ISSN 1119-4618

Laboratory diagnosis of pulmonary tuberculosis: a comparison of different methods.

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Received: 13 7 2000. Accepted: 31 1 2001

ABSTRACT

A laboratory study designed to compare the sensitivity of microscopy (direct and concentrated smears); Direct and Concentrated sputum culture as well as comparing Ziehl-Neelsen and Auramine-Phenol staining methods in the diagnosis of pulmonary tuberculosis was carried out. The survey involved one hundred and Eighty Six (186) new patients seen at the chest and General Out-patient clinics of the Jos University Teaching Hospital (JUTH) and the out-patient Department of the Evangel Hospital Jos, with symptoms of broncho-pulmonary disorders and who were sent to the Microbiology Laboratory for Acid fast bacilli (AFB) Microscopy between February, 1996 and November, 1997. Also included were one hundred and thirty (130) sputum smears made from different samples sent to the laboratory within the same period. A total of 55 (29.57%) patients were positive for acid fast bacilli by both microscopy and culture methods. Thirty-eight, 38 (69.1%) of the 55 positive cases were detected by direct smear, 43 (78.2%) by concentrated smear, 16 (29.1%) by direct sputum culture and 33 (60.0%) by concentrated sputum culture. The difference between a single concentrated smear, 78.2% and 3 consecutive direct smears (69.1%) for identifying tubercle bacilli was not statistically significant ($P>0.05$) but was significantly different from 60.0% for concentrated culture and 28.57% direct culture ($P<0.05$). Concentrated sputum smear examination for routine diagnosis of pulmonary tuberculosis is indicated.

INTRODUCTION

Tuberculosis (TB) still constitutes a serious public health problem in Nigeria and other parts of the world inspite of the excellent guidelines available for prevention and treatment of the disease. The main thrust of all TB control programmes is adequate case finding and effective chemotherapy (Porter, 1991; Porter & McAdam, 1992; Godfrey- Fausett, 1993). This is the only way to break the chain of transmission within communities.

Laboratory based TB surveys in Nigeria are very few (Alausa *et al* 1977; Idigbe and

Onwujekwe 1983; Idigbe *et al* 1996, Nwofor *et al* 1997) This is probably due to inadequate facilities in majority of the countries (Idigbe and Onwujekwe 1983). However, Sputum smear examination by microscopy for acid fast bacilli still remains the cornerstone of TB case finding in Low-income countries (Yahaya *et al*, 1996). The sensitivity of sputum smear is of interest being the cheapest and often only practical method of bacteriological confirmation of TB. It is often recommended in many low income countries that examination of two consecutive sputum smears is as efficient as culture in detecting cases of pulmonary TB (Mitchison, 1968). The relative merits of culture and direct smear examination methods is still very controversial (Idigbe and Onwujekwe 1983). Some workers believe that microscopy is slightly superior and will detect 72 - 77% of positive sputum (Alausa *et al* 1977, Idigbe and Onwujekwe 1983). Culture method has been established to be expensive and requires highly trained personnel (WHO, 1982).

Diagnosis of pulmonary TB at the Jos University Teaching Hospital (JUTH) is usually based on the patient's clinical and radiological findings as well as presence of acid fast bacilli in direct, unconcentrated sputum smear of 3 consecutive early morning sputa. This study was therefore undertaken to compare direct sputum smear microscopy as is done in the hospital with concentrated smear; Direct and Contentrated sputum culture in the diagnosis of pulmonary TB. It also aims to compare Ziehl-Neelsen (ZN) and Auramine Phenol staining techniques.

MATERIALS AND METHODS

Study population

The study population was drawn from new patients attending the chest clinic and General outpatient Departments of the Jos University Teaching Hospital and the out-patient Department of the Evangel Hospital Jos with symptoms of bronchopulmonary disorders. One hundred and Eighty Six of such patients were included in this study which was conducted between February, 1996 and November, 1997. The patients comprised of 107 males and 79 females. Patients already on any antituberculosis regimen were excluded from the study. Table 1 show the age and sex distribution of the study population.

Table 1: Age and sex distribution of the study population:

(Years)	Males	Females	Total (%)
<15	4	2	6 (3.22)
15 – 24	18	13	31 (16.67)
25 – 34	37	39	76 (40.86)
35 – 44	22	11	33 (17.74)
45 – 54	15	7	22 (11.83)
55 – 64	5	4	9 (4.84)
>65	6	3	9 (4.84)
Total	107 (57.53%)	79 (42.47%)	186 (100)

Laboratory studies

(i) **Collection of Specimens**: - Three consecutive early morning sputum samples were collected from each of the patients into clean containers. Only mucoid sputum specimens were accepted.

(ii) **Bacteriological Analysis:** - The sputum samples were examined for acid fast bacilli using microscopy and culture.

Direct smears were made from all the three specimens received per patient while only one sample was concentrated using Petroff's method (Cruickshank *et al* 1975). All such smears were stained using the ZN staining technique and examined.

Loopfuls of each of the various sputum specimens (Direct and concentrated) were evenly spread onto surfaces of a pair of Lowenstein - Jensen medium. Culture media were prepared according to the method described by Cheesbrough (1988). The slopes were incubated at 37°C and observed weekly for 8 weeks. Slopes without visible growth after 8 weeks were discarded and recorded as negative while slopes showing mycobacterial growth were recorded as positive. A case was taken as positive if visible growth of acid fast bacilli was obtained from at least one of the three sputum samples per case. All such growths were checked for acid fast properties by ZN microscopy.

(iii) Camparison of ZN and Auramine Phenol Staining Technique

One hundred and thirty (130) smears were made from different sputum samples received at the microbiology laboratory of the Jos University Teaching Hospital for Acid Fast Bacilli (AFB) microscopy and examined using the ZN staining technique and the results recorded. The same slides were again counter-stained using the Auramine-phenol staining technique as described by Cheesbrough (1988) and examined using fluorescent microscope. The result were recorded independently and later collated.

RESULTS

Out of the one hundred and Eighty Six sputum samples examined, 55 (29.57%) were found to be positive for acid fast bacilli using the different methods, (Table 2).

Table 2: Age and sex distribution of positive cases;

Age (Years)	MALES		FEMALES		Total Positive (Rate)
	No Examined	No Positive	No Examined	No Positive	
15	4	0	2	0	0 - 9 (16.36%)
15 24	18	5	13	4	24 (43.64%)
25 34	37	15	39	9	14 (25.45%)
35 44	22	9	11	5	5 (9.09%)
45 54	15	3	7	2	1 (1.82%)
55 64	5	1	4	0	2 (3.64%)
65	6	2	3	0	
Total	**107**	**35 32.71%**	**79**	**20 25.32%**	**55 29.57%**

* Based on total number of cases positive for Acid fast bacilli

Further analyses of these positive cases show that direct smear microscopy detected 38 (69.17%) cases while 43 (78.20%) were detected by concentrated smear microscopy. On the other hand, 16 (29.1%) cases were detected by direct Sputum culture, while 33 (60.0%) cases were detected by concentrated sputum culture (Table 3).

Table 3. Bacteriological examination of sputum samples using direct and concentrated smears; and direct and concentrated sputum culture:

N = 186

Method	No. Positive	Percentage
Direct Smear	38	(69.1)
Conc. Smear	43	(78.2)
Direct Culture	16	(29.1)
Conc. Culture	33	(60.00)

N.B Total No. Positive = 55

Table 4. Number Positive by Direct Smear, Concentrated Smear, Culture, Relative to Total Number of Positive Cases:

N = 186

Description of Method	No. Positive relative to Total No of Positive cases	Percentage
Direct smear and concentrated smear positive	38	69.1
Concentrated smear positive but direct smear negative	5	9.1
Culture positive but direct smear and concentrated smear negative	12	21.8
TOTAL	55	100.00

Table 4 correlates the results of direct smear, concentrated smear microscopy and culture. 55 (29.57%) of the study population were positive for AFB by the different methods. Of the recorded positive cases, 38 (69.17%) were identified by both direct and concentrated smear microscopy, 5 (9.17%) were detected by a single concentrated smear but not by three different direct smears. 12 (21.82%) were positive by culture but negative by microscopy.

Table 5 shows a comparison carried out between Ziehl-Neelsen (ZN) and Auramine-Phenol staining techniques. The total number of slides examined was one hundred and thirty (130). The result of the comparison showed that the number of smears, which were negative by ZN and positive by Auramine-Phenol, was 11 (19.64%) and in all positive cases, there was a 100% agreement except that the intensity was higher using the Auramine Phenol staining technique. The difference is statistically insignificant using the Mann Whitney test ($P>0.05$).

DISCUSSION

Detection of patients with open cases of pulmonary TB based on specific diagnosis, followed by adequate and effective chemotherapy remains the backbone of any meaningful TB control programme (Porter, 1991; Poter & McAdam 1992; Godfrey - Fausett 1993; Idigbe *et al.* 1996). In Nigeria and many other developing countries, diagnostic facilities are inadequate mainly due to poor resources and or lack of the desired political will to fund such programmes which invariably accounts for the few reports of laboratory based tuberculosis surveys (Alausa *et al.* 1977; Idigbe & Onwujekwe, 1983; Idigbe *et al* 1996; Nwofor *et al* 1997) Consequently open cases of TB which have been estimated to be able to infect 10 - 14 persons per year per case are constantly disseminating tubercle bacilli into the

Table 5. Comparison between ZN and Auramine Phenol Staining Techniques:

ZN STAINING RESULTS	URAMINE PHENOL STAINING RESULTS				
	NEG.	+	++	+++	++++
NEG.	45 (80.36)	9(16.07)	2(3.57%)	0	0
+	0	20(74.07)	7(25.93%)	0	0
++	0	0	20(76.92%)	6(23.08%)	0
+++	0	0	0	8(66.67%)	4(33.33%)
++++	0	0	0	1(11.11%)	8(88.89%)

NEG- Negative
\+ - Occasional bacilli seen
++ - 1 - 10 bacilli Phf seen
+++- 11 - 20 bacilli Phf seen
++++- > 20 bacilli Phf seen.

environment. Since TB is characterised by a high infection rate with an equally high morbidity and mortality rate, a very sensitive diagnostic method aimed at adequate case finding and treatment becomes pertinent.

The cultivation and isolation of micro-organisms in the laboratory in disease conditions have remained the main thrust of definitive diagnosis in microbiology world-wide, however, the slow growth rate of *Mycobacterium tuberculosis* in culture, the high cost as well as requirement for highly trained personnel have not allowed rapid diagnosis of TB. In our study, 69.17% of confirmed positive cases were diagnosed by 3 consecutive direct smears, 78.2% by a single concentrated smear, 29.1% direct sputum culture and 60.0% by concentrated sputum culture.

This indicates that concentrated smear is superior to the other methods employed. This finding is similar to those of earlier workers (Alausa *et al*, 1977, Idigbe & Onwujekwe 1983, Nwofor *et al* 1997) Direct sputum culture for *M. tuberculosis* recorded the least number of positive cases in this study followed by concentrated sputum culture. Some of the sputum samples which were clearly positive on microscopy could not be grown on culture due to contamination by fungi and other bacterial species probably resulting from mixed infections. Mixed infections has been found to be a common occurrence in Nigeria and elsewhere (Hosty *et al* 1961, Osoagbaka 1984, Nwobu *et al* 1989,

Idigbe *et al,* 1996). It is also possible that some of the patients had access to some antituberculosis drugs and did not disclose this fact. The examination of sputum samples for other pathogens including fungi in cases of broncho-pulmonary disorders is therefore indicated. The use of microscopic examination of stained concentrated sputum smears should also be encouraged owing to the high cost of culture work.

Reports in the literature by Cheesbrough (1988) suggest that Auramine - Phenol staining is equally cheap and very sensitive such that it can replace the ZN staining technique where a fluorescent microscope is available. Our experience has however shown that the technique though capable of detecting most positive cases, can result in quite a few false positives. The difference was not however statistically significant ($P>0.05$).

False positively may be as a result of the presence of other organism in sputum that take up the flourecein dye (Auramine) used in the study.

ACKNOWLEDGEMENTS

We are thankful to Messrs. Audu Matawal and Monday Danung for their assistance. Grant Support by the University of Jos Senate (RGC/1993/94/009) is gratefully acknowledged.

REFERENCES

Alausa, K. P., Osoba, A.O., Montefiore, D., Sogbetun, O.A. 1977. Diagnosis of Tuberculosis in a Developing country. 1968–1975. *African Journal of Medical Sciences* **6:** 103-108.

Cheesbrough, M. 1988: *Medical Laboratory Manual for Tropical Countries;* Butterworth Ltd., Kent, England.

Cruickshank, R., Duguid, M. B., Swain, R.H.A. 1975. *Medical Microbiology,* Vol. II, 390p, Churchill Livingstone, London.

Godfrey-Fausett, P. 1993. Of Molecules and Men: The Detection of Tuberculosis, Past Present and Future, In: Porter J.D.H. & McAdam K.P.W.J. (Eds.) *Proceedings of the Third Annual Forum on Tuberculosis: Back to the Future.* London School of Hygiene and Tropical Medicine, John Wiley & Sons, 79 - 95.

Hosty, T.S., Dumonte, M.C., Ajello, A. 1961. Prevalence of *Nocardia aseroides* in Sputa examined by a Tuberculosis Diagnostic Laboratory. *Journal of Laboratory and Clinical Medicine* **58:** 107-114.

Idigbe, E.O., Onwujekwe, D.I. 1983. Clinico-Laboratory Study of Pulmonary Tuberculosis in Lagos, Nigeria. *Nigerian Journal of Microbiology.* **3:** (2): 107-113.

Idigbe, E.O., Sofola, T.O., John, E.K.O., Okoye, R., Onubogu, C., Begg, O., Giwa-Amu, J. 1995. The trend of pulmonary tuberculosis in Lagos Nigeria, 1982 - 1992. *Biomedical Letters* **51:** 99-105.

Idigbe, E.O., John, E.K.O., Dabiri, M.A., Onubogu, C., Okoye, R., Giwa-Amu, J., Begg, O., Nwobu, R.U. 1996. Clinical and Microbiological Reassessment of some classified chronic cases of Pulmonary Tuberculosis in Lagos, Nigeria. *Nigerian Journal of Medical Research* **1:** 1-5.

Mitchison, D.A. 1968. Examination of Sputum by Smear and Culture in Case Finding. *Bulletin of the International Union against Tuberculosis & Lung Diseases* **41:** 139-147.

Nwobu, R.A.U., Odugbemi, T.O. Eneje, R.C., Bamdia, E.O. 1989. Mycologic and Bacteriologic Investigations of Clinical Sputum Specimens in Lagos, Nigeria. *West African Journal of Medicine* **84:** (4), 274-278.

Nwofor, A.C., Onile, B.A., Oluboyo, P.O. Idigbe, E.O. 1997. Comparison of Microscopic and Cultural Methods for the Diagnosis of Pulmonary Tuberculosis. *Nigerian Quarterly Journal of Hospital Medicine* **7:** (3) 227-229.

Osoagbaka, O.U. 1984. Bacteriological Evidence of Occurrence of Nocardia Species in Pulmonary Infections in Nsukka, Nigeria. *West African Journal of Medicine* **3:** 135-144.

Porter, J.D.H. 1991. Tuberculosis in Developing Countries. *Communicable Disease Report* **1:** R135 - 139.

Porter, J.D.H., McAdam, K.P.W.J. 1992. Aspects of Tuberculosis in Africa. 1 Tuberculosis in Africa in the AIDS era - the role of Chemoprophylaxis. *Transactions of Royal Society of Tropical Medicine and Hygiene.* **86:** 467 - 469.

Yahaya, A, Ipuge, Hans L. Relder, Donald O.A. Enarson. 1996. The Yield of acid fast bacilli from serial smears in routine Microscopy Laboratories in Rural Tanzania. *Transactions of Royal Society of Tropical Medicine and Hygiene 90: 258-261.*

W.H.O. 1992. Tuberculosis Control Report *of a Joint IUAT/WHO Study Group, Technical Report Series No. 671, WHO Geneva*