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Research Article

MYCOBACTERIUM TUBERCULOSIS INFECTION AMONG HOUSEHOLD MEMBERS OF PULMONARY TUBERCULOSIS PATIENTS IN ANMBRA STATE, NIGERIA

Oluboyo Bernard[1*]., Enweani Ifeoma[2]., Ekejindu Ifeoma[2] and Oluboyo Adeola[1]

[1]Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria
[2]Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria

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A B S T R A C T

Tuberculosis is spread to people through inhalation of minute flakes of protein that bears living tubercle bacilli in the air. This study investigated the prevalence of *Mycobacterium tuberculosis* among household members of pulmonary tuberculosis patients. Two hundred sputa from 96 males and 104 females between ages 10 and 50 years were collected from symptomatic household members of pulmonary tuberculosis patients. The samples were examined for the presence of acid fast bacilli using Ziehl Neelsen staining technique and cultured on Lowenstein-Jenson medium for the isolation of *Mycobacterium tuberculosis*. Sputa culture recorded a total prevalence rate of 8.0% (3.5% males and 4.5% females). Total prevalence rate of 5.5% was recorded in Ziehl Neelsen staining (2.5% males and 3.0% females). Thus, 31.2% of culture positive sputa remained undetected by smear staining. The prevalence rate recorded in sputa culture was significantly ($p<0.05$) higher than that recorded for Ziehl-Neelson staining. No significant increase ($p>0.05$) in prevalence was recorded between gender groups and across age ranges respectively. An unacceptably high rate of tuberculosis infection occurs among the household members of Tuberculosis patients. More of public enlightenment crusades on the mode of Tuberculosis transmission are urgently needed to curb the spread of tuberculosis to household members.

INTRODUCTION

Tuberculosis is a widespread and often lethal infectious disease caused by different strains of mycobacterium; usually *Mycobacterium tuberculosis* (MTB) in humans (Kumar *et al*., 2007). Record has it that one third of the world's population is thought to be infected with MTB (Jasmer, 2002). The incidence of infections by the bacteria are said to happen at the rate of about one infection per second (World Health Organization [WHO], 2007). Tuberculosis (TB) is one of the top 10 causes of death worldwide (WHO, 2017). Six countries accounted for 60% of the new cases of tuberculosis globally and Nigeria ranked 4th on the list. The statement should read Nigeria rank forth on the list - India (1st), Indonesia (2nd), and China (3rd). Nigeria equally ranked 4th of the top 20 countries in absolute numbers and tuberculosis burden severity based on 2014 estimate published in the 2015 global report on tuberculosis (WHO, 2015). The incidence of tuberculosis in Nigeria is falling but at a slow rate. It fell gradually from 331 cases per 100,000 people in 2001 to 322 cases per 100,000 people in 2015 (Trading Economics, 2017).

Corresponding author:* **Oluboyo Bernard
Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread through the air, when people with active pulmonary tuberculosis (PTB) cough, sneeze, speak, sing or spit expelling aerosol droplets (Azadeh, 2015). A single sneeze is estimated to release up to 40,000 droplets (Cole and Cook, 1998, Azadeh, 2015). Each of these droplets is capable of transmitting the disease, since the infectious dose of 10 bacteria may cause tuberculosis (Nicas, *et al*., 2005, Azadeh, 2015). Individuals with protracted illnesses or in close contact with tuberculosis patients are particularly at high risk of becoming infected, with an estimated 22% infection rate (Ahmed and Hasnain, 2011, Azadeh, 2015). An individual with active but untreated tuberculosis may infect 10-15 other people through close contact over the cause of one year (WHO, 2017).

People with latent infection are not thought to be contagious (Kumar *et al*., 2007) but can transmit infection if the bacteria become active in them. Transmission of infection from person to person can be circumvented by isolating patients with active tuberculosis and placing them on anti-tuberculosis drug regimens. After about two weeks of treatment, subjects with nonresistant active infections generally do not remain contagious to others (Ahmed and Hasnain, 2011). It typically takes three to four weeks before the newly infected person

becomes infectious enough to transmit the disease to others. Identifying such persons and treating them in time would be one of the ways of minimizing the spread of the disease and reducing its prevalence. Household members of TB patients are at risk of contracting the disease due to close contacts especially in overcrowded accommodation with little or no proper ventilation. Not many researchers have investigated household members of pulmonary tuberculosis patients in Anambra State for the prevalence of *Mycobacterium tuberculosis*. This study therefore investigated the prevalence of *Mycobacterium tuberculosis* among symptomatic household members of tuberculosis patients in Anambra State.

MATERIALS AND METHODS

A total of 200 individuals consisting of 96 males, 104 females of ages between 10 and 50 years were examined, in this explorative cohort study of PTB infectivity among household members of tuberculosis patients. Coughing co-inhabitants of PTB patients were selectively investigated for PTB using smear AFB staining and sputum culture. This investigation was done in Anambra State, Nigeria. Anambra State is within latitude 6.2758^{o}N and longitude 7.0068^{o}E with population of 4,055,048 and land area of 4,844km^{2} (thegpscoordinates.net/Nigeria/anambra).

Ethical approval

The ethical approval to conduct this research was given by the ethical committee of the Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. The protocol for the research project was approved by the committee.

Informed consent

The consent of participants in this study was sought for and obtained. Only sputa from the participants were required.

Sample collection

Tuberculosis patients were identified at the Nnamdi Azikiwe University Teaching Hospital where they access medication for their ailment. The household of the patients were visited and coughing members who were not yet diagnosed of tuberculosis were investigated. Three sputa samples collected in three consecutive days into sterile wide-mouth bottles (Cheesbrough, 1994) were taken to Mega Diagnostic Laboratories, Nnewi, for analysis within one hour of collection.

Sputum AFB staining

Using sterile applicator stick, purulent part of the sputa were transferred to slides and made into about 30 mm round smear. The smear were allowed to air-dry completely and fixed with 2 drops of 70% ethanol and stained with Ziehl-Neelsen stain (Cheesbrough, 1994).

Sputum culture

The sputa were decontaminated by mixing equal volume of sputum and sodium hydroxide (40g/l) solution and shaken at intervals to homogenize the sputum. Using a sterile Pasteur pipette, 200µl of the well-mixed homogenized sputa were inoculated on the slope of acid Lowenstein-Jenson medium in McCartney bottles and allowed to run down the slope (Cheesbrough, 1994). The inoculated slopes were incubated at 37^{o}C and occurrence of growth was observed at weekly intervals for 8 weeks in a rack placed at an angle of about 45^{o}C to ensure that the specimen is in contact with the full length of the slope (Cheesbrough, 1994, Adler *et al*., 2005, Satapathy *et al*., 2014).

Mycobacterium tuberculosis identification

MTB was identified through colony characteristics and confirmed as acid fast bacilli through Ziehl-Neelsen staining technique (Venkataswamy *et al*., 2007). Test for pigment production was done by leaving the culture in the light for 2 hours and reincubating at 37^{o}C overnight and the colonies were then examined for pigmentation (Cheesbrough, 1994). Colonies that do not produce pigment in light or darkness were identified as *Mycobacterium tuberculosis*. The subculture of the organisms were further incubated at 25^{o}C and examined for growth. MTB was further identified through failure to grow at 25^{o}C and on Lowenstein-Jensen medium containing 500µg/ml of 4 (p) – nitrobenzoic acid medium (Cheesbrough, 1994, Satapathy *et al*., 2014).

Statistical analysis

The data generated were analyzed using statistical package for social sciences version 17. The frequencies were recorded in percentages and compared using chi square at significant level of $p< 0.05$

RESULTS

Sputa culture recorded total prevalence rate of 8.0%. This consisted of 3.5% males and 4.5% females (Table 1).

Table 1 Prevalence of *Mycobacterium tuberculosis* in sputum culture in relation to smear AFB staining (n=200).

Investigation	Positive n (%)	Negative n (%)	χ^2	p-value
Sputum culture	16(8.0)	184 (92)		
Smear AFB Staining	11 (5.5)	189 (94.5)	133.862	0.000

$p < 0.05$, significant
NB: 68.8% (11 out of 16) of *Mycobacterium tuberculosis* culture positive sputa were detected using smear AFB staining living 31.2% undetected.

Total prevalence rate of 5.5% was recorded for Ziehl-Neelsen staining. This consisted of 2.5% males and 3.0% females (Table 1). Of the 16 culture positive sputa, 11 (68.8%) were smear positive; 31.2% of the culture positive sputa remained undetected. Among the males tested using culture, prevalence rate was 7.3% while among the females prevalence rate of 8.7% was recorded (Table 2).

Table 2 Prevalence of *Mycobacterium tuberculosis* in sputum culture and smear AFB staining in relation to gender.

Investigation	Gender	Total	Positive n(%)	Negative n(%)	χ^2	p-value
Sputum culture	Male	96	7(7.3)	89 (92.7)		
	Female	104	9 (8.7)	95 (91.3)	0.126	0.723
Smear AFB staining	Male	96	5 (5.2)	91(94.8)	0.03	0.862
	Female	104	6 (5.8)	98 (94.2)		

In Ziehl-Neelson staining technique, prevalence rate among males was 5.2% while among females, the prevalence rate 5.8% was recorded. The prevalence rate recorded in sputa culture was significantly ($p<0.05$) higher than that recorded for Ziehl-Neelson staining technique. No significant increase in prevalence ($p>0.05$) was recorded between gender groups (Table 2) and across age grades (Table 3) respectively.

Table 3 *Mycobacterium tuberculosis* in sputum culture and smear AFB staining in relation to age grade of subjects

Age grade	Sputum culture				
	Total	Positive	Negative	χ^2	p-value
10-19	62	4 (6.5)	58 (93.5)		
20-29	87	8 (9.2)	79 (90.8)		
30-39	41	3 (7.3)	38 (92.7)	0.451	0.929
40-49	10	1 (10.0)	9 (90.0)		
Smear AFB staining					
10-19	62	3 (4.8)	59 (95.2)		
20-29	87	5 (5.7)	82 (92.3)		
30-39	41	2 (4.9)	39 (95.1)	0.483	0.925
40-49	10	1 (10.0)	9 (90.0)		

DISCUSSION

Infection rate among household members of MTB patients in this study was 8.0% with sputum culture from symptomatic household members. This is higher than records of similar studies such as 6.9% (Singh *et al*., 2013), 5.3% (Nair *et al*., 2016), 1.15% (Gupta *et al*., (2016) all in India and 3.76% (Xu and Hu, 2008) in China. The TB patients in the present study were all undergoing anti-tuberculosis medication. After about two weeks of treatment, subjects with nonresistant active infections are expected to be generally non-contagious to others (Ahmed and Hasnain, 2011). The prevalence recorded in this study may therefore be considered high. However, infections may have taken place among some of the household members before the sick individuals reported to the hospital for medical assistance. Infectivity rate of *Mycobacterium tuberculosis* among people in close contact with active tuberculosis patients is said to be 22% (Ahmed and Hasnain, 2011, Azadeh, 2015) in cases before drug intervention. Early TB diagnosis and subsequent early chemotherapy is vital in the prevention of the spread of PTB to household members of TB patients. Of the total culture positive sputa, 68.8% were smear positive living 31.2% positive samples undiscovered. This is however lower than 40-70% range of undetected tuberculosis using smear test (Pio and Chaulet (2003).

There was no gender related selective infection in susceptibility to *Mycobacterium tuberculosis*. Though higher number of females in the household of TB patients tested positive to both smear AFB and sputum culture, the difference is not statistically significant ($p>0.05$). Borgdorff *et al* (2000) however observed that TB is diagnosed more often in men than in women in both routine notifications and prevalence surveys. The deviation in this study could be because greater symptomatic females were investigated as there are generally more females in the households visited.

TB infects people of all ages. Age 20-29 recorded the highest prevalence of PTB in this study. However, prevalence is not statistically significant ($p>0.05$) across the age ranges. While Borgdorff *et al* (2001) recorded peak incidence of PTB infection of 5.9 per 100,000 populations per year at age range of 25-34, Blaser *et al* (2016) recorded peak incidence of PTB infection of 463 per 100,000 people at age range 25-29. The age range corresponding to the peak levels of infection in this study (20-29) appear similar to peak age ranges (25-34, 25-29) recorded by the above researchers. This age range (20-35) may be having more contact with PTB patients in the household and could be targeted in a wider society for TB prevention enlightenment programme.

PTB prevalence of 8.0% warrants TB education for people with PTB on how to make sure that they do not transmit TB to other people. Household members of PTB patients also need TB education to prevent infection by the bacterium while caring for their sick relations. While strict adherence to TB medications is recommended, other standard recommendations demand that smear positive TB patients spend as much time as possible outdoors, and (if possible) sleep alone in a separate, sufficiently ventilated room (WHO, 2008). Cough etiquette and respiratory hygiene are further measures against spreading of pulmonary TB in public places (WHO, 2009).

In conclusion, this study presents the need to target household members of PTB patients for TB education as a step to prevention of TB spread in the general public. About 31.2% of PTB patients may not be detected early if reliance for diagnosis is only on smear investigation. PTB spread is generally neither gender related nor age selective. Identification of infected household members of PTB patients and subsequent treatment of such individuals will help in reducing the spread of the disease among household members.

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Conflict of interest

The authors express no conflict of interest in this research.

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