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Short Communication

CHARACTERİSATİON OF MYCOBACTERİUM ISOLATED FROM CASES OF TUBERCULOSİS IN HUMANS IN SOKOTO STATE, NİGERİA

GARBA[1], H. S., AHMED, H. AND GALADİMA[2], M.

Faculty of Veterinary Medicine [1]College of Health Sciences, Usmanu Danfodiyo University Sokoto and [2]Department of Microbiology Ahmadu Bello University Zaria

Correspondence: P.M.B. 2346 Sokoto

SUMMARY

A study was conducted to determine the species of Mycobacterium involved in cases of human tuberculosis in Sokoto State, Nigeria. Specimens from 39 of the 106 samples collected yielded Mycobacterium on Loweinstein-Jensen media and were characterized. *Mycobacterium tuberculosis* was isolated from 27(69.23%) mostly from cases of pulmonary tuberculosis while *M. bovis* and atypical mycobacterium in 8(20.51%) and 4(10.23%) respectively principally from cases of extra-pulmonary tuberculosis. These are significant findings to be considered in planning epidemiological studies and tuberculosis control in this and other regions of the country.

Key words: Mycobacterium, tuberculosis, atypical, extra-pulmonary

INTRODUCTİON

Mycobacterium has been implicated as a cause of death of several hundreds of people world-wide (Spingett, 1972). When the tubercle bacilli were first classified into human, bovine and avian types and subsequently named *M. tuberculosis*, *M. bovis* and *M. avium* respectively, it was believed that each was specific for the class of host in which it was prevalent (Boughton 1969). Whereas *M. tuberculosis* was known to be the most frequent cause of human tuberculosis, some human cases are reported to be caused by *M. bovis* and to a lesser extent *M. avium* (Kolo 1991). In Nigeria, there are reports on the incidence of the disease caused by the tubercle bacilli complex comprising primarily *M. tuberculosis*, *M. bovis* and *M. avium* isolated from both human and animal sources (Alhaji 1976). Reports have shown that the less virulent atypical mycobacteria are recognized to pose a serious health threat to human (Kolo 1991; Shehu 1992) In Sokoto State, there exist a close contact between livestock and their owners and a rise in the incidence of tuberculosis (Garba, 2002). It is, therefore, expected that certain cases of tuberculosis in the region may arise from infection with species other than *M. tuberculosis*. Thus, there is the need to isolate and characterize mycobacteria from human patients in this area.

This paper reports the characterization of *Mycobacterium species* isolated from patients diagnosed with tuberculosis in Sokoto State of Nigeria.

MATERİALS AND METHODS

Pathological specimen (37 lungs; 5 kidney; 3 spinal abscess; 5 endometral scrapings 3; skin lesions; 5 abdominal lesions) were

collected from patients presenting with emaciation, coughing, laboured breathing, fatigue and diagnose by radiography (Chest x-ray) Mantoux test and or microscopic demonstration of acid – fast bacilli as being tuberculosis (Tsukamura, 1967; Abdulkadir, 1989; Kolo, 1991; Griffin and Buchan, 1994; Harries *et al* 1997). Urine, sputum and pus from abscesses were also collected from diagnosed cases of extra pulmonary tuberculosis for microbiological examination and species identification using culture, biochemical tests and animal inoculation.

Sputum was collected in clean, sterile wide-mouth screw-capped containers. Method of collection varied with age of patient and type of tuberculosis diagnosed for adults with pulmonary tuberculosis. Sputum was collected by the patient in sterile wide-mouth, screw-capped containers. For patients less than 10 years of age, sputum was collected as above with the aid of the patients relations, or in some cases by gastric aspiration if symptoms gets swallowed. In case of renal tuberculosis, urine was collected. Endometral scrapping was collected in cases of genital tuberculosis and pus in the cases of abscess. Bloody or serous effusion was prevented from clotting using 1 ml of one percent (1%) sodium citrate as described by Kolo (1991).

Samples were transported immediately after collection in ice pack to the Infectious Disease Laboratory at City Campus of the Usmanu Danfodiyo University, Sokoto, for processing. When the samples could not be processed immediately after collection, they were stored under refrigeration at 4°C until processed.

Sputum specimens were processed as described by Cruicshank *et al.* (1975). Five millilitres of each specimen were transferred to sterile 50ml screw-capped centrifuge tubes and 10mls of sodium hydroxide containing phenol red indicator added. Tubes were tightly capped, shaken and allowed to stand for 30 minutes and then neutralised using ammonium chloride. The specimen was centrifuged at 3000 relative centrifugal force (RCF) for 20 minutes and the supernatant discarded into 4% formalin leaving about 2mls of sediments. A smear from the sediment was stained using Ziehl-Nelsen technique and examined microscopically for acid-fast bacilli.

Other specimens were processed as described by Shehu (1992). Samples were diluted to 40ml using buffered saline and centrifuged in a 50ml screw capped tube at 3000 RCF for 20 minutes. The supernatant was discarded and the sediment re-suspended in 15mls of 4 % sodium hydroxide (NaOH). The rest of the procedure was as described above for the sputum sample.

The processed specimens were investigated by Zeihl-Nelson staining technique for acid-fast bacilli. Specimen containing acid fast bacilli were decontaminated using the methods described by Milian-Suazo *et al* (2000) as follows: Specimen was homogenized for 3 to 4 minutes in 5mls of 4% phenol red. Seven ml of the homogenized tissue suspension were placed in a test tube and 5ml of 0.5N sodium hydroxide was added and the mixture kept for 10 minutes. With the use of a pipette, 10 to 15 drops of hydrochloric acid 6N were added to the tube with the macerated tissue suspension until the mixture turned yellow indicating neutrality. The mixture was then

centrifuged at a speed of 3000 RCF for 20 minutes and 90% of the supernatant discarded into 4% formalin. Two drops of the residual fluid were then used to inoculate Lowenstein-Jensen medium enriched with pyruvate/glycerol. Biochemical tests were conducted on the isolates to confirm the specific infecting organism.

The organisms were then characterized based on established biochemical properties of various species of the organisms (Kolo 1991)

RESULTS

Of the 106 samples cultured, only 39(36.79%) produced growth. Biochemical characterization revealed that 27(69.23%) of the isolates were *M. tuberculosis*, 8(20.51%) *M. bovis* while 4(10.26%) were the atypical Mycobacterium species. The isolates from sputum samples from patients with pulmonary tuberculosis showed that 23 were *M. tuberculosis;* 3 *M. bovis* and 2 of atypical Mycobacterium.

Table I: **The frequency of occurrence of Mycobacterium species isolated from human patients with various forms of tuberculosis**

Organ Infected	*M. tuberculosis*	*M. bovis*	Atypical mycobacterium	No of cases
Lungs	23	3	2	28
Kidney	1	1	1	3
Spine	2	0	0	2
Genitalia	1	2	0	3
Skin	0	0	1	1
Abdomen	0	2	0	2
TOTAL	**27(69.23)**	**8(20.51)**	**4(10.26)**	**39**

Figures in parenthesis are percentages

For extra pulmonary tuberculosis, the 3 cases diagnosed with renal tuberculosis, yielded one each of *M. tuberculosis*, *M. bovis* and atypical Mycobacterium. The two cases of tuberculosis of the spine both yielded *M. tuberculosis* while the three cases of genital tuberculosis, yielded one *M. tuberculosis* and *two M. bovis.* Tuberculosis of the skin produced atypical tubercle bacilli, while the two cases of tuberculosis of the gastrointestinal tract both yielded *M. bovis.*

DİSCUSSİON

This study has shown that *M. tuberculosis* has remained the major causative organism causing tuberculosis in humans in Sokoto state. The study however identified other members of the tubercle bacilli complex such as *M. bovis* and some atypical tubercles as causative agents of tuberculosis. This isolation was made from samples collected from both pulmonary and extra pulmonary tuberculosis. Whereas *M. tuberculosis* was found to be responsible for majority of the pulmonary tuberculosis, *M. bovis* was implicated in a good number of extra pulmonary cases. The involvement of species of tuberculosis other than *M. tuberculosis* in human infection has been reported world wide (Grifith 1937; Christensen 1981; Silber *et. al.*1987; Kolo 1991) Similarly in Nigeria Idigbe *et. al.* (1986) and Kolo (1991) had isolated *M. bovis* from human infection in Lagos and Zaria respectively. The isolation of atypical tubercle bacilli and indeed *M. bovis* from human cases calls for strategic planning for the control of the disease in view of the importance of livestock as sources of these organisms. This region is endowed with a lot of livestock resources and man and livestock are known to be closely associated (Garba 2002). For effective planning of a tuberculosis control programme, proper education needs to be

mounted so as to highlight the mechanism of transmission between man and animals.

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