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Epidemiology of Clinical Isolates of *Mycobacterium tuberculosis* at Ibadan, Nigeria

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Summary: Despite the huge burden of tuberculosis (TB) in Nigeria, case detection rate of infectious cases still remain low, thus constituting obstacle to eradication of the disease in the community. We carried out a 15 month (1st January 2008 to 30th March 2009) retrospective review of epidemiology of clinical isolates of *M. tuberculosis* isolated at TB regional reference laboratory at the department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria**.** Fifty isolates were recovered from 720 specimens during the period of study with a recovery rate of 6.9%. Sixty-two (8.6%) of the specimens were contaminated. Thirty eight (76.0%) isolates were from the specimens of male subjects and 12 (24.0%) from female subjects giving a male to female ratio of 3.2: 1.0 Majority (62.0%) of the isolates were from subjects aged 20 years and above with an isolation rate of 7.3% while only two clinical isolates (4.0%) were recovered from specimens from children. A high yield of 20.8% was recovered from specimen collected from Hausa ethnic group who predominantly domiciled in a particular part of the metropolis. In terms of socio-economic status, clinical isolates recovered from specimens from unskilled workers (76.0%) was more than thrice from that obtained from the professionals (24.0%). Seven (14.0%) of the total isolates were recovered from extra-pulmonary lesions while the majority 43 (86.0%) were for pulmonary TB. The isolation rate from children and extra-pulmonary sites are low. This suggests a need to pay more attention to diagnosis of childhood and extra-pulmonary TB in Ibadan, Nigeria.

Keywords*: M. tuberculosis*, Isolates, Epidemiology, Ibadan

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INTRODUCTION

In spite of substantial success in implementing standardized care and improving rates of cure in recent years, the global burden of tuberculosis (TB) remains enormous (WHO, 2009). With nearly nine million people infected and an estimated 1.6 million deaths from the disease each year, TB is considered as an important public health issue worldwide (WHO, 2009). Despite this immense global burden, case detection rates are low (WHO, 2009), posing serious hurdles for global TB control. Nigeria has one of the highest TB burdens in the world and one of the lowest case detection rates among the World Health Organization (WHO) designated 22 high burden countries (WHO, 2006). Directly Observed Treatment Short Course (DOTS), the WHO recommended strategy for global eradication of TB was started in 1993 in Nigeria by the National TB and Leprosy Control Program (NTBLCP) (Open Society Institute, 2006). The percentage of DOTS coverage in Nigeria is 75% as at 2006 (WHO, 2008).

Four technologies are commonly used in TB diagnosis in disease-endemic countries including Nigeria. They include radiography, bacteriology, histopathology and tuberculin skin test. As one of the components of DOTS strategy, quality-assured bacteriology is regarded as gold standard for TB diagnosis all over the world. In populations with high TB incidence as seen in sub-Saharan Africa, identification of acid-fast bacilli (AFB) in samples from suspected site of disease provides immediate diagnosis with high specificity and is regarded as the practical gold standard. Sputum microscopy has two important drawbacks- it is laborious and is of low sensitivity especially in situations where there is high prevalence of TB/HIV co-infections. Isolation of *Mycobacterium tuberculosis* on selective medium is considered as bacteriological confirmation of TB. Where quality-assured laboratories for TB cultures exist, sputum cultures should be done along with microscopy. This would facilitate adequate monitoring of treatment and also pave way for early detection of resistant organisms. The scarcity of

information on the epidemiology of clinical isolates of *M. tuberculosis* in Nigeria might be explained by lack of adequate infrastructure and trained manpower (Grange and Zumla, 2002; Kehinde et al, 2005). This study was therefore carried out to provide baseline epidemiological data on clinical isolates of *M. tuberculosis* isolated at TB regional reference laboratory in Ibadan, Nigeria.

MATERIALS AND METHODS

This was a 15 month (1st January 2008 to 30th March 2009) laboratory- based retrospective study that was carried out at the TB laboratory, department of Medical Microbiology and Parasitology, University College Hospital (UCH), Ibadan. TB laboratory in UCH is a regional reference laboratory in Southwestern part of Nigeria with facilities for isolation of *M. tuberculosis*. It is supported by Damien Foundation, Belgium through the NTBLCP of the Federal Ministry of Health, Abuja. It receives specimens within and outside the hospital including adjoining health care centers. Epidemiologic information such as age, gender, tribe, profession, history of chronic cough, history of smoking and site of infection of subjects who submitted samples during the study period were retrieved from the available hospital register while culture results were sought out from the laboratory register.

For suspected cases of pulmonary TB, three sputum samples from each subject were collected onto a well –labeled wide mouth container covered with lid while only one specimen –blood, cerebrospinal fluid, urine, etc was collected from those with extra pulmonary lesions. Specimens containing saliva were discarded. The specimens were transported to the TB laboratory for immediate processing. Each specimen was smeared using Zeihl-Neelsen (Z-N) reagents hot method (Isenberg, 1992). The staining technique was standardized by using known AFB slide and slide stained with egg albumin as positive and negative controls respectively. Apart from that, quality control of the Z-N reagents was included in every staining slide. The procedure was read according to grading system of the International Union Against TB and Lung Disease as –ve, scanty, 1+, 2+, 3+ (Enarson, 2000). The three sputum specimens from each subject were pulled together and then decontaminated using 4% NAOH solution. One ml of the resulting solution was inoculated onto freshly prepared modified egg- based Ogawa medium (Saito et al, 1978) and incubated at 37^{o}C for six weeks. *M. tuberculosis* strain H37Rv and sterile Ogawa medium were used as positive and negative controls respectively. Contamination of culture was determined by looking for visible growth on Ogawa medium on or before two weeks of incubation and also by repeat Z-N staining reaction. Isolates were confirmed as *M. tuberculosis* by standard biochemical tests (Barrow and Feltham, 1995). Data were analyzed by using frequency tables and percentages

RESULTS

Table 1 shows epidemiology of clinical isolates of *M. tuberculosis* isolated in Ibadan, Nigeria during the study period. Fifty isolates were obtained from 720 samples processed giving an isolation rate of 6.9%. Thirty eight isolates (76.0%) were from specimens from male subjects while specimens from female subjects constituted 12 (24.0%), giving a male to female ratio of 3.2: 1.0

Table 1:
Epidemiology of *M. tuberculosis* isolates

VARIABLE	Clinical Isolates (n)	% Isolation
Gender		
Male (n= 498)	38	7.6%
Female (n=222)	12	5.4%
Total (n=720)	50	6.9%
Age (in yrs)		
<10 (n= 78)	02	2.6%
11-20 (n=216)	17	7.9%
>20 (n=426)	31	7.3%
Total (n=720)	50	6.9%
Tribe		
Yoruba (n=564)	28	5.0%
Hausa (n=96)	20	20.8%
Others (n=60	02	3.3%
Total (n=720)	50	6.9%
Profession		
Unskilled (n=516)	38	7.4%
Skilled (n=204)	12	5.9%
Total (n=720)	50	6.9%
History of chronic cough		
Yes (n=624)	46	7.4%
No (n=96)	04	6.7%
Total (n=720)	50	6.9%
History of smoking		
Yes (n=204)	31	15.2%
No (n=516)	19	3.7%
Total (n=720)	50	6.9%
Site of infection		
Pulmonary (n=546)	43	7.9%
Genitourinary (n=45)	02	4.4%
Bone and joint (n=36)	02	5.6%
Miliary (n=42)	02	4.8%
Central nervous system (n =51)	01	2.0%
Total (n=720)	50	6.9%

Only two isolates (2.6%) were obtained from children less than 10 years of age while 31 clinical isolates were from patients aged 20 years and above giving an isolation rate of 7.3%.

A higher yield for *M. tuberculosis* (20.8%) was obtained from samples collected from the Hausa ethnic group compared with 5.0% obtained from samples collected from the Yoruba ethnic group.

Concerning the profession of subjects from whom the samples were collected, 38 (76.0%) of the isolates were from unskilled workers while 12 (24.0%) were recovered from professionals. More than 90% of the total number of isolates were from patients who gave positive history of chronic cough while a lower percentage (8.0%) were from those with negative history. Thirty one *M. tuberculosis* isolates (62.0%) were obtained from samples collected from smokers while 19 (38.0%) were from non-smokers. Only one isolate was recovered from cerebrospinal fluid giving an isolation rate of 2.0% while the majority (86.0%) was obtained from the pulmonary. Of the total specimens cultured, 62 (8.6%) were contaminated.

DISCUSSION

Bacteriological isolation of *M. tuberculosis* in pure culture from an infected site is regarded as confirmation for diagnosis of TB. Furthermore, the culture method of isolation which was founded in the early days after Koch's discovery of tubercle bacilli has since established itself as gold standard for TB diagnosis worldwide. The overall isolation rate of 6.9% obtained in this study is lower than 10.7% obtained by the same author and his co-workers in their ten year retrospective review of laboratory reports of pulmonary TB in Ibadan some years ago (Kehinde et al, 2006). This may be due to shorter period of study compared with 10 year review in the previous work.

The isolation rate of 2.6% recovered from children with TB in this study was low. This may be due to the fact that most of the times, children with TB have closed caseous lesions with antecedent low numbers of viable mycobacteria when compared with adults who often have open cavities which contain large number of organisms (Starke, 2001).Globally, establishing a definitive diagnosis of childhood TB has remained a challenge for NTBLCP especially in resourced-limited countries of Africa and Asia which harbour significant burden of the disease (WHO, 2008).

A high yield of 20.8% recovered from samples collected from Hausa ethnic group may be culturally ascribed to the fact that they tend to be their brothers' keepers by living together in large groups. This situation favours transmission of droplet air-borne infections including pulmonary TB. The close relationship between low socio-economic class vis a vis poverty and TB has been well documented (WHO, 2005). This may explain higher recovery rate of 7.4% from unskilled workers compared with 5.9% obtained from the professionals. The NTBLCP in many of the disease endemic countries should find ways by which DOTS program would be more accessible to the poor.

Only four *M. tuberculosis* isolates were recovered from samples collected from patients who gave negative history of chronic cough. This emphasizes difficulty involved in the diagnosis of extra-pulmonary TB in which chronic cough which is one of the cardinal symptoms of TB may be absent. The diagnostic dilemma becomes more worrisome in life-threatening situations such as TB meningitis in which diagnoses are often made at post mortem especially, in communities with inadequate facilities.

The fact that high yield of 15.2% was recovered from samples collected from patients with positive history of chronic smoking supports the assertion that chronic smoking is a risk factor for pulmonary TB (Kehinde et al, 2010) and is often regarded as *sine qua non* to acquisition of other respiratory tract diseases such as bronchial asthma and lung cancer.

Nearly 90% of the culture isolates were from patients with pulmonary TB with isolation rate of 7.9% while only 7 (14.0%) were from extra-pulmonary lesions. This is not surprising because pulmonary TB is the commonest presentation of TB but diagnostic dilemma associated with extra-pulmonary TB may be responsible for its' underreporting. Ige and his co-workers (Ige et al, 2005) reported 22.4% prevalence of extra-pulmonary TB cases in Ibadan in 2005 while 12.3% prevalence was documented at Ile Ife (Erhabor et al, 2003).

In conclusion, though culture method is regarded as gold standard for TB diagnosis worldwide, the need to fast-tract development of better tools for diagnosis of extra-pulmonary TB and childhood TB is urgent in order to reduce their morbidity and mortality in the community.

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