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SHORT COMMUNICATION

EVALUATION OF THE SODIUM HYPOCHLORITE CONCENTRATION METHOD OF DETECTING ACID-FAST BACILLI FROM SPUTUM OF PATIENTS IN CENTRAL HOSPITAL, DELTA STATE, NIGERIA

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ABSTRACT

The use of sodium hypochlorite (NaOCl) was revalidated in the laboratory diagnosis of acid fast bacilli (AFB) against the long existing direct smear Zeihl-Neelsen method to ascertain the more sensitive and reliable method. Sputum from 260 patients, aged 20-52 years attending the Chest Clinic of the Central Hospital Agbor, Delta State, Nigeria were examined for acid-fast bacilli by direct microscopy and centrifugation following treatment with 3.5% sodium hypochlorite. The percentage positive cases rose from 6.92% for direct smear to 28.85% after treatment with sodium hypochlorite. This was found to be statistically significant ($p<0.0001$). The sodium hypochlorite concentration improved specificity from 43.33% to 56.67% and sensitivity from 19.35% to 80.65%. Thus, the sodium hypochlorite concentration method may be more sensitive and reliable for the diagnosis of tuberculosis in laboratories. Hence, it could be recommended for use in Tuberculosis Control Programme in Nigeria and can be adapted to suit local needs of rapid diagnosis.

Keywords: Tuberculosis, Acid-fast, sodium hypochlorite, Bacilli, rapid diagnosis

INTRODUCTION

The resurgence of tuberculosis (TB) is one of the most serious global public health challenges of the twenty-first century (Gandy and Zumla, 2002). Tuberculosis affects about one-third of the world's population and is a leading cause of death worldwide, with majority of the deaths occurring in developing countries (Kochi, 1991; Hall, 1996). In Nigeria, there has been an upsurge in new cases of TB since 1992 (UNAIDS, 2000).

This increase in TB cases and the associated increase in TB suspects have had an adverse effect on TB diagnostic services. Laboratories in Nigeria are being overburdened with specimens for sputum smear microscopy. Sputum smear microscopy is a critical element of a TB control programme. Acid-fast microscopy is still the simplest and most rapid procedure that could be performed in the laboratory to detect the presence of acid-fast bacilli (Cheesbrough, 2000). It will take 5,000 to 10,000 bacilli per ml of sputum to detect their presence in smears. The microscopic examination for Acid-fast bacilli (AFB) remains the main bacteriological tool for the diagnosis of TB (Aber *et al.*, 1980).

In many laboratories in Nigeria, due to limited resources, only direct microscopy is used in the diagnosis of TB cases. Although Acid-fast microscopy is highly specific test for mycobacterial organisms, it however, has sensitivity as low as 8.8% (Aber *et al.*, 1980; Corbett and De-Cock, 1996; Miorner *et al.*, 1996). In order to improve the concentration of Acid-fast bacilli in samples, centrifugation of the specimens is needed to concentrate mycobacterium in clinical specimens (Ratnam and March, 1986).

Some studies have shown that liquefaction of sputum with sodium hypochlorite (NaOCl) (the household bleach) and subsequent centrifugation increases the sensitivity of direct sputum microscopy (Oliver and Reusser, 1942; Corper and Nelson, 1949; Gebre *et al.*, 1995; Githui *et al.*, 2007; Makunde *et al.*, 2007). The use of sodium hypochlorite has been shown to increase the number of samples positive for acid-fast bacilli by as much as 100% and has the advantage of lowering the risk of laboratory infection (Gebre *et al.*, 1995).

In this study, we revalidated the sensitivity of the concentration by centrifugation of sputum samples using sodium hypochlorite and compared this with the direct microscopy of sputum from patients attending the Central Hospital Agbor, Delta State, South-South Nigeria.

MATERIALS AND METHODS

A total of 260 patients aged 20-52 years and comprising males and females were recruited for this study. Sputum samples from each patient attending the Chest Clinic of the Central Hospital Agbor, Delta State, Nigeria, from January to October 2008 were examined for Acid-fast bacilli (AFB) in line with the National Guidelines for Tuberculosis Control Programme. The study was approved by the Research and Ethical Review Committee of the Ministry of Health, Asaba, Delta State, Nigeria.

The samples were collected in plastic universal containers. The methods of Aber *et al.* (1980) were employed in the treatment of the samples. The samples were subjected to direct smear (Ziehl-Neelsen) method and thereafter concentrated, following treatment with 3.5% sodium hypochlorite in order to compare the sensitivity and the reliability of the two methods

The smears were prepared from the mucopurulent portion of the samples. The slides were air dried, fixed with heat and stained according to ZN method (Cheesebrough, 2002). In the liquefaction (decontamination and centrifugation) method, 1.0 ml of sputum was transferred to a tube containing 1.0 ml of 3.5% Sodium hypochlorite (JIK®, Nigeria), mixed and allowed to stand on the bench for 10 min., after which 8.0 ml of distilled water was added. The contents were then centrifuged at 4000 rpm for 15 min (Perera and Arachchi, 1999). After decanting the supernatant, the slides were coded, air-dried, heat fixed, and stained by the Ziehl-Neelsen technique. The stained slides were examined under oil immersion (X 100).

Smears were reported negative, when no AFB was observed in at least 100 microscopic fields and reported positive, when 1 to 9 AFB were seen in 100 microscopic fields (scored as few bacilli); when 10 to 99 AFB were seen in 100 fields (scored as 1+); when 1 to 10 AFB were seen per field in at least 50 fields (2+); and when more than 10 AFB were seen per field in at least 20 fields (3+) (IUATLD, 1996).

Data generated was subjected to statistical analyses using the StatsDirect software Version 2.4.1.

RESULTS AND DISCUSSION

This study showed positive results for both the direct smear ZN technique and the sodium hypochlorite method (Table 1). Of the 260 sputum samples examined, 75(28.85%) were positive for the sodium hypochlorite centrifugation method. In comparison, the direct smear method detected 18(6.92%). This difference in sensitivity was found to be statistically significant ($p < 0.0001$).

The sodium hypochlorite method had a sensitivity of 80.65% with a specificity of 56.67%, while the sensitivity of direct microscopy was lower (19.35%) with a specificity of 43.33% (Table 2).

Table 1: Comparison of results for the recovery of Acid-fast bacilli in 260 sputum samples using Direct Smear and Sodium hypochlorite

Treatment Method	Positive	Negative
Direct Smear	18 (6.92%)	242 (93.08%)
NaOCl+ Centrifugation	75 (28.85%)	185(71.15%)

Chi-square test with Yates-correction = 41.06, $P < 0.0001$; considered extremely statistically significant.

Table 2: Sensitivity and specificity of AFB detection by different smear preparations

Treatment Method	Sensitivity	Specificity
NaOCl + Centrifugation	80.65%	56.67%
Direct Microscopy	19.35%	43.33%

Tuberculosis has emerged as the leading cause of death due to single infectious agent in recent times. Early identification and isolation of tuberculosis in patients is of utmost importance to minimize the risk of further epidemic spread of the disease. Sputum microscopy forms the main stay of diagnosis, as it is the most reliable, specific and objective method available especially in developing countries (Saxena *et al.*, 2000).

The present study was carried out to evaluate the sodium hypochlorite concentration method as a means of improving early case detection of pulmonary tuberculosis as was previously reported (Githui *et al.*, 2007; Makunde *et al.*, 2007). The recovery rate of AFB (6.92%) for the direct smear and 28.85% following treatment of samples with 3.5% Sodium hypochlorite and centrifugation; and the improved sensitivity of 19.35% to 80.65% and specificity of 43.33% to 56.67% obtained in this study are similar to those reported in Holland and Ethiopia (Van Deun *et al.*, 2000; Yassin *et al.*, 2003).

The increased sensitivity has been attributed to the significantly higher density of bacilli per microscopic field obtained by centrifugation and reduction of debris leaving a clear field for microscopy. There is therefore enough evidence to recommend the introduction of bleach method in most settings, where

mycobacterial culture is not performed routinely (Angeby *et al.*, 2004) as is the case in TB Control Programme in Nigeria.

CONCLUSION

Since control programmes rely heavily on smear microscopy, treatment with sodium hypochlorite and subsequent centrifugation of samples, is recommended for use in the National Tuberculosis Control Programmes in developing countries as the method is safer, cheaper and could be adapted to suit local needs.

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