

Comparison between Bleach Concentration Method and Conventional Ziehl Neelsen Technique of Determination of Mycobacterium Tuberculosis in Sokoto Metropolis

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ABSTRACT

Tuberculosis (TB) is a re-emerging infectious disease of international health priority. It is particularly worrisome in Africa, which informed the declaration of public health emergency by the World Health Organization. This study was aimed to compare between bleach concentration method and conventional ziehl Neelsen technique. Out of 150 patients attending Sokoto specialist hospital who present symptom of tuberculosis screened for the infection, only 64(42.7%) were positive for tuberculosis when using bleach concentration method and 33 (22.0%) were positive with conventional Ziehl Neelsen technique with highly statistical significant difference of bleach concentration method over conventional Ziehl Neelsen stain technique ($P < 0.05$). Socio-demographic variables such age, marital status, sex and socio-economic status were considered in this study, in which bleach concentration method showed significant difference over conventional Ziehl Neelsen technique in all the variables tested except the socio-economic status.

Key Words:

TB; Ziehl Neelsen; Bleach Concentration; Socio-demographic variables and Infection

INTRODUCTION

Tuberculosis (TB) is an infectious disease which is caused by an acid-fast bacillus, which belongs to the *Mycobacterium tuberculosis* complex. TB transmission begins with a human source, most often a person with cavitary, pulmonary TB. When an infectious patient coughs, sneezes or talks, aerosols are formed in the lungs and expelled. These aerosols contain the micro-particles that carry the bacilli, and can be inhaled by others (Comstock, 2000). The disease affects the lungs in approximately two thirds of cases, but almost all other organs can be the site of TB infection. It is estimated that about one third of the world's population is infected with TB. However, the infection is contained by the immune system in about 90 % of those infected. The TB bacilli can lie dormant for years, being protected by a thick waxy coat. If the immune system is weakened, for example by an HIV infection or treatment with immunosuppressive agents, the chances of developing active TB become much higher (Comstock, 2000). Tuberculosis is both preventable and curable. One third of the world's population (two billion people) carries the TB bacteria. More than nine million of these become sick each year with active TB that can be spread to others, but the latent disease cannot be spread. It

disproportionately affects people in resource-poor settings, particularly in Africa and Asia. It also poses significant challenges to developing economies as it primarily affects people during their most productive years. More than 90% of new cases and deaths occur in developing countries (United States Embassy in Nigeria, 2012). Bacteriological diagnosis of tuberculosis (TB) worldwide is to a large extent dependent on direct microscopy of sputum smears after Ziehl-Neelsen (ZN) staining. This method is rapid, specific and reasonably easy to perform, but its sensitivity is not optimal when used in control programmes (Aber *et al.*, 1980). As it is likely to diagnose the most infectious patients, the World Health Organization (WHO) nevertheless recommends it for screening patients with cough lasting for more than 2 weeks for tuberculosis infection (WHO, 2000). Nevertheless, only 42% of 3.8 million notified cases were detected by microscopy in 2001 (WHO, 2000). Mycobacterial culture on solid media is sensitive and specific, but it takes weeks to perform and requires biosafety level III laboratories. It is therefore usually only performed on selected cases (if at all) in countries with limited resources. Newer molecular techniques, such as polymerase chain reaction (PCR), although rapid, are also too costly to be used routinely in those settings where most TB cases occur (Angeby *et al.*, 2004).

Microscopy clearly has many advantages when it comes to speed and feasibility, and if sensitivity could be improved it has the potential to become an even more valuable tool for National Tuberculosis Control Programmes (NTPs) around the world. In the last decade many researchers have suggested that the performance of sputum smear microscopy can be significantly improved if sputum is liquefied with one or other chemical reagent and then concentrated by centrifugation or sedimentation prior to acid-fast staining

(Heifets and Good, 1994). The most widely studied procedure is liquefaction of sputum with sodium hypochlorite (NaOCl), usually known as household bleach. Briefly, sputum is mixed with an equal amount of 5% NaOCl, and the mixture is incubated at room temperature for 10–15 min. Distilled water is added and the sample is concentrated, either by centrifugation for 15 min or by sedimentation overnight. The NaOCl reagent has the advantage of being available almost everywhere. Moreover it is an effective disinfectant, which kills off *Mycobacterium tuberculosis*, and thus probably improves safety in laboratories that lack adequate biosafety facilities. NaOCl is also effective against the human immunodeficiency virus (HIV), and although most HIV particles in sputum are non-infectious it might be of at least psychological importance to laboratory staff, especially if the sputum is haemoptoic (Angeby *et al.*, 2004). Tuberculosis (TB) is a re-emerging infectious disease of international health priority. It is particularly worrisome in Africa, which informed the declaration of public health emergency by the World Health Organization in 2005 (Omoleke, 2012). Approximately, one-third of the world population is infected and about three millions die each year from this disease. It remains the principal cause of death in the developing countries. In Sokoto State, according to the Sokoto TB Control Program (STCP) report, 2115 people were infected in 2012 and 40 died of the disease. The estimated cases of all forms of TB are 203 per 100,000 populations. The new smear positive is 110 per 100,000 populations while the estimated prevalence is 463 per 100,000 populations. However, the diagnosis of Tuberculosis (TB) relies on bacteriological examination of sputum, and the microscopy of smear made directly from sputum has a low sensitivity and there is urgent need for improved method. Thus, an alternative method of microscopy smear made directly from sputum after liquefaction

of the sputum with household bleach (NaOCL) and concentration of bacteria by centrifugation is now available. The NaOCL method increases the number of samples positive for acid- fast bacilli by more than 100% (Bonnet *et al.*, 2008). In spite of the potential benefits, bleach concentration techniques have not made it into routine practice in resource-poor settings. It is unclear whether this is because the published studies are not convincingly enough, their design is not relevant for the setting where the technique is supposed to be used or the results of the studies are not well known among people working in National Tuberculosis Programme (NTP). The results of this study can be applied in prevention, raising awareness of Tuberculosis infection as well as setting priorities in health services planning.

MATERIAL AND METHOD

STUDY AREA

The study was carried out in Sokoto Specialist Hospital, Sokoto State, Nigeria. It is a one of the major referral centre for a number of privately owned hospitals and local government specialist hospitals within the state. Hence it was thought suitable to use this center as a study site among other hospitals in the metropolis. It was located at Sokoto south and was established by colonial master since 1937. Most of the patients seen at the hospital who came from the city and surrounding districts belong to the lower socio-economic class. The Hospital has Laboratory for Tuberculosis and receives sputum specimens for routine examination for Acid fast bacilli (AFB). Examinations are carried out for diagnostic work-up and follow-up of bacteriologic response to anti-tuberculosis chemotherapy in initially sputum smear-positive patients re-examine at 2 months, 5 months, and at the end of an 8 month treatment regimen. The definition of bacteriologically confirmed pulmonary tuberculosis is based on two

consecutive, spontaneously produced sputum specimens positive for AFB, using a Ziehl-Neelsen technique and Bleach Concentration Method.

Ethical Approval

Ethical approval for this study was obtained from the Sokoto specialist hospital ethical committee.

STUDY POPULATION

The study population consists of all patients who present symptoms of the TB, but with no history of prior treatment against TB, visited Sokoto specialist hospital, were simultaneously included in the study.

Inclusion and Exclusion Criteria

Patients of different age and sex who attending the hospital during the period of the study after seek a verbal permission to participate in the study were included. However, patients who are not willing to participate in the study or who receiving medical treatment, against Tuberculosis at the time of the study were excluded from the study.

Questionnaire

Questionnaires were used for the retrospective study to obtained data on Socio- Demographical characteristic which include age, marital status, sex and educational qualification.

SPECIMEN COLLECTION

The screw-cap containers were given to the patients to cough into. Collection of samples was done in a secluded and open air area. All collections were done in the morning and immediately screened. The screw-cap containers were labeled carefully using serial numbers ranging from 01 – 400, as described by Ekkrakene and Igeleke (2010).

Conventional Ziehl Neelsen method

Each patient sputum sample was collected using the screw-cap container and a sterilized wire loop was used to collect the purulent part of the sputum and a smear made on a sterilized microscopic slide, as described by (WHO, 2009).

Bleach Concentration method

The decontamination of the sputum and smear preparation was done as described by (WHO, 2009), followed by ZN technique.

Smear Examination

All heat-fixed sputum smears were examined for the presence of AFB using Z-N staining technique, and a 100 x oil immersion objective microscopy confirmed the presence of AFB as described by (WHO, 2009).

Data analysis:

The data was subjected to statistical analysis t-test was to determined whether there is significant difference between bleach concentration between method and conventional ziehl neelsen technique with the level of significance set at $p < 0.05$ using statistical package (R version 2.13.1).

RESULTS

Total number of 150 patients who showed the symptoms of Tuberculosis, but prior to treatment was included in this study. Their ages ranged from 1 to 80 years, majority of the patients were seen between 20 to 30 years of age. There was male predominance, the male to female sex ratio being 1:0.85. Majority of the patient’ belonged to poor and lower middle class groups. Also most the patients do not undergone formal education. Independent t – test was used to

determined as of whether, there is statistical significant different between bleach concentration method and conventional ziehl neelsen technique of determine tuberculosis. The distribution of tuberculosis among different age groups show that, higher prevalent was recorded among patients when using bleach concentration method with statistical significant difference at 5% ($p < 0.05$) (Table 1). In relation to marital status bleach concentration method was found more sensitive in both married (55.1%) and single (29.2%) than conventional ziehl neelsen stain technique with highly statistical significance at 5% ($p < 0.05$) (Table 2).

In relation to sex, bleach concentration method was highly sensitive than ziehl neelsen stain technique in both male (54.3%) and female (29.0%) at 5% significant level ($P < 0.05$) (Table 3). In relation to educational level also bleach concentration method was highly significant ($p < 0.05$) than conventional ziehl neelsen method (Table 4). In relation to socio-economic class, bleach concentration method was not significantly difference ($p > 0.05$) than conventional ziehl neelsen method (Table 5).

Table 1: Comparison between Bleach concentration method and Ziehl neelsen stain technique based on Age

Age	Bleach Concentration method +Ve	Bleach Concentration method +Ve (%)	Conventional ziehl neelsen method +Ve	Conventional ziehl neelsen method +Ve (%)
1-19	6	46.2	2	15.4
20- 39	31	41.9	16	21.6
≥ 40	27	42.9	15	23.8

($t = 3.3109$, $df = 5$, $p\text{-value} = 0.02122$)

Table 2: Comparison between Bleach concentration method and Ziehl neelsen stain technique base on marital status

Marital Status	Bleach Concentration method +Ve	Bleach Concentration method +Ve (%)	Conventional ziehl neelsen method +Ve	Conventional ziehl neelsen method +Ve (%)
Married	43	55.1	23	29.5
Single	21	29.2	10	13.9

(t = 3.5286, df = 3, p-value = 0.03868)

Table 3: Comparison between Bleach concentration method and Ziehl neelsen stain technique base on Sex

Sex	Bleach Concentration method +Ve	Bleach Concentration method +Ve (%)	Conventional ziehl neelsen method +Ve	Conventional ziehl neelsen method +Ve (%)
Male	44	54.3	25	30.9
Female	20	29.0	8	11.6

(t = 3.2387, df = 3, p-value = 0.0479)

Table 4: Comparison between Bleach concentration method and Ziehl neelsen stain technique base on Educational level

Education	Bleach Concentration method +Ve	Bleach Concentration method +Ve (%)	Conventional ziehl neelsen method +Ve	Conventional ziehl neelsen method +Ve (%)
Primary	7	58.3	4	33.3
Secondary	8	21.1	4	10.5
Tertiary	7	30.4	5	21.7
Non-formal education	42	54.5	20	26.0

(t = 2.6118, df = 7, p-value = 0.03482)

Table 5: Comparison between Bleach concentration method and Ziehl neelsen stain technique base on Socio-economic status

Socio- economic class	Bleach Concentration method +Ve	Bleach Concentration method +Ve (%)	Conventional ziehl neelsen method +Ve	Conventional ziehl neelsen method +Ve (%)
High class	6	54.5	3	27.3
Middle class	9	25.0	5	13.9
Lower class	49	47.0	25	24.3

(t = 2.2076, df = 5, p-value = 0.07834)

DISCUSSION

A total of 150 patients who presented with symptoms of tuberculosis were diagnosed for the presence of *Mycobacterium tuberculosis* using both bleach concentration and conventional Ziehl Neelsen methods at Sokoto State Specialist Hospital, Sokoto, Nigeria. Of the 150 patients tested, only 33 (22%) were positive using conventional Ziehl Neelsen method while 64 (42.7%) were positive when bleach concentration method was used, with highly statistical difference at (p<0.05). The improved recovery of mycobacteria after treatment with sodium hypochlorite (NaOCL) might be attributable to changes in surface properties of the Mycobacteria (i. e., charge and hydrophobicity) and/or denaturation of sputum constituent leading to flocculation and subsequent increased sedimentation rate of the mycobacteria (Daffe and Etienne, 1999).

The result of this study correlates with the work of Angeby *et al.* (2000) who showed a statistically significant ($P < 0.05$) improvement in sensitivity with the bleach method as compared to the direct or conventional Ziehl neelsen method. The result of this study is also consistent with the work of Aung *et al.* (2001) at TB Institute in Myanmar who reported significant improvement (p<0.05) of the bleach concentration method in the detection of

tuberculosis. Similarly, it correlates well with the report of Bruchfeld *et al.* (2000) that reported sensitivity of bleach concentration over conventional Ziehl Neelsen method. Similar work by Farnia *et al.* (2002) of hospitalized patients at a National Tuberculosis Research Institute in Iran that showed a higher significant difference (p<0.05) of the bleach concentration method over conventional ziehl neelsen technique. However, the result obtained in this work is different from the report of Wilkinson and Sturm (1997) who showed no improvement in sensitivity when bleach concentration method was compared with conventional Ziehl Neelsen staining technique. The significant difference of the bleach concentration method over Ziehl Neelsen stain technique might be attributed to the liquefaction of sputum *with* sodium hypochlorite (NaOCl), usually known as household bleach, unlike in conventional Ziehl Neelsen staining technique which involves direct microscopy of the purulent of portion the sputum after staining, which might not necessarily contain the bacterium in that portion. The bleach concentration method increases the detection rate; it makes the sputum safer in terms of infection control since the bacilli are deactivated with sodium hypochloride (NaOCL) and also more sensitive in terms of number of positivity (Angeby *et al.*, 2004)

The distribution of tuberculosis among different age groups showed that higher prevalence (46.2%) was recorded among patients between age of 1- 19 (46.2%), while low prevalence (41.9%) was recorded among those between the ages range of 20-39 years when using bleach concentration method (Table 1). Similarly, the distribution of *Mycobacterium tuberculosis* among different age groups using conventional Ziehl Neelsen technique, showed that patients within the ages of 20- 39 years had the highest prevalence of TB while those within the age range of ≥ 40 years had the lowest prevalence of 3.8%. There was also with significant difference when using bleach concentration method over conventional Ziehl Neelsen technique ($p < 0.05$) (Table 1). The implication of these findings is that successful implementation of the bleach concentration method might improve case detection rate and thereby reduce tuberculosis incidence among the different age groups in the State

The most likely risk factors that makes young age group to be at higher risk of developing tuberculosis is the frequent rate of transmission of the disease to such groups, due to smoking, alcohol abuse which may result in decreased immunity, HIV infection and lack of awareness on the mode of transmission which predisposes them to the disease.

CONCLUSION

A total of 150 patients attending Sokoto specialist hospital who present symptom of tuberculosis were screen for the infection, only 64(42.7%) were positive for tuberculosis when using bleach concentration method and 33 (22.0%) were positive with conventional Ziehl Neelsen technique with highly statistical significant difference of bleach concentration method over conventional Ziehl Neelsen stain technique. Socio-demographic variables

such age, marital status, sex and socio-economic status were considered in this study, in which bleach concentration method showed significant difference over conventional Ziehl Neelsen technique in all the variables tested except the socio-economic status.

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