BBL Taxo TB (Becton, Dickinson and Company, USA), absorbent paper strips and TB Niacin Positive Test Control paper discs were used according to the manufacturer's instruction. Profusely grown four week old sub-cultures were used, following the manufacturer's instructions. Briefly explained: Using a sterile dropping pipette, approximately 2.5ml sterile double distilled pyrogen-free water was added to each culture tube. Each surface growth was gently and carefully punctured with 1 ml sterile pipette to permit extraction of niacin. With the help of sterile dropping pipette, approximately 0.6ml of the fluid extract was carefully removed and transferred to the bottom of 20x125 mm screw cap test tube. A negative control was also prepared and included. The strips (made up of positive and negative controls) and test cultures were dropped with arrow signs downward into the tubes and stoppered immediately. Developed colours of the extracts were then compared after 15 minutes. Niacin accumulation was indicated by a vivid appearance of yellow coloration in the extract.

Nitrate Reduction to Nitrite Test [32]**:** Mycobacterial cellular membranes harbour the enzyme, Nitrate Reductase, which is capable of reducing nitrates to nitrites. Mycobacteria can use this enzyme as a means to source nitrogen [31]. Thus, the presence of nitrate (usually as sodium nitrate) in liquid cultures of MTBC can be reduced to nitrite by Nitrate Reductase. The formation of the nitrite can be confirmed by the formation of a furchsia colour [33] following the addition of a thio-amide, sulfalinamide, and the amine salt, dihydrochloride-N-naphthyl ethylenediamine. This test was conducted on four-week old sub-cultures using BBL Taxo TB Nitrite Test Strips (Becton and Dickinson, USA), and absorbent paper strips following the manufacturers' instruction. About 0.5 ml sterile distilled water was transferred to each test tube. Using a sterile 1ml pipette, two clumps of growth were harvested from each culture tube and added to the distilled water and the contents swirled gently into a suspension. The strips were then transferred into the tubes and incubated at 37ºC for 2 hours. The colours of the top portion of the strips were noted immediately after the incubation. Positive and negative controls tests were included. Reduction of nitrate to nitrite, was indicated by a change in coloration of the top portion of the test strip from white to dark blue.

Capilia™ TB-Neo assay

The Capilia™ TB-Neo assay [18,34] is used in distinguishing members of the MTBC from other mycobacteria on the basis of the presence or absence of the 24-kDa immunogenic protein, MPB64. While members of the MTBC produce this protein other mycobacteria do not. All the harvested sub-cultures on Coletsos® medium were subjected to the Capilia™ TB-Neo assay by following the manufacturer's instructions (TAUNS, Numazu, Japan, and TAUNS Laboratories, Inc., Shizuoka, Japan), with slight modifications. Briefly explained: a volume of 200µL each of the supplied extraction buffer was dispensed into appropriately labelled 0.5mL screw-capped tubes using an Eppendorf repeater pipette. A loop full (1mm diameter platinum micro-loop) each of grown sub-cultured colony was placed into a tube containing extraction buffer. The screw cap of each tube was then fixed firmly, and vortexed vigorously but briefly (10s) to obtain a uniform suspension. An aliquot of 100µL of each suspension was pipetted into the sample hole in each cartridge. The reading area was then observed and recorded. A purple-red line in both the test area (T) and the control area (C) was recorded as a positive result while a purple-red line in the control area only was recorded a negative result. Positives were confirmed as being members of the MTBC.

DNA Isolation from Culture slants

All the harvested isolates after sub-culture on Coletsos® medium were treated to yield DNA of sufficient purity for the 16S rDNA PCR Assay as follows: A loop-full each of sub-cultured mycobacteria from the Coletsos slants was harvested into 150µl lysis buffer of 10mM TRIS-HCl, pH8 (Sigma-Aldrich, USA), with 1mM EDTA (Sigma, USA) and 1% Triton X-100 (BDH Laboratory Supplies, Poole, England) and then vortexed briefly for 10 seconds. Cells were lysed (95ºC for 30 minutes), vortexed again (10 seconds) and then spun down at 5,000 x G for 3 minutes. Subsequently, 135µl of the supernatant was removed and incubated on a heating block at 37ºC for 30 minutes after the addition of 2µl of 5mg/ml DNase-free RNase (Roche Diagnostics, Mannheim, Germany). The samples obtained were then stored at 4ºC until required for downstream analysis.

Diagnostic PCR for the MTBC (THE 16S rDNA PCR ASSAY)

All harvested isolates after sub-culture were screened for their MTBC status by subjecting them to the 16S rDNA MTBC diagnostic PCR. The 16Sr DNA PCR design is based on the amplification of sequences (using primers pMyc14F-unlabelled and pMyc7R) in the insertion element *IS*6110, which is characteristic of the members of the MTBC. The presence of a 208bp fragment confirms an isolate as a member of the MTBC [20,21]. The 16S rDNA PCR Assay was performed on the lysed DNA samples for categorisation as members of the MTBC, or otherwise, using the primers: pMycF 5'-GRGRTACTCGAGTGGCGAAC-3', and pMycR – 5'-GGCCGGCTACCCGTCGTC-3' [20]. The diagnostic fragment was amplified using a Promega PCR kit (Promega Corporation, USA), following the manufacturer's instructions with slight modifications. Briefly explained: The PCR mix was prepared by aliquoting and mixing in a DNA-free room 5µL of 10X Gold Star Buffer, 5.4 µL dNTP's (150mM) containing $MgCl_2$, 0.3µL Gold Star Taq Polymerase and 38.3µL Baker HPLC-grade water (Sigma-Aldrich, Il, USA). DNA template, 1µL, was then added to give a total reaction volume of 50µL. The amplification reaction was programmed in 'hot start' mode as follows: 10 minutes at 95°C; then 34 cycles of (30 seconds at 95°C; 30 seconds at 55°C; 30 seconds at 72°C) and then finally held at 72°C for 5 minutes. The success of the PCR was verified by the presence of a 208bp fragment after 1.5% agarose

gel (Seakem, Lonza) electrophoresis in 1X TBE (pH 7.4) run at 80V for 30 minutes (EC135-90 Power Pack, E-C Apparatus Corporation, USA).

Results and Discussion

The population of 214 coughing inmates comprised 108 convicted inmates and 106 remanded inmates and its descriptive statistics are displayed in Table 1.

Table 1. Age characteristics of coughing inmates sampled (n=214)

Category	Number of inmates sampled	Age range (years)	Median age (years)	Mean age (years)
Convicts	108	18 - 78	30.0	34.0
Remands	106	16 – 83	31.0	32.3
Total	214	16 – 83	30.0	30.6

Table showing age characteristics of coughing inmates sampled (n=214)

A total of 29 decontaminated sputum samples were acid-fast ante-culture. Culture yielded a total of 54 growths, out of which 43 were acid-fast post-culture. After subjecting this panel of 43 acid-fast (post-culture) isolates to biochemical tests of niacin production, nitrate reduction and growth-inhibition on LJ-medium by p-Nitro-benzoic acid (PNB), results obtained are instructive. Thirty (30) of these 43 isolates were characterised as being members of the MTBC after successfully producing niacin. Thirteen (13) failed to produce niacin. Regarding the nitrate reduction test, while 29 isolates successfully reduced nitrate to nitrite, 14 isolates failed to reduce nitrate to nitrite. This indicated that these 14 isolates could be made up of NTM's or even *Mycobacterium africanum*. This was confirmed by repeating the nitrate reduction experiment on only the 30 isolates which successfully produced niacin. It was observed that only one isolate failed to reduce nitrate indicating it could be *Mycobacterium africanum* (see Table 2). Also, all these 30 isolates were further confirmed as probably MTBC since they could not grow on LJ media impregnated with *p*-Nitro benzoic acid (PNB). Not surprisingly, 13 out of the 43 acid-fast post-culture isolates grew on LJ media impregnated with *p*-Nitro benzoic acid (0.5mg/ml PNB-containing LJ medium) (see Table 2). As a general observation, most classical *M. tuberculosis* strains are able to reduce nitrate to nitrite. This activity is mediated by the presence of the nitrate reductase enzyme. It is worth commenting on two (2) very important members of the MTBC *viz M. bovis* and *M. africanum*. Strains of *M. bovis* are negative or very weakly positive nitrate reductants. Approximately 20% of *M. africanum* strains successfully reduce nitrate and the rest are negative. Results of the Biochemical tests indicate that though all the 30 isolates realised after culture can be categorised as MTBC, one isolate may be further classified as *M. africanum* (see Table 2).

Table 2. Results of Biochemical Tests

Biochemical Test			**Mycobacteria sub-specie**		
Niacin Production	**Nitrate Reduction (n=43)**	**PNB Test (n=43)**	**MTB**	***M. africanum***	**Non-tuberculous mycobacteria (NTM)**

(n=43)								
+	-	+	-	+	-			
30	13	29	14	13	30	29	1	13

Table showing results obtained from Niacin Production, Nitrate Reduction, Para-nitro benzoic acid (PNB) test for speciation into classical *Mycobacterium tuberculosis*, *Mycobacterium africanum* and NTM or atypical (mycobacteria).

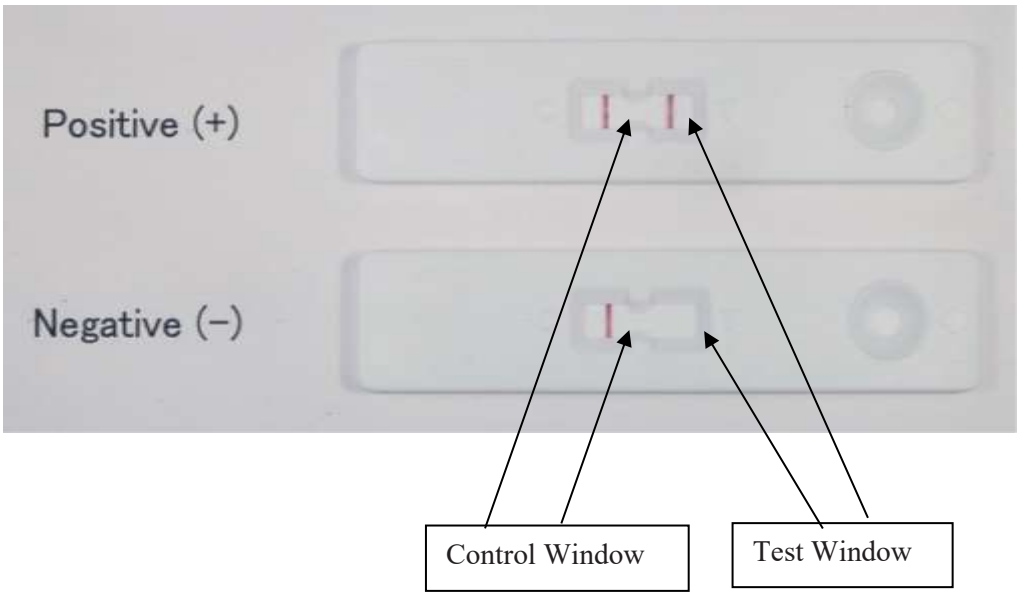
To reiterate, the 43 post-culture acid-fast isolates realised out of the total number of 54 growths harvested from LJ-slants were subjected to the Capilia™ TB-Neo assay to categorise them into either MTBC or non-tuberculous mycobacteria based on the presence or absence of the 24-kDA immunogenic protein MPB64 (see Table 3). For 30 out of the 43 isolates a reddish-purple line was indicated in both the test area (T) and the control area (C) of the Capilia TB-Neo cartridge (see Figure 1 and Table 3).

Table 3. Results of Capilia™ TB-Neo assay

	Presence of MPB64 protein	Absence of MPB64 protein	Mycobacterial species	
			MTBC	**NTM**
Capilia™ assay (n=43)	30	13	30	13

Table displaying results of the Capilia™ TB-Neo assay. Assay categorises Mycobacteria into *Mycobacterium tuberculosis*-complex or Non-tuberculous Mycobacteria (NTM).

Figure 1. A Cartridge of the Capilia® TB-Neo Test. Legend: Figure displaying Positive and Negative results. In the Negative test result, only one reddish-purple band appears in the control (C) window. In the Positive test result, in addition to the control band, a clear distinguishable reddish-purple band also appears in the test (T) window.



All the 43 isolates, out of the 54 harvested growths, which were acid-fast post-culture and subjected to the 16S rDNA PCR assay also confirmed the outcome of the Capilia™ TB-Neo assay (see Table 4).

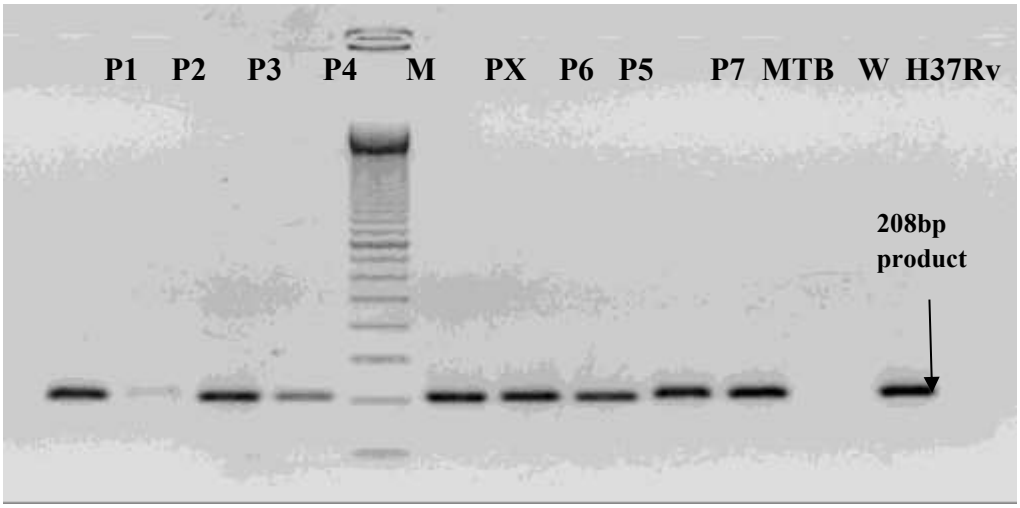
Table 4. Results of 16S rDNA PCR Assay

PCR Assay	Presence of 208bp fragment	Absence of 208bp fragment	Mycobacterial species	
			TBC	NTM
16S rDNA PCR Assay (n=43)	30	13		13

Table showing the results of 16S rDNA PCR Assay based on the IS6110 insertion element to distinguish *Mycobacterium tuberculosis*-complex from other Mycobacteria.

A total of 30 isolates were confirmed as MTBC by the appearance of the 208bp fragment after electrophoresis on agarose while 13 isolates were categorised as NTM or atypical mycobacteria (see Figure 2).

Figure 2. A representative electrophoregram of 16S rDNA PCR products. (Legend: Figure shows 208bp fragment for positive MTBC. P1 – P9 = samples; M= 100bp Molecular Weight ladder; MTB=TB strain +ve control; W=water HPLC-grade as –ve control)



The results in Table 5 shows coughing inmates (and their age characteristics) yielding a positive MTBC isolate as categorised by the Capilia™ TB-Neo assay.

Table 5. Age characteristics of coughing inmates yielding a positive MTBC isolate

Inmate Category	Number	Age Range/yrs	Median Age/yrs	Mean Age/yrs (s.d.)
Convicts	6	22 - 55	35.5	37.0 (12.6)
Remands	24	19 - 53	28.0	30.9 (8.2)
Total	30	19 - 55	28.5	32.1 (9.6)

The outcome of this study is instructive juxtaposing the results obtained from screening the isolates with the Capilia™ TB-Neo test with those obtained from screening with the 16S rDNA PCR assay and assuming the latter to be the 'Gold Standard' (see Table 6).The 16S rDNA PCR assay is an extremely sensitive test. The IS*6110* sequence is one of the more common targets for molecular-based diagnosis of MTBC. The test involves the amplification of a 208 bp fragment of *IS6110* and its sensitivity allows it to be able to detect as little as 100fg ($100x10^{-15}$g) MTB DNA (equivalent to 20 bacterial cells) [20,21]

contained in a sample. The primers pair, pMyc14 and pMyc7, described in the 'Materials and Methods' section offers an accurate diagnostic PCR method for the MTBC because of its high sensitivity. The sensitivity and specificity of the Capilia™ TB-Neo test in diagnostically categorising MTBC isolates is appreciated in the 2X2 contingency Table 6, using 16S rDNA PCR assay as the 'Gold Standard'. The Capilia™ TB-Neo exhibited both a maximum sensitivity (100%) and specificity (100%). The advantages in the application of the Capilia™ test include its sensitivity and specificity being comparable to nucleic acid amplification methods, and the facts that no specialised equipment is required, and speed and ease of performance. As such, it is increasingly finding use in clinical practice [15,16].

Table 6: Comparison of the Capilia™ TB-Neo assay and the 16S rDNA PCR assay in differentiating MTBC from NTM or atypical mycobacteria.

16S rDNA PCR Assay ('Gold Standard')			
Capilia™ Test	**MTBC**	**NTM**	**Total**
MTBC	30 (A)	0 (C)	30
NTM	0 (B)	13 (D)	13
Total	30 (A+B)	13 (C+D)	43

Capilia™ TB-Neo assay is assumed as the test method while 16S rDNA PCR is considered as the 'Gold Standard'): (Total, N=43)

Sensitivity=$\frac{A}{A+B} = \frac{30}{30} = 1.00 \; or \; 100\%$ Specificity $= \frac{D}{C+D} = \frac{13}{13} = 100\%$

Discussion

The prevalence of MTBC amongst coughing prison inmates was determined as 14.0% (30/214). The prevalence of culture-active tuberculosis was determined as 0.7% (45/6,132). Approximately 80% (24/30) of all the culture-active TB identified in this study emanated from remanded inmates. In real terms, however, the prevalence values amongst coughing inmates are 0.06% (6/108) and 22.6% (24/106) for the convicted and remanded populations respectively. For the remand population, this is obviously a high latent infection rate and with the practice of moving batches of remand prisoners between different police cells and other prisons, the remanded inmate population could serve as a reservoir of TB infection within the penal system. Furthermore, with a lethargic and over-burdened legal system in operation, remanded inmates constitute an important segment in any TB management and control strategy. This indicates that the remand population should be given utmost attention in the control and management of TB in the Ghanaian penal environment. That the convicted inmate population accounted for approximately 20% (6/30) of all the culture-active TB identified points to the fact that the existing TB control and management protocol being undertaken by the Ghana Prison Service is fairly efficient and effective in obviating micro-epidemics of the disease. The percentages of MTB growths on culture in the sputa from coughing inmates domiciled in individual cells of selected prisons are interesting and instructive. The highest number of coughing inmates sampled in a single individual cell was 9 out of which 55.6% (5/9) had sputa giving a positive growth of MTB on culture and confirmed as such by 16S rDNA PCR assay and the Capilia™ TB-Neo assay. The number of inmates in each cell ranged from 18 to 25. Out of these 5 inmates, 4 of who were on remand posed a grave threat to inmates in the same prison cell or dormitory.

Additionally, 13 samples elicited from these coughing inmates were found to contain agents other than classical MTB; 8 were NTM and one was *Nocardia nova* (*Nocardiaceae*). The isolate, *Nocardia nova*,

is an unusual isolate in this setting, and it was obtained from the sputum of a 44 year old convicted artisanal metal worker who complained of intense coughing for a week prior to sampling. This convict could recall no previous history of hospitalisation for his cough, but explained that the cough varied in intensity from the mild to the serious and could be described as 'on and off'. This sample was acid-fast on ZN staining and exhibited a creamy, moist, sticky and mildly slimy morphology on LJ slants. Apart from members of *Mycobacteriaceae*, exhibition of acid-fastness on ZN-staining is a well-known feature of members of *Nocardiaceae*, *Gordoniaceae*, *Tackamunellaceae* and *Dietziaceae*. Clearly, this inmate had been infected by *Nocardia nova* and, therefore, may have been exhibiting a subclinical form of Nocardiosis. Nocardiosis is a disease caused by *Nocardia* species found either in the soil or air [35,36]. It can affect such organs as the lungs, brain and the skin [34]. Immuno-compromised individuals and those on anti-cancer medications are particularly susceptible to Nocardia infections. However, whether this inmate was immuno-compromised or not was outside the scope of this study. Norcardia also infects birds [37,38]. This finding may indicate an underlying problem of the possibility of NTM infections occurring together with TB infections in densely populated and cramped institutionalised sections of the Ghanaian population such as the prisons.

Conclusion

The results obtained in this study emanated from techniques which can be easily be employed in resource-stretched settings. These techniques *viz,* microscopy, culture, biochemical speciation tests, the Capilia™ TB-Neo assay and the 16S rDNA PCR assay, are sensitive, easily adaptable and yield sufficiently accurate results for the effective management and control of tuberculosis in Ghanaian prisons. The Capilia™ TB-Neo assay, as demonstrated, can be a useful additional test to include as part of the traditional routine first-line screening tools in a resource-stretched setting. For this study, the detection of a member of *Nocardiaceae,* preliminarily mimicking members of the *Mycobacteriaceae,* indicates the necessity to include a more specific genetic test in the routine tuberculosis screening protocol for populations in institutionalised settings such as the prisons.

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Supplementary files legends

Figures

Figure 1. Two Cartridges of the Capilia® TB-Neo Test

Positive and Negative results of the Capilia® TB-Neo Test. In the Negative test result, only one reddish-purple band appears in the control (C) window. In the Positive test result, in addition to the control band, a clear distinguishable reddish-purple band also appears in the test (T) window.

Figure 2. A representative electrophoregram of 16S rDNA PCR products

Diagnostic 16S rDNA PCR for MTBC. P1 – P9 = samples; M= 100bp Molecular Weight ladder; MTB=TB strain +ve control; W=water HPLC-grade as –ve control legend text.

Tables

Table 1. Age characteristics of coughing inmates sampled

Age characteristics of coughing inmates sampled (n=214).

Table 2. Results of Biochemical Tests

Results obtained from Niacin Production, Nitrate Reduction, Para-nitro benzoic acid (PNB) test for speciation into classical *Mycobacterium tuberculosis* and *Mycobacterium africanum*.

Table 3. Results of Capilia® TB-Neo Test

Results of the Capilia® TB-Neo Test. Test categorises Mycobacteria into *Mycobacterium tuberculosis*-complex or Non-tuberculous Mycobacteria (NTM).

Table 4. Results of 16S rDNA PCR Assay

Results of 16S rDNA PCR Assay based on the IS6110 element to distinguish *Mycobacterium tuberculosis*-complex from other Mycobacteria.

Table 5. Age characteristics of coughing inmates yielding a positive MTBC isolate

Age characteristics of coughing inmates yielding a positive *Mycobacterium tuberculosis*-complex sputum sample.

Additional files

Additional file 1 – Sample additional file title

Additional file descriptions text (including details of how to view the file, if it is in a non-standard format).

Additional file 2 – Another sample additional file title

Additional file descriptions text (including details of how to view the file, if it is in a non-standard format).