http://dx.doi.org/10.4314/bajopas.v12i1.33S

Bayero Journal of Pure and Applied Sciences, 12(1): 209 - 214
ISSN 2006 – 6996

A SURVEY OF CO-INFECTION OF SOME PATHOGENIC BACTERIA WITH TB IN PATIENTS ATTENDING FEDERAL MEDICAL CENTER KATSINA, NIGERIA

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ABSTRACT
Tuberculosis (TB) is known to be one of the oldest forms of human diseases which still remain the leading cause of death worldwide. It is characterized as a pulmonary disease which occurs due to accumulation of Mycobacterium tuberculosis (MTB) onto the lungs alveolar surfaces. M. tuberculosis is an implicated pathogenic bacteria associated with T.B. It is the chronic infectious disease caused by the tubercle bacillus (M. tuberculosis). This study was aimed to determine co-infection of other pathogenic bacteria within MTB patients attending Federal Medical Centre (FMC), Katsina. The study design was cross-sectional, conducted to isolate some pathogenic bacteria that co-infected TB patients who are Acid fast bacilli (AFB) positive and AFB negative. The samples obtained were cultured on Blood, Chocolate and MacConkey agar and incubated at 37°C for 24 hours. Pure isolates were confirmed using Grams Staining and biochemical reaction. Data obtained were presented as simple percentage and using statistical analysis which revealed that the incidence was common among the age categories 51-60 with 28%, followed by those ≥60 years with 20%. The lowest prevalence was recorded the at age category 11-20 with 10%. Based on gender, males presented with the higher incidence (35/50) i.e. 70% than female (15/50) i.e. 30%. Klebsiella pneumoniae, Staphyllococcus aureus and Pseudomonas aeruginosa were the most prevalent organisms isolated with 21.9%, 19.66% and 19.10% respectively. E. coli with 05.62% being had the least isolates. The research demonstrated the existence of both Gram positive and Gram negative bacterial pathogens that co-infect with TB patients, especially among the elderly males. Further research should be tailored towards investigating other pathogens that co-infect with MTB patients, such as fungi and viruses, in diversified hospitals in Katsina state and beyond.
Keywords: AFB, Pathogenic bacteria, Tuberculosis and Mycobacterium and Federal Medical Centre, Katsina.

INTRODUCTION

Tuberculosis (TB) is known to be one of the oldest forms of human diseases. Accordingly, it is known to cause mortality of nearly two million people each year (Pranita *et al.,* 2016). In spite of the effective treatment strategies, the disease still remains the major cause of death among the different curable infectious diseases (Kavita *et al.,* 2016). TB could affect different places e.g. bones, the nervous system or many other organ systems, but basically, it is characterized as a pulmonary disease which occurs due to accumulation of *Mycobacterium tuberculosis* (MTB) onto the lungs alveolar surfaces. *M. tuberculosis* is a constrained pathogenic bacterial species which belong to the family of mycrobacteriaceae. It was first discovered by Robert Koch in 1882 (Christina *et al.,* 2016). It can form acid stable complexes made up of peptidoglycan during Gram staining. It mainly infects the lungs of humans. There are many methods to diagnose tuberculosis, among which are tuberculin skin tests, acid-fast stain, and chest radiographs (Kaiming, 2016).

M. tuberculosis has developed resistance over time against the drugs used for its treatment hence drug resistance has become a serious problem around the world. The effective treatment regime for TB must contain multiple drugs to which the organism is susceptible to. This is because using a single particular drug for its treatment may lead to the development of drug resistant bacterial populations. Using two or more drugs simultaneously will help each other in order to counter the problem of resistance synergy towards a particular drug. During the initial stages of TB, it is difficult to select the particular drug to which the patients' isolates would be susceptible. This selection criterion is important because improperly selected drug would subsequently lead to the development of additional drug resistant organisms. The antibiotics commonly used for TB treatment include isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB) or streptomycin (SM). The course of the drug therapy normally last for at least 6-9 months. It is important to take the medication as per the guidance of the Physician and adherence to the full course of the medication (Shams *et al.,* 2014).

Co-Infection of tuberculosis with other bacteria has not been widely reported. Although suppressed bacterial infections can occur in TB patients, the simultaneous occurrence of both infections leads to delayed diagnosis and inadequate treatment. Tubercular bacterial co-infection needs to be considered, especially if TB occurs in atypical pulmonary or extrapulmonary locations. Tubercular and bacterial co-infection is uncommon in patients with intact immunity, but has been described in immunodeficient hosts such as those with HIV–AIDS. There are a few reports about the co-occurrence of TB with organisms like *Mycobacterium leprae*, *M.* intacellulare, *Streptococcus pneumonia*, *Salmonella typhi*, *Streptococcus milleri*, *Psedumonas species* and *Klebsiella species* (Arora *et al.,* 2015).

Tuberculosis patients are found to have co-infection with HIV, likewise tuberculosis patients are suspected to have co-infection with fungi (Candida spp.) and viral infection like HIV is normally believed to be the predisposing agent of tuberculosis, because of the suppression of immunity that it brings about. Candida infections also normally manifest after immunosuppression, and are normal consequences of TB infections (Ndukwu, 2016). Therefore, the aim of this work is to look at the possible pathogenic bacteria co-infecting with MTB patients in the study area.

MATERIALS AND METHODS

Sample Population/Size

A prevalence rate of 17.3% from a previous study in Kebbi state by Abdulkadir and Ibrahim (2018) was used to determine the sample size (220 samples), using an equation described by Naing *et al.,* (2006). A total of fifty (50) samples were used during the research in order to minimize error.

Collection and Transport of Sputum

Sputum for microbiological investigation is collected and transported as follows. Clean dry, wide-necked, leak-proof container, was given to the patient and requested him or her to cough deeply to expectorate and produce a sputum specimen. The sample was immediately transported to the Microbiology Laboratory in the Federal Medical Center for processing.

Inclusion criteria

This study included all those patients that present to be AFB positive and AFB negative.

Also it included patients from the age range 11 - ≥60.

Exclusion criteria

This study does not include patients below age 11 category.

It also does not involve patients presented with other infection like HIV/AIDS patient.

Ethical Approval and Clearance

Ethical approval for the study was obtained from the office of medical director Federal Medical Center Katsina State, with the HREC assigned number (FMCNHREC. REG. NO. 3/082012), before the commencement of the study.

Acid Fast Stain

A smear was made on a clean glass slide, and was then allowed to air dried and then fixed in alcohol. To raise the smear, it was then covered with carbon fuchin stain and heated until vapour just begins, the stain was washed off with clean water and then covered with 3% v/v acid alcohol for five minutes it's then washed well with clean water and covered with malachite green stain for two minute then washed with clean water. The prepared smeared was then drained using draining rack to air dried. The slide was examined under X100 (oil immersion) objective lense (Sagar, 2015).

Culturing and isolation

Specimens were inoculated onto prepared MacConkey Blood agar and Chocolate agar were inoculated and incubated at 37^0C for 24 hours.

The discrete colonies formed were used in obtaining the pure isolates, by sub-culturing the colonies in a purely prepared MacConkey Blood agar and Chocolate agar for microbial characterization.

Morphological characterization

The colonies of the isolates were examined and recorded based on their colonies colour, size, margin, edge, consistency, opacity and colour change in the medium at the end of 24hours.

Gram staining

Bacterial smears of 24 hrs old cultures were made on clean glass slides, heat fixed and stained as follows: The slide was flooded with crystal violet solution (primary stain) for one minute, drained and rinsed with water; followed by Grams iodine solution for one minute, drained and rinsed with water. Decolourised with ethyl alcohol for 30 Seconds and later counterstained with safranin for one minute and observed under an oil immersion microscope (Michael and Burton, 2010).

Biochemical Test

Indole test

The cultured media was inoculated into tryptone broth and incubated at 37^0C for 24 hours. About 0.2 ml of Kovac's reagent was then added to the test tube, shaken and allowed to stand. The formation of red ring on the surface of the broth confirmed the production of indole (Michael and Burton, 2010).

Methyl red test

The cultured media was inoculated with Methyl red – Vogues Proskauer (MR-VP) broth and incubated for 24 hours at 370C. The appearance of a red colour on addition of methyl red solution was considered as positive (Michael and Burton, 2010).

Voges – Proskauer test

Cultured was inoculated with MR-VP (glucose broth) medium and incubated at 37^0C for 24 hours. After incubation, 3 ml of Barrit's reagent A (5% alpha naphthol: 5.0 g Absolute ethanol: 95 ml) and one ml of Barrit's reagent B (Potassium hydroxide: 40g; Creatine: 3g; Distilled Water: 1000 ml) were added. The tubes were shaken and allowed to stand for 15 minutes and observed for colour change. The development of pink colour was considered as positive (Michael and Burton, 2010).

Citrate Utilization test

The test isolates were streaked over the slant of Simmon's citrate agar and incubated for 24hrs at 370C. Growth on the slant and change in colour to blue of the medium indicates positive result (citrate is been utilized by the organism) (Michael and Burton, 2010).

Catalase Test

A drop of hydrogen peroxide was put at the center of glass slide and the colony of 24 hour cultured media was picked and smeared. The formation of bubbles within 3-4 seconds was recorded as positive (Michael and Burton, 2010).

Coagulase Test

A 0.1 ml of the plasma was dropped at the center of the slide and the colony of 24 hour cultured media was picked and smeared. Agglutination is formed within 1 minute which was recorded as positive result (Michael and Burton, 2010).

Oxidase test

A piece of filter paper was soaked with a few drops of oxidase reagent. A colony of the test organism was then smeared on the fiter paper. A deep purple colour on the smeared portion indicates the presence of cytochrome enzyme oxidase (Michael and Burton, 2010).

Statistical analysis

Data was presented using descriptive statistics and analyzed using statistical tool; GraphpadInstat statistical software version 3.0.

RESULTS

From Table 1 the study revealed the incidence of TB which occurs mostly within the age range of 51 - 60 with 28%, followed by ≥ 61 with 20%, 31-40 with 16%, 21-30 with 14%, 41 % 41 - 50 with 12 %, the lowest prevalence was observed in the 11-20 age category with 10% only. Statistically there is no significant difference between age group category with x^2= (3.358), df= (5) and p-value (0.6449).

Result shows that, 35 were males and 15 were females 23 (46%) males were TB positive while 12 (24%) were males TB negative also 6 (12%) were females and TB positive while 9 (18%) were females and TB negative. Statistically, there is no significant difference with x^2 (1.892), df (1) and p-value (0.1689).

Table 1. Occurrence of TB based on age

Age group	No. of patients examined	No. of patients with pathogens Positive	negative
11-20	5	02 (6.90%)	03 (14.29%)
21-30	8	03 (10.34%)	04 (19.05%)
31-40	8	04 (13.80%)	04 (19.05%)
41-50	6	05 (17.23%)	01 (04.76%)
51-60	14	09 (31.03%)	05 (23.80%)
≥ 61	10	06 (20.69%)	04 (19.05%)
Total	**50**	**29 (100%)**	**21 (100%)**

Table 2. Occurrence of TB based on Gender

Gender	No. of patients examined	No. of patients with pathogens positive	negative
Males	35	23 (46%)	12 (24%)
Females	15	06 (12%)	09 (18%)

A total of 178 isolates were obtained from both TB negative 85 (47.75%) and positive 93 (52.25%) patients. Hence more bacteria are found from TB positive than from TB negative with *K. pneumoniae* been the most commonly isolated organisms than any other bacterium, while *S. pneumoniae* and *E. coli* are the least.

Table 3: Bacterial pathogens isolated from the patients

Pathogens isolated	**No. of isolates**		**Total**	**Occurrence (%)**
	Positive	**Negative**		
K. pneumonia	21	18	39	21.9%
S. aureus	18	17	35	19.66%
P. aeruginosa	13	17	34	19.10%
H. influenza	13	13	26	14.61%
M. catarrhalis	09	04	13	07.30%
S. pneumonia	07	14	21	11.80%
E. coli	08	02	10	05.62%
Total	**93**	**85**	**178**	**100.00%**

DISCUSSION

In this study, 35 male patients (70%) and 15 in female patients (30%) present with co-infection. This finding was closely related with the previous study reported by Amaryllis *et al.,* (2016) with slight difference in the number of male patients. Out of the 50 samples screened, all the samples were significant with the target organism that is 100%. Among the organism isolated are two Gram positive bacteria (*S. aureus* and *S. pneumoniae*) and the rest are Gram negative (*K. pneumoniae, P. aeruginosa, H. influenza, M. cattarrhallis* and *E. coli*). This occurrence is higher the than previous studies in Kano, Nigeria Taura *et al.,* (2013) and in Benin City Egbe *et al.,* (2011). The bacterial pathogens isolated are similar to that of the work of Vijay and Dalela (2016) in Jhalawar, India.

In this research *K. pneumoniae* was found to be the most predominant pathogen isolated from the sample, this is in agreement with the previous studies conducted by Egbagbe and Mordi (2006); Egbe *et al.,* (2011); Vijay and Dalela (2016). However, the study of Taura *et al.,* (2013) reported *S. pneumoniae* as the most predominant pathogen followed by *K. pneumoniae.*

Also, the study of Egbagbe and Mordi (2006) reported the absence of *S. aureus as* the second most leading pathogen. Furthermore, based on gender screened during the study, we found male (70%) recorded high percentage than female (30%) in our research which is similar to the previous study conducted by Taura *et al.,* (2013) and Vijay and Dalela (2016).

The prevalence based on age group category showed that those in the age range 51-60 and ≥ 60 are the most susceptible co-infection. It also agreed with the findings of Panda *et al*., (2012), where they recorded a higher occurrence among patients ranging from 51-60 and 60-70 years. Millet (2013) working with older adults in the United Kingdom also recorded an increase in prevalence of lower respiratory tract infections with increase in age. A diminishing immunity due to age as well as other health complications is probable reasons for this trend Egbe *et al*., (2011) as recorded in previous study.

CONCLUSION

This research demonstrated the existence of numerous Gram negative and Gram positive organisms co-infection among TB positive and negative patients. In the study, the incidence was observed among elderly patients than younger ones. Males demonstrated higher co-infection with the pathogenic microbes than the females. Furthermore, *K. pneumoniae* and *S. aureus* are the most predominant organisms isolated with *E. coli* being the least prevalent.

RECOMMENDATIONS

1. This study restricts itself to pathogenic bacteria that co-infected TB patients, thus the need emanates for the conductance of further studies on other aetiological agents, such as fungi and viruses.
2. Future effort should be tailored towards other areas and health centres in Katsina State and Nigeria at large.
3. Hospital should be encourage to incorporate identification of aetiological agent of T.B and the presence of other pathogens so as to avoid the failure of therapy. This will help in tackling the danger of co-infection as seen this survey.

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