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First Nationwide Survey of the Prevalence of TB/HIV Co-Infection in Ghana

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Abstract

Background: To better understand the extent of the magnitude of tuberculosis (TB) and Human Immunodeficiency Virus (HIV) co-infection in Ghana, a baseline study was conducted to establish the national prevalence of the dual infection. The study aimed to determine the most prevalent HIV serotype (HIV-1 or HIV-2) in TB patients (new and old cases); genotype mycobacterial species causing TB/HIV co-infection and determine their drug susceptibility patterns. **Methods:** Sputum and dried blood samples were collected from 503 TB patients from 67 health facilities nationwide between December 2007 and November 2008. All samples were processed for mycobacterial and HIV testing using conventional and molecular methods. **Results:** A total of 517 paired sputum samples were received from 517 patients. A total 503 patients [335 (66.6%) males; 168 (33.4%) females] had at least one culture positive sample. Majority (93.0%) of the patients were new cases while 7.0% were old cases. All 503 TB isolates were *Mycobacterium tuberculosis* complex. Of 503 blood samples, 74 were positive for HIV (14.7%), comprising 71 (14.1%) and 3 (0.6%) for HIV-1 and HIV-1 & 2 respectively; none was positive for HIV-2 alone. The seroprevalence of HIV in newly diagnosed TB patients and those already on treatment, was 69/468 (14.7%) and 5/35 (14.3%) respectively ($p > 0.05$). Differentiation of isolates from TB/HIV co-infected patients showed that 70/74 (94.6%) were *Mycobacterium tuberculosis* while 4/74 (5.4%) were *Mycobacterium africanum*. Monoresistance to isoniazid and rifampicin were 4/74 (5.4%) and 1/74 (1.4%) respectively; resistance to both drugs (multi-drug resistant-MDR) was not observed. Sixty nine (93.2%) isolates were susceptible to both drugs. **Conclusion:** The prevalence of HIV infection in TB patients

was 14.7%. TB/HIV was common among the sexually active age group (25 - 34 years). Majority of the TB isolates were *M. tuberculosis* which were susceptible to both isoniazid and rifampicin. HIV-1 was the common serotype infecting TB patients in Ghana.

Keywords

TB/HIV, Co-Infection, *Mycobacterium tuberculosis* complex, Drug Resistance, Ghana

1. Introduction

The syndemic relationship between tuberculosis (TB) and human immunodeficiency virus (HIV) infection has contributed to high morbidity and mortality worldwide [1]. The World Health Organization (WHO) Africa region is the hardest hit where about 74% of the estimated 1.2 million TB patients co-infected with HIV occurred in 2014 [2]. For many years, efforts to tackle TB and HIV have been largely independent of each other, despite their overlapping epidemiology. The risk of progression from latent to active TB is increased by infection with HIV [3]. Likewise, TB is the most important opportunistic disease that increases progression to acquired immune deficiency syndrome (AIDS) and the number one killer in HIV-positive individuals [4] [5]. Thus effective TB control can contribute to better HIV/AIDS control by reducing the TB burden in people with HIV as well as providing an entry point to HIV prevention and care for people with TB [6]. The HIV prevalence in the general population in Ghana was 1.47% in 2014 which is an indication of a downward trend over a fifteen-year period from 2.3% in 2000 [7]. Studies in Africa and Asia have reported high HIV prevalence in TB patients than are observed in the general population [8]. Routine programme data from TB/HIV sites showed that in 2005, 40 percent of TB patients who were tested for HIV were HIV-positive and in 2006 these went down to 33 percent [9] [10]. Ghana is a high burden TB/HIV country [11], however very few studies have reported on the prevalence of TB/HIV co-infection in Ghana [12] [13] [14]. These previous studies were single hospital-based in the two big cities in Ghana—Accra and Kumasi. There was the need to conduct a nationwide study to establish the national prevalence of this dual infection. This study was therefore conducted to determine the prevalence of HIV in TB patients in Ghana.

2. Materials and Methods

2.1. Study Design

The study was cross-sectional in accordance with WHO Guidelines for HIV surveillance among TB patients [15].

2.2. Study Population and Sampling Strategy

All TB patients diagnosed within the study period between December 2007 and

November 2008 in selected health facilities were eligible for participation. Using a list of all health facilities in the country, the number of registered TB patients in the treatment centres per year was noted. A cumulative population list was then compiled from which participating facilities were selected from all the ten regions of Ghana. Thirty clusters consisting of 67 health facilities were selected and grouped into northern and southern sectors (**Table 1**). Selection of study participants was done using the consecutive sampling method to minimize bias. Hence, every patient who met the eligibility criteria was selected and after obtaining his/her informed consent was interviewed using a structured questionnaire.

2.3. Sample Size Calculation

The minimum sample size was calculated using the formula: $N = PQ/(E/Z)^2$ [15] where N = the minimum sample size required; P = the maximum expected prevalence rate; Q = 100 − P; E = the margin of sampling error tolerated; Z = the centile of the standard normal

If the confidence level is chosen at 95% then Z = 1.96

With the expected HIV prevalence rate among TB patients *(P)* at 30%

Q will be 100 − 30 = 70; and margin of error at 5%, the **minimum sample size** was:

$$N = 30 \times 70 / (5/1.96)^2 = 2100/6.51 = 323$$

To allow for losses, a 60% adjustment was included: 323 × 60% = 194

(These losses included patients diagnosed as having TB who did not return to the centres and from whom it was not possible to obtain 2 sputum samples, patients whose cultures were contaminated or did not grow and patients who refused HIV testing after counselling). Maximum Sample Size = 323 + 194 = **517**

2.4. Sample Collection and Transport

For each eligible patient, an equal volume of 1% Cetylpyridinium Chloride (CPC) was added to two sputum samples for homogenization and decontamination on transit and to maintain the viability of the bacilli if present. Blood was collected onto blood spot collection card to prepare dried blood spot according to the study specific protocol [16]. All the samples were stored at 4˚C and transported within one week to Noguchi Memorial Institute for Medical Research (NMIMR) TB Laboratory for analysis.

2.5. Mycobacterial Identification and Drug Susceptibility Testing (DST)

Preliminary identification was made by the detection of acid fast bacilli (AFBs) in sputum samples by smear examination, using the Ziehl-Neelsen (ZN) staining technique. Subsequently, sputum samples were processed according to WHO guidelines [17]. Briefly, decontaminated sputum samples were centrifuged at 3000 g for 15 minutes and the supernatant discarded. The sediments obtained

Table 1. Selected health facilities (N = 67) and number of isolates obtained (N = 503).

Region	Health facility	Number of isolates
	Northern sector	
Ashanti		38
	1. Komfo Anokye Teaching Hospital	
	2. Obuasi Government Hospital	
	3. Anglogold Ashanti Hospital, Obuasi	
	4. Bryant Mission Hospital, Obuasi	
	5. Agogo Mission Hospital	
	6. Manyhia Hospital	
Brong Ahafo		23
	7. Sunyani Regional Hospital	
	8. Duayaw Nkwanta Catholic Hospital	
	9. Berekum Hospital	
	10. Dormaa Ahenkro Hospital	
	11. Goaso Hospital	
	12. Hwiediem Hospital	
	13. Techiman Catholic Hospital	
	14. Kintampo Hospital	
	15. Nkoranza Hospital	
Northern		42
	16. Tamale Teaching Hospital	
	17. Baptist Medical Center, Nalerigu	
	18. Saboba Hospital	
	19. Salaga Hospital	
	20. Yendi Hospital	
Upper West		25
	21. Wa Hospital	
	22. Nadowli Hospital	
	23. Jirapa Hospital	
	24. Nandom Hospital	
	25. Lawra Hospital	
Upper East		50
	26. Bolgatanga Hospital	
	27. Bawku Hospital	
	28. Navrongo Hospital	
	29. Sandema Hospital	
	30. Wiaga Hospital	
	31. Zebilla Hospital	
	32. Talensi Hospital	

Continued

Southern sector		
Volta		40
	33. Ho District Hospital	
	34. Ho Regional Hospital	
	35. Dzodze Catholic Hospital	
	36. Aflao Hospital	
	37. Amfoega Catholic Hospital	
	38. Kpando Mary Maiguate Hospital	
Central		40
	39. Winneba Hospital	
	40. Swedru Hospital	
	41. Cape Coast Regional Hospital	
	42. Cape Coast District Hospital	
	43. Ankaful Psychiatric Hospital	
	44. St Xavier Hospital, Assin Fosu	
Eastern		74
	45. Regional Hospital, Koforidua	
	46. St Joseph's Hospital, Koforidua	
	47. Nsawam Hospital	
	48. Atua Government Hospital	
	49. St Martin's Hospital, Agomanya	
	50. Akuse Hospital	
	51. Holy Family Hospital, Nkawkaw	
	52. Atibie Government Hospital	
Western		55
	53. Effia Nkwanta Regional Hospital	
	54. Tarkwa Government Hospital	
	55. Eikwe Catholic Hospital	
	56. Axim Hospital	
Greater Accra		116
	57. Tema General Hospital	
	58. Tema Polyclinic	
	59. Tema Adom Clinic	
	60. 37 Military Hospital	
	61. La General Hospital	
	62. Maamobi Clinic	
	63. Kaneshie Polyclinic	
	64. Ussher Polyclinic	
	65. Achimota Hospital	
	66. Ridge Hospital	
	67. Korle-Bu Teaching Hospital	

were reconstituted with sterile phosphate buffer saline (PBS) and about 2 - 3 drops inoculated onto two slopes of Lowenstein-Jensen (LJ) media; one containing glycerol, the other containing 0.5% sodium pyruvate. The inoculated tubes were incubated at 37˚C and monitored weekly for a maximum of 8 weeks for growth and subsequent isolation of mycobacterial species. Final identification of the species were based on acid fastness, colony morphology and ability to grow on LJ media containing 500 mg/ml p-nitrobenzoic acid (PNB) [18]. Line probe assay (LPA) was done to differentiate and determine isoniazid and rifampicin susceptibility profile of mycobacterial isolates from TB/HIV patients using Geno Type MTBC and Geno Type MTBDR *plus* (Hain Lifescience, Nehren Germany) respectively according to manufacturer's instructions [19].

2.6. HIV Testing

All patients were offered pre and post counselling for HIV test according to national guidelines [6]. Initial screening for HIV was done by eluting blood from filter paper and testing with First Response anti-HIV 1 & 2 (Premier Medical Corporation, India). This test simultaneously detects and indicates specific antibodies against HIV-1 and HIV-2. Next, all HIV reactive samples were subjected to a line immunoblot assay, the Inno-Lia Score HIV-I & II (Innogenetics, Belgium) to confirm the presence of HIV-1 and HIV-2 antibodies. Quality control of the HIV test was performed by random testing of 10% of HIV non-reactives from the rapid assay on the line immunoblot assay.

2.7. Data Management and Analysis

Raw data on-site were double entered into a data entry file—Microsoft Office Excel 2003 (Microsoft Excel, Palisade Corp, Newfield, NY, USA). Missing values, inconsistencies and outlier values were checked and corrected where necessary. Analysis of the data was done using Statistical Package for Social Sciences (SPSS) version 15.0 software for windows (SPSS Inc., Chicago, IL, USA). The threshold for statistical significance was $p \leq 0.05$.

2.8. Ethical Considerations

The study was approved by the Scientific and Technical Committee and the Institutional Review Board of Noguchi Memorial Institute for Medical Research, Federal Wide Assurance 00001824 (NMIMR-IRB CPN 022/02-03). All patients involved in the study gave their informed consent prior to participation.

3. Results

Sixty seven health facilities from the northern and southern sectors of Ghana were involved in the study (**Table 1**). A total of 517 paired positive sputum samples were received from 517 patients. Out of this, a total of 503 TB patients [335 (66.6%) males and 168 (33.4%) females] had at least one culture positive sample (**Table 1**). Most patients, 136 (27.0%) were within the age group 25 - 34 years ($p < 0.001$). There were more new cases 468/503 (93.0%) than old cases 35/503

(7.0%) ($p < 0.001$). Majority (73.6%), 370 out of 503 were employed in the informal sector ($p < 0.001$). The difference in HIV seroprevalence rates between males, 36/335 (10.75%) and females, 38/168 (22.6%) was statistically significant ($p < 0.05$). The seroprevalence of HIV in newly diagnosed TB patients and those already on treatment, was 69/468 (14.7%) and 5/35 (14.3%) respectively ($p > 0.05$) (Table 2). Out of the 503 dried blood spots tested for HIV, 74 (14.7%) were seropositive. This comprises 71 (14.1%) HIV-1 and 3 (0.6%) HIV-1 & 2. None of the samples were positive for HIV-2 infection alone (Table 3). All the 503 isolates were *Mycobacterium tuberculosis* complex (MTBC) based on phenotypic identification. The LPA run for all 74 isolates from TB/HIV co-infected patients showed that 70 (94.6%) were *Mycobacterium tuberculosis* while the remaining 4 (6%) were *Mycobacterium africanum* (Table 4). Monoresistance to isoniazid and rifampicin were 4 (5.4%) and 1 (1%) respectively. However, resistance to both drugs (multi-drug resistant-MDR) was not observed. Sixty nine (93%) isolates were susceptible to both drugs (Figure 1).

Table 2. Socio-demographic characteristics of patients whose culture were positive (N = 503).

Variable	Frequency (%)	P value	HIV seroprevalence	P value
Sex				
Male	335 (66.6)	$p < 0.001$	36	$p < 0.05$
Female	168 (33.4)		38	
Age group (years)				
< 15	7 (1.4)		$p < 0.001$	
15-24	71 (14.1)			
25-34	136 (27.0)			
35-44	107 (21.3)			
45-54	111 (22.1)			
55-64	30 (5.9)			
65+	41 (8.2)			
TB Status				
New case[a]	468 (93.0)	$p < 0.001$	69	$p > 0.05$
Old case[b]	35 (7.0)		5	
Occupation				
Informal[c]	370 (73.6)			
Formal[d]	26 (5.2)			
Unemployed[e]	42 (8.3)			
Student[f]	65 (12.9)			

[a]Pulmonary TB patients in the selected diagnostic centres who have never been treated for TB or have been treated for a period less than one month. [b]All patients who have been previously treated for TB for a period more than one month. [c]Jobs performed outside the formal structures that govern taxes, workplace regulations and social protection schemes. [d]Jobs performed within formal structures that govern taxes, workplace regulations and social protection schemes. [e]Not engaged in any gainful occupation. [f]Someone who attends an educational institution and not engaged in any gainful occupation.

Table 3. HIV seroprevalence in 503 TB patients.

Region	[a]First Response		[b]Inno-Lia		
	HIV-1	HIV-2	HIV-1	HIV-2	HIV- 1&2
Greater Accra	12	0	12	0	0
Eastern	14	0	13	0	1
Western	8	0	8	0	0
Central	4	0	4	0	0
Volta	7	0	7	0	0
Ashanti	4	0	3	0	1
Brong-Ahafo	2	0	1	0	1
Northern	7	0	7	0	0
Upper East	10	0	10	0	0
Upper West	6	0	6	0	0
TOTAL (%)	74 (14.7)	0 (0)	71 (14.1)	0 (0)	3 (0.6)

[a]First Response—a rapid discriminatory assay for HIV-1 and HIV-2 antibodies. [b]Inno-Lia—a line immunoblot assay used to confirm the presence of HIV antibodies in reactive HIV samples from initial rapid screening.

Table 4. Differentiation of MTBC isolates (N = 74) from TB/HIV co-infected participants using GenoType MTBC Assay.

MTBC Species	Number	Percentage (%)
Mycobacterium tuberculosis	70	94.6
Mycobacterium africanum	4	5.4

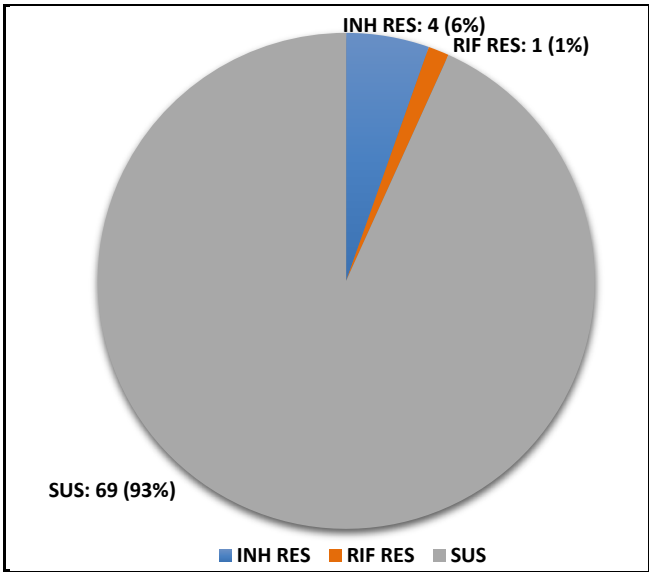

Figure 1. Drug Susceptibility Test (DST) profile of isolates (N = 74) obtained from the TB/HIV co-infected participants. INH RES: isolates that are resistant to isoniazid. RIF RES: isolates that are resistant to rifampicin. SUS: isolates that are susceptible to both isoniazid and rifampicin.

4. Discussion

TB/HIV co-infection is of great public health concern worldwide. This study sought to provide baseline data to inform health policies and actions to address this deadly disease duo in Ghana. Testing for HIV antibodies in TB patients as used in this study seemed an appropriate strategy to determine the burden of TB/HIV. While screening HIV-positive individuals for TB infection may be another option, HIV patients already burdened with stigmatisation may be unwilling to undertake a TB test as well. This assertion corroborates a report [15] showing that TB patients are more likely to accept HIV testing than HIV patients testing for TB. HIV prevalence in TB patients can be used as an indicator of the degree of spread of HIV in the general population. This information is also important for the provision of comprehensive HIV/AIDS care and support. HIV infection rates of up to 48% have been reported among newly diagnosed TB cases in Ghana [20], but the burden of TB among HIV-infected individuals in Ghana is barely acknowledged. Consistent with the global trend, more males (66.6%) than females (33.4%) were diagnosed with TB in this study [1]. The reasons for gender disparity with regards to TB infection is not well understood though it is believed that some cultural and biological factors may play a role [21]. Majority 70/74 (94.6%) of the TB/HIV co-infected patients were infected with *M. tuberculosis* which is very similar to what other studies have reported in Ghana [22] [23]. On the contrary, the number of *M. africanum* (5.4%) isolates was relatively low. Almost 93% of isolates obtained from TB patients were susceptible to both isoniazid and rifampicin. This result may be due to the fact that majority of the patients were new cases and as such had not taken any anti-TB drug before. The overall TB/HIV co-infection prevalence was 14.7% which was consistent with the 14.8% reported earlier in Ghana [9]. However, it was relatively lower than previous rates of 23.2%, 16.8 % and 46.2% respectively reported in Ghana [12] [13] [14] as well as the average rate for Africa (32%) [24]. These previous studies from Ghana were conducted in a very limited number of sites and the study population may not exactly reflect the general population. In Ghana infection with HIV-1 is most common, accounting for over 90% with very few cases of mixed infection of both HIV-1 and HIV-2 reported [25]. Hence it was not surprising to find similar results in this present study. There is an indication that there has been a steady change in the prevalence of TB/HIV co-infection over the last decade. This change in co-infection prevalence might be the result of improvements in public awareness, better treatment regimens and a general decline in HIV prevalence in Ghana [7]. Conversely, TB prevalence in Ghana is reported to be about three times higher than the estimates from WHO prior to the conduct of a TB prevalence survey in 2013 [2]. This suggests that many people are at an increased risk of HIV infection which necessitates a continuous collaborative plan for TB and HIV control activities to ensure comprehensive care for TB/HIV patients.

Some possible limitations in this study were that while TB patients will benefit from this study by knowing their HIV status and thus access treatment and care

for both diseases, the same cannot be said for HIV patients who may be harbouring TB since the study focused on TB infected persons and not HIV infected persons. That notwithstanding, the results have been useful in strengthening the implementation of the joint TB/HIV strategy in Ghana. It has also been a vital biological marker of the HIV/AIDS epidemic in Ghana and indicating the impact on TB infection. Funds for the molecular testing part of the study became available in 2017 hence the delay in the publication of the findings.

5. Conclusion

The prevalence of TB/HIV co-infection was 14.7%, and co-infection was more common in the sexually active age group of 25 - 34 years. Majority of these TB/HIV co-infected patients were infected with *M. tuberculosis* which were susceptible to both isoniazid and rifampicin. HIV-1 was the most common HIV strain infecting these patients. Further research is needed to monitor the trend and level of interaction between TB and HIV in Ghana.

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Competing Interest

Authors declared that they have no competing interests.

Authors' Contribution

KKA and WKA came out with the study design and supervised the laboratory work. KKA contributed to the writing and editing of the manuscript. RO, CB and NN conducted the conventional TB laboratory work. JK and KB performed the HIV testing. SOA performed the molecular laboratory work, contributed to the manuscript writing and data analysis. GIM assisted with the proposal writing and data analysis. AH edited the study protocols. NAA and FAB contributed to the study implementation by providing reagents and equipment. All authors read and approved the final manuscript before submission.

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List of Abbreviations

AFB: Acid fast bacilli
AIDS: acquired immune deficiency syndrome
A-LYS: Alcalic lysis
Anti-TB: Anti-tuberculosis
CPC: Cetylpyridinium Chloride
DOTS: Directly Observed Treatment Short course
DST: Drug susceptibility testing
HIV: Human Immunodeficiency Virus
LJ: Lowenstein-Jensen
LPA: Line probe assay
MDR: Multi-drug resistance
MTBC: *Mycobacterium tuberculosis* complex
NMIMR: Noguchi Memorial Institute for Medical Research
TB: Tuberculosis
PBS: Phosphate buffer saline
PCR: Polymerase chain reaction
PNB: Para-Nitrobenzoic acid
WHO: World Health Organization
ZN: Ziehl-Neelsen