

Effect of 1.5% sodium hydroxide final concentration on recovery rate of Mycobacterial Species and decontamination of other Bacterial and Fungal contaminants on sputum

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Abstract

Background: Digestion and decontamination of non-sterile clinical specimens such as sputum are an essential step in the isolation of mycobacteria. Masking of mycobacteria in Mycobacterial growth indicator tube (MGIT) 960 liquid culture system by fungi and bacteria other than mycobacteria is a major problem.

Objective: To assess the effect of 1.5% sodium hydroxide final concentration on recovery rate of mycobacterial species and decontamination of other bacterial and fungal contaminants from sputum sample.

Methodology: Laboratory based cross sectional study with convenient sampling technique was carried out on subjects referred to the National Tuberculosis Reference Laboratory of Ethiopian Public Health Institute from November 2015 to February 2016. Single morning sputum was collected from each patient and analyzed.

Results: A total of 264 subjects were enrolled in the study. The mean age of participant was 31 (SD 20.14 - 41.42) years old. The majority (61%) were male. Increasing the final concentration of NaOH from 1% to 1.5% reduced the contamination rate from 22.4% to 6.8% ($P < 0.001$) without affecting mycobacterial recovery ($P = 1.00$). A total of 26 different species of microbial contaminants were identified as being associated with BACTEC MGIT 960 culture system.

Conclusion: Results presented in this study demonstrated that the use of a final concentration of 1.5% NaOH with NALC method aids in reducing culture contamination rate for decontaminating sputum samples referred for tuberculosis culture diagnosis. Among the identified microbial contaminants, the most predominant was coagulase negative *Staphylococcus* species.

Key words: Decontamination methods; Mycobacteria; NALC- sodium hydroxide

Introduction

Tuberculosis (TB) is a disease of major public health concern globally with an estimated 9.6 million new cases of TB and about 1.5 million deaths in 2014 (Global Tuberculosis Report, WHO 2015). It is caused mainly by a single infectious agent, namely, *Mycobacterium tuberculosis*, which is transmitted easily in overcrowded settings and in conditions of malnutrition and poverty (Pereira *et al.*, 2005). There has been an increase of cases in multidrug resistant (MDR) tuberculosis as well as extensively drug resistant tuberculosis at global and national level.

In 2014, 3.3% of new TB cases and 20% of previously treated cases are estimated to have MDR-TB; among this 9.7% of people with MDR-TB are expected to have extensively drug resistant TB (XDR). Globally, out of 9.6 million estimated TB cases only 6 million TB cases were reported. This indicates that above 3 million estimated TB cases were undiagnosed and not reported. Of the 480,000 estimated multidrug-resistant TB cases, only 120,000 cases were detected. So far, more TB patients were tested for drug resistance in 2014 than ever before. Rapid case detection is critical, and attempts to shorten the time for detection deserve attention (Global Tuberculosis Report, WHO 2015;

TB DOTS Strategy Coordination 2014, Republic of South Africa). According to 2015 WHO TB report, Ethiopia is one of the high TB burden countries. The estimated country TB prevalence and incidence is 200 and 207 per 100 000 population in 2014, respectively (Global TB Report, WHO 2015).

Demonstrating acid fast bacilli by Ziehl Neelsen (ZN) smear microscopy procedure from various clinical samples has been the mainstay for the diagnosis of mycobacterial infections (Chakravorty *et al.* 2005). Although many new molecular diagnostic methods have been developed, AFB smear microscopy is widely applied and TB culture remains the “gold” standards for the diagnosis of active TB. These old methods have been routinely used for the confirmation of TB in patients particularly in Africa (Della Latta P 2004). Definitive diagnosis of mycobacterial disease demands that the causative agent be recovered and identified in culture. Specimens collected from sterile body sites of presumptive TB patients can be inoculated directly onto primary isolation mycobacterial growth supporting media without prior decontamination as long as they are properly handled and homogenized

before inoculation. However, most of clinical specimens submitted to the mycobacteriology laboratory for cultural confirmation of the TB are non-sterile; contain microbes other than mycobacterial (contaminants). *Mycobacterium tuberculosis* culture usually requires sputum decontamination and centrifugation to prevent cultures from being overgrown by contaminating bacteria and fungi(Grandjean *et al.* 2008).

The use of sodium hydroxide (NaOH) -sodium citrate along with the mucolytic agent N-acetyl L-cystein (NALC) (NALC-NaOH method) is one of the most widely used methods, as it is rapid and relatively effective in reducing the number of contaminating organisms(Yajko *et al.* 1993). Decontamination of clinical samples submitted for the diagnosis of mycobacteria has two objectives: destruction of bacteria other than mycobacteria and homogenization of the specimen to allow for adequate exposure of the bacilli. Decontaminating clinical samples enables killing much of the contaminants while harming as few mycobacteria as possible(Global Tuberculosis Report, WHO 2009).

The increase in the concentration of NaOH has effect on the mycobacterial recovery rate due to its killing effect. In addition, high final NaOH concentration leads to false positive results in MGIT liquid culture system. Since, detection of growth in MGIT is based on an oxygen sensor system, as a result high concentration of NALC or NaOH might result in false fluorescence (Peres *et al.* 2009; Becton Dickinson 2006).

In fact, the high contamination rate affects the diagnosis of TB; and has a direct impact on the sensitivity and specificity, time for the delivery of laboratory results, and the capacity to trace people with positive culture results. Therefore, an economical, straightforward decontamination and concentration method would improve case detection rate in a setting such as Ethiopia. Hence, in this study 1.5% final concentration of NaOH decontamination solution were evaluated in comparing with 1% standard decontamination method, for the successful cultivation of *Mycobacterium species*. Then contaminants prominent in TB culture laboratories were identified. Therefore, the main aim of this study was to compare 1.5% final concentration of NaOH with the standard 1% NaOH decontamination method for the successful cultivation of *Mycobacterium species* and to identify the most common contaminants in TB culture laboratories.

Methods and Materials

Study design and period: A cross sectional study design with convenient sampling technique was used to assess the effect of 1.5% NaOH final concentration on detection and recovery rate of mycobacterial species and decontamination of bacterial and fungal contaminants from sputum collected in patients referred to the Ethiopian Public Health Institute from November 2015 to February 2016 Addis Ababa, Ethiopia.

Study area and population: The study was conducted at the National TB Reference Laboratory (NTRL) of Ethiopian Public Health Institute (EPHI). All patients referred from 12 health facilities (Addis Hiwot Health Center, Armed Force General Teaching Hospital, Alem Tena Higher Clinic, ALERT, Ambo Hospital, Kara Health Center, Fiche Hospital, Police Hospital, St Paul Hospital, St Peter Hospital, Tullu Bolo Health Center, and Universal Higher Clinic) for routine MDR-TB diagnosis and MDR-TB treatment follow-up culture diagnosis were enrolled in the study. Specimens were collected at the referring health facilities.

Specimen Collection, Storage and Transportation: Morning sputum was collected from 264 patients. Each sputum was spilt into two. Half were processed for culture using 1% final concentration of sodium hydroxide-sodium citrate with NALC, and the remaining half were processed by 1.5% final concentration of sodium hydroxide-sodium citrate with NALC. Clinical specimens were collected in wide-mouth containers at the referring health facility. Specimens were either processed in the same day of collection or refrigerated at -4°C for an average of 3 to 5 days until transported to the EPHI. Specimens were transferred using cold chain through the network of national sample transportation system.

Specimen Digestion and Decontamination: Each specimen was mixed and equally divided into two parts. One part of the specimen was decontaminated with NaOH-NALC and sodium citrate solution to make a final concentration of 1.5% NaOH while the other half of the specimen was processed with same solutions to have a final concentration of 1%. The standard procedure is recommended by the Centers for Disease Control and Prevention (Patricia *et al.*,1985), while the comparative procedure with 1.5% NaOH final concentration is recommended by WHO(GLI 2014). All specimens were processed and inoculated into the MGIT tube for cultivation using the MGIT-960 system according to the manufacturer's recommendations, including the use

of PANTA (Polymixin B, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin) antibiotic supplement (Becton Dickinson 2013).

Equal amounts of solution containing 0.5% (w/v) NALC, 3% (w/v) NaOH and 1.47% (w/v) sodium citrate (final concentrations (w/v): 0.25% NALC, 1.5% or 1% NaOH, 0.735% sodium citrate) were added to a sputum in a 50 ml BD Falcon centrifuge tube. All tubes were incubated at room temperature for 15 min. After incubation, the mixture was neutralized with PBS (Phosphate Buffer Saline), bringing the total volume to 50 ml. The homogenate was centrifuged with a relative centrifugal force of 3,000xg for 15 minutes. The supernatant was decanted/discarded into a splash-proof container containing a tuberculocidal disinfectant. The sediment was re-suspended using 1ml phosphate buffer (pH 6.8) and was used for smear preparation and inoculation of MGIT tube containing modified Middlebrook 7H9 medium. All inoculated MGIT tubes were incubated in BACTEC MGIT 960 system at 37°C for 42 days. All tubes that flag positive after incubation in BACTEC MGIT 960 system were considered positive for microbial growth. All steps and procedures used for processing samples with the standard 1% NaOH final concentration were the same in both methods except for higher NaOH concentration added for improved decontamination purposes.

Materials required for collection and culture processing were taken from the National TB Reference Laboratory, and an experienced laboratory technologist have performed the work at the National TB Reference laboratory in a Class IIB biosafety cabinet. All possible efforts were made to prevent laboratory cross-contamination; including the collection of clinical specimens with a leak proof sterile container. The stock of decontamination fluid was sterilized before use. The specimens in the laboratory were processed by batch and two negative (starting and ending) controls were included in each batch. All reagents used for sample processing were checked for sterility.

Identification of Mycobacteria: The BACTEC MGIT 960 system is designed for the rapid detection of mycobacteria in all types of clinical specimens except blood. The system consists of a culture tube containing modified Middlebrook 7H9 medium with a fluorescent growth indicator embedded in silicone on the bottom of each tube. This compound is sensitive to the presence of dissolved oxygen in the broth medium. As microorganisms grow, the oxygen in the medium is depleted with a subsequent increase in the fluorescence of the indicator. The enhanced fluorescence in tubes can be monitored automatically

over time using the BACTEC 960 detection module (TB DOTS Strategy Coordination 2014, Republic of South Africa; Lu *et al.* 2002).

Smears were prepared from both the sediment and culture positive MGIT tubes. Mycobacteria in all tubes that flag positive with MGIT-960 system were identified by performing acid-fast staining and SD Bioline MPT64 TB antigen tests (SD Bioline Kit, Standard Diagnostics, Inc., South Korea) to rapidly confirm the presence of MTB complex (TB DOTS Strategy Coordination 2014, Republic of South Africa). One drop from suspended pellet and an aliquot of broth from culture positive MGIT tube about 100 µl (2 drops), were stained with Ziehl-Neelsen as WHO recommendations (Mycobacteriology laboratory manual, GLI 2014). Each slide was coded and read blindly by a qualified technologist. When comparing the results of AFB smears, only their negativity or positivity for AFB was used because of the expected difference in quantitative results between the concentrated and un-concentrated portions of the same sample.

TB Ag MPT64 Rapid test can detect MPT64 antigen (Immunogenic Protein (alternate gene name: mpb64) specifically secreted from tuberculosis bacteria. The MPT64 antigen is the secretory protein (24 kDa) that is one of the secreted major antigens from *Mycobacterium tuberculosis* complex (MTBC). Then, 100 µl of liquid cultures were added to the sample pore and incubated for 15 minutes. **Identification of bacteria other than mycobacteria:** A portion of positive culture (10 µl) was inoculated on Blood Agar (Titan Biotech, Ltd, of India), Chocolate Agar using soluble hemoglobin powder (Oxoid, Ltd, United Kingdom) and MacConkey Agar (Oxoid, Ltd, United Kingdom). The inoculated media were incubated at 37°C for at least 48 hours. Colonies typically of bacteria were identified by Vitek 2 compact system (BioMérieux Diagnostics 2007, of France).

Fungal Identification: All positive tubes were also sub-cultured onto Sabouraud Dextrose Agar (Central Drug House (CHD), Ltd, of India) and Brain Heart Infusion Agar (Carl Roth, Ltd, of Germany) for fungal growth. Culture plates were examined twice a week for any fungal growth for about four weeks. Cultures of mycelia fungi (molds) were identified by examining macroscopic and microscopic characteristics of their colony. Texture, rate of growth, topography and pigmentation of the front and the reverse side of the culture were employed. Microscopic identification of mold isolates was performed by placing pieces of a colony from Sabouraud Dextrose Agar to clean microscopic slide and staining with lacto phenol cotton blue.

After placing a cover slip, each preparation was observed microscopically.

Quality control: The sterility of the sample processing reagents was tested by inoculating the portion of the reagent onto blood agar media and incubated at 37°C for 72 hours (Mycobacteriology laboratory manual, GLI 2014). Similarly, the sterility of culture media was also checked by incubating 5% of the batch media. The performance of culture media was tested by using control strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228) and H37Rv (ATCC 27294). For instance, 2% of a batch of 100 tubes of MGIT Culture media was tested with 10^{-2} , 10^{-3} and 10^{-4} dilutions of *M. tuberculosis* control strain (H37Rv) for monitoring the performance of the new lots media. A positive and negative panel slides were used for assessing the quality of newly prepared staining reagents (ZN stains). Data were captured at each and every activity of quality control process.

Data collection and analysis: The values for Positive percent agreement, Negative percent agreement and overall accuracy were calculated using a 2x2 table.

The degree of association was calculated using the kappa statistic and difference of proportions and their significance were calculated using X^2 and the Z test using SPSS version 20 and Stata/SE Ver. 13. The time to positive culture were compared between the two concentrations of sodium hydroxide methods using the

Wilcoxon signed-ranks test. The following variables were collected using a structured questionnaire from registers and patient request form such as categorical data: sex, medical history, referring health facility, previous treatment, co-infection, reasons for test requested and microbial profile (bacterial and fungal contaminants), and as continuous variables: age, time for positive test results. Categorical data were presented using percentage, and continuous variables were presented using mean and standard deviation. P value < 0.05 was considered to be statistically significant.

Results

Demographic and clinical characteristics: A total of 264 study participants were enrolled in the study. Majority (61.0%) were male. The mean age was 30.78 ± 10.64 years old. The co-infection (TB/HIV) rate in both sexes was 5.3%. Among the participants, 241 (91.3%) of them were referred for follow-up culture examination of patients on MDR-TB treatment (Table 1).

Effect of 1% and 1.5% Sodium Hydroxide final concentration in processed samples: AFB smear positivity rate was 13.6%. There is no difference between the two concentration (with 1.5% and 1% NaOH) in the isolation of mycobacterial species from both smear positive and negative samples, include MTBC (n=51), and NTM (n=3).

Table 1: Sex and age category of study participants referred for tuberculosis culture diagnostic tests at EPHI NTRL in the period from November 2015 to February 2016

Variables		Co-infections		Total N (%)
		No N (%)	Yes N (%)	
Age category	18 to 24	82(31.1)	3(1.1)	85(32.2)
	25 to 34	94(35.6)	5(1.9)	99(37.5)
	35 to 44	42(15.9)	5(1.9)	47(17.8)
	45 to 54	22(8.3)	1(0.4)	23(8.7)
	> 55	10(3.8)	0(0.0)	10(3.8)
Gender	F	99(37.5)	4(1.5)	103(39.0)
	M	151(57.2)	10(3.8)	161(61.0)
Previous TB history	New Case	6(2.3)	1(0.39)	7(2.7)
	Retreatment	14(5.03)	2(0.98)	16(6.1)
	MDR-TB	230(87.1)	11(4.2)	241(91.3)
Total no. of participants				264

Majority of the study participants (51%) were from ALERT, followed by St Peter (36.4%) (Figure1).

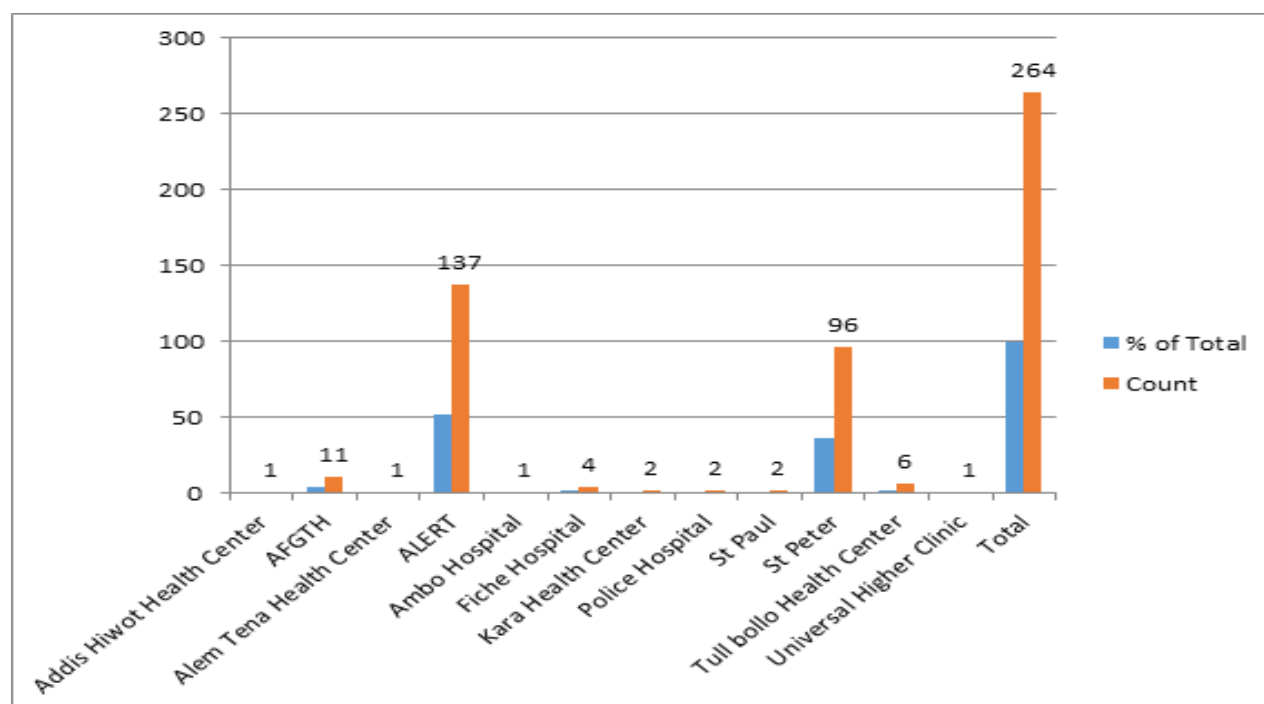


Figure 1: Distribution of study participants with referring health facility for mycobacterial culture at EPHI NTRL in the period from November 2015 to February 2016

Table 2: Classification of culture results and AFB results among sputum's collected from patients referred to EPHI NTRL in the period from November 2015 to February 2016.

AFB from Sputum	1.5%NaOH				1%NaOH mmm			
	Contaminated N (%)	Negative N (%)	Positive		Contaminated N (%)	Negative N (%)	Positive	
			NTM N (%)	MTBC N (%)			NTM N (%)	MTBC N (%)
Negative	15(5.7)	187(70.8)	3(1.1)	23(8.7)	58(22.0)	146(55.3)	3(1.1)	21(8.0)
Positive	3(1.1)	5(1.9)	0(0.0)	36(13.1)	1(0.4)	5(1.9)	0(0.0)	30(11.3)
Total	18(6.8)	192(72.7)	3(1.1)	51(19.3)	59(22.4)	151(57.2)	3(1.1)	51(19.3)

The overall accuracy of MGIT culture was 92.68% and 77.24%, when specimens decontaminated with

1.5% and 1% NaOH with NALC, respectively (Table 3).

Table 3: Positive percent agreement, Negative percent agreement and overall accuracy of the two decontamination methods (1% and 1.5% NaOH) at EPHI NTRL in the period from November 2015 to February 2016.

Decontamination methods (Concentration of NaOH)		Positive Percent Agreement	Negative Percent Agreement	Over all Accuracy
1.5% NaOH		85.71	98.01	92.68
1% NaOH		93.33	95.48	77.24
Differences	Z value	7.62	1.24	4.48
	P value	0.225	0.214	<0.001

The difference between the two methods in total was statistically not significant ($P < 0.001$) and the two

decontamination methods were moderately associated ($Kappa = 0.562$) (Table 4).

Table 4: A cross tabulation for the 1% and 1.5% NaOH decontamination methods evaluated at EPHI NTRL in the period from November 2015 to February 2016.

			1%NaOH			Total
			Positive	Negative	Contaminated	
1.5% NaOH	Positive	N (%)	42(15.9%)	3(1.1%)	9(3.4%)	54(20.5%)
	Negative	N (%)	7(2.7%)	148(56.10%)	37(14.0%)	192(72.70%)
	Contaminated	N (%)	5(1.90%)	0(0.0%)	13(4.90%)	18(6.80%)
Total		N (%)	54(20.40%)	151(57.20%)	59(22.40%)	264(100%)
Statistics		χ^2	184.12			
		Kappa	0.562			

The percentage of culture correlation (smear positivity versus culture positivity rate) when using either the final concentration of 1% sodium hydroxide or 1.5%

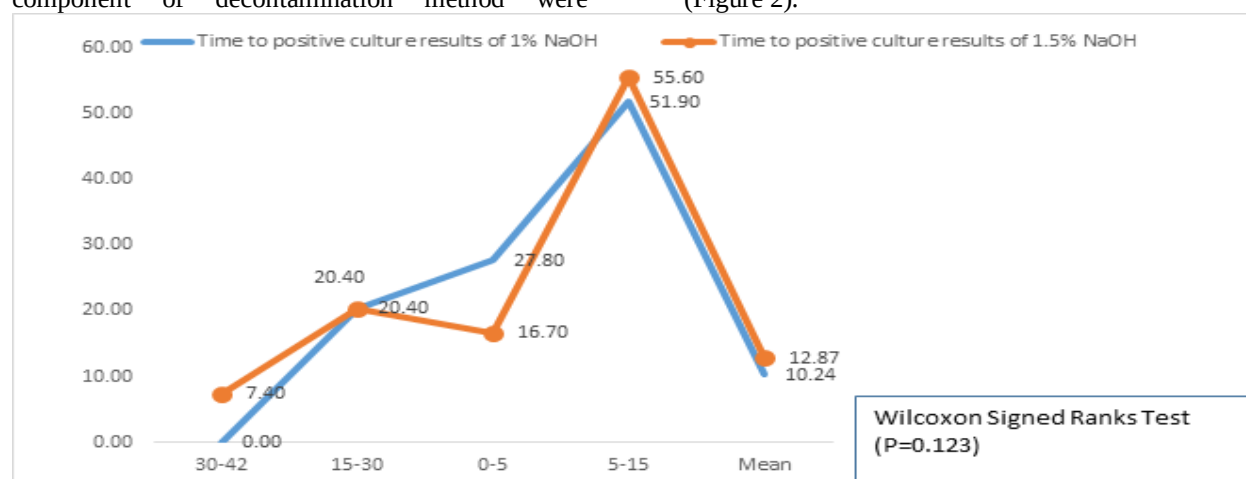
sodium hydroxide were 83.3% and 77.8%, respectively, as shown in table 5.

Table 5: Comparison of decontamination methods on contamination rate, recovery rate, culture correlation and culture contribution (smear negativity versus culture positivity rate).

Indicators	Decontamination methods			Statistics	
	1% NaOH (%)	1.5%NaOH (%)	Difference	Z value	P value
Contamination	22.4	6.8	15.6	5.08	<0.001
Recovery	20.45	20.45	0	0	1.000
Correlation	83.33	77.78	5.56	0.59	0.552
Contribution	10.53	11.40	0.877	0.31	0.785

The mean incubation period to positive culture determined with the 1% and 1.5%NaOH as a component of decontamination method were

(10.24±6.59 and 12.87±9.65 days), respectively, however, this difference was not significant (P=0.123) (Figure 2).

**Figure 2: The mean incubation period of culture positive isolated from smear positive and negative cases at EPHI NTRL in the period from November 2015 to February 2016**

The AFB negative versus culture negative, and AFB negative versus culture contaminated results with the 1% and 1.5%NaOH concentration method were

(55.3% and 22.0%;70.83% and 5.68%), respectively, as shown on table 6.

Table 6: Smear examination (AFB) and culture correlation for sputum specimens processed by the NALC-NaOH method using standard (1%) and (1.5%) final concentrations of NaOH from patients referred to EPHI NTRL in the period from November 2015 to February 2016.

AFB and MGIT Culture results	Decontamination methods (Concentration of NaOH)				Statistics	
	1.5% NaOH		1% NaOH		Z	P value
	N	%	N	%		
AFB positive Vs Culture positive	28	10.61	30	11.3	-0.2538	0.7996
AFB positive Vs Culture negative	5	1.93	5	1.9	0.0251	0.9799
AFB positive Vs Culture contaminated	3	1.1	1	0.4	0.9322	0.3513
AFB negative Vs Culture positive	26	9.85	24	9.1	0.2942	0.7686
AFB negative Vs Culture negative	187	70.83	146	55.3	3.697	0.002
AFB negative Vs Culture contaminated	15	5.68	58	22.0	8.1824	<0.001
Total	264	100	264	100		

Common bacterial and fungal contaminants:

The most prevalent bacterial and fungal contaminants in the MGIT were identified. A total of 73 microbial contaminants were isolated from 59 (22.4%) contaminated MGIT tubes of 264 sputum's processed using the standard 1% NaOH final concentration. A

total of 26 different microbial species were identified from contaminated MGIT tubes. Of the 26 identified microbial contaminant 24(92.3%) were bacterial and 2(7.7%) were fungal contaminants. From 59(22.4%) contaminated MGIT tubes, 10(16.9%) were isolated with more than two microbial contaminants (Figure 3).

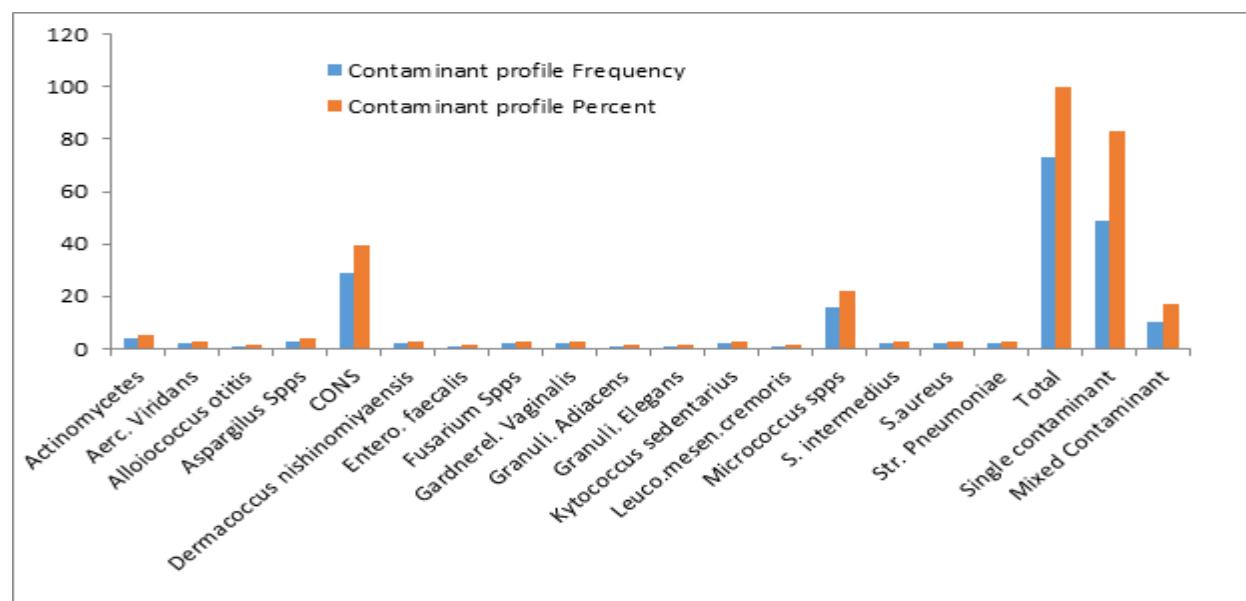


Figure 3: Distribution of bacterial and fungal contaminants associated with MGIT culture at EPHI NTRL in the period from November 2015 to February 2016

Discussion

The BACTEC MGIT 960 culture system improves the duration of time for detection and identification of mycobacterial species. Despite the fact that, the MGIT liquid culture system is associated with a high

contamination rate compared to the conventional solid culture. Currently, an increased contamination rate with MGIT 960 culture system is a major challenge in tuberculosis culture laboratories. However, in this study and other similar studies (Bradner & Stabel

2012) the 1.5% sodium hydroxide final concentration with NALC were found to reduce the culture contamination rate when compared to the commonly used standard decontamination method 1% sodium hydroxide with NALC.

Treating clinical specimens by 1.5% sodium hydroxide with NALC have reduced the contamination rate from 22.4% to 6.8% compared to 1% sodium hydroxide with NALC. A statistically significant ($P < 0.001$) reduction in the contamination rate was observed after increasing the final concentration of sodium hydroxide to 1.5% for samples incubated on MGIT 960 culture system. The contamination rate recorded in this study using the 1.5% NaOH-NALC was also reasonably lower compared to those reported by Peres *et al.* (2009) with a final concentration of 1.25% NaOH-NALC.

The contamination rate recorded in a study done at St John's Laboratory in Canada, and a multi-center study in Italy by the modified NALC-NaOH method with 1.5% NaOH were relatively lower, i.e., in the range 4-5% (Ratnam *et al.* 1987; Piersimoni *et al.* 2001), and decreased to non-detectable levels (Chien *et al.* 2000; Bradner *et al.* 2013). The varied performance of decontamination methods might be due to the slight selectivity of culture medium used. This selectivity is as a result of different; modifying ingredients, antibiotics, and the state of the medium either solid or liquid as described before (Chien *et al.* 2000; Somoskövi *et al.* 2000; Lu *et al.* 2002; Hines *et al.* 2006).

Findings in this study showed neither harmful nor beneficial effect on the mycobacterial recovery ($P = 1.000$). Likewise, other studies on comparisons of decontamination methods reported the same rate of mycobacterial viability (Bradner *et al.* 2013; Ratnam *et al.* 1987; Piersimoni *et al.* 2001). In contrary, evaluation of culture results for specimens decontaminated with either 1.25% or higher concentration of NaOH-NALC showed a significant difference in the viability of mycobacterial species (Peres *et al.* 2009; Corner *et al.* 1995). A reduction in mycobacterial recovery might be due to the culture method used or the specimen type. Supporting our findings, above twenty (20.2%) differences in recovery of mycobacterial isolate was also observed between the MGIT 960 versus solid media in a study done by Hines *et al.* (2006).

No statistical significant differences was observed ($P = 1.000$) when mycobacterial isolate detection on MGIT cultures from both smear positive and smear negative clinical specimens when decontaminated with 1% final concentration of NaOH-NALC compared to 1.5% NaOH-NALC. Similarly, in a study

by Bradner and Stabel (2012), no significant difference was seen on the cultures contributions and recovery rate for the clinical specimens in a study conducted to optimize methods for the detection of *Mycobacterium avium* subs p. In contrast, better performance in isolate detection was observed by comparing four decontamination methods for recovery of *Mycobacterium avium* complex from stools (Yajko *et al.* 1993). This study was done to evaluate the four decontamination methods that the entire nine smears positive were culture positive for *Mycobacterium avium* complex with the standard concentration of NaOH with NALC. But, in a study conducted in Brazil, specimens with paucibacillary and negative smear results were found to reduce mycobacterial isolation (Peres *et al.* 2009). Increasing the concentration of NaOH to the level evaluated in this study might not significantly affect the culture contribution rate.

The mean incubation period to positive culture determined with the 1% NaOH as a component of decontamination method (10.24 ± 6.59 in days) was shorter than 1.5% NaOH (12.87 ± 9.65 in days), however, the mean time for detection observed in this study between the two decontamination methods has no significant difference ($P = 0.123$). as indicated elsewhere, specimens subjected to sodium hydroxide concentration ranging from 0.5% to 2% had no effect on the time to positive culture result (Bradner *et al.* 2013), nevertheless, a relative increment of mean time with 1.5% was seen compared to studies done abroad (Piersimoni *et al.* 2001).

The two decontamination methods, concentrated 1.5 % and 1% NaOH, have showed moderate association ($Kappa = 0.562$). Increasing concentration of sodium hydroxide from 1% to 1.5% had improved the performance characteristics of BACTEC MGIT 960 culture system for successful yield of valid results on the cultivation of mycobacterial species. Overall accuracy of the two methods was 77.24 and 92.68, respectively.

In this study, the false positive (instrument positive and smear/sub-culture negative) and false negative (instrument negative and smear /sub-culture positive) results were not identified. This might be due to the total specimens included in this study could not be sufficient to detect the instrument false positive or false negative culture results. The most frequently isolated contaminants observed were coagulase negative staphylococcus (CONS) followed by *micrococcus species*. The most prominent bacterial contaminants associated with MGIT system, Coagulase-negative staphylococci, isolated in this study were similar with a rate of 34.8% recorded in a

study done in Philadelphia in 1997(Cornfield *et al.* 1997) and by McClean (2011). In the same way, gram-positive organisms were highly favored in the MGIT system(Piersimoni *et al.* 2001).

Many of the isolates were grown on 5% Sheep Blood Agar, Brain Heart Infusion Agar and Sabouraud Dextrose Agar but none of the isolates were culture positive on MacConkey agar. This result showed that the standard decontamination method used and the concentration of antibiotics consisting; Polymixin B, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin (PANTA), added to the MGIT tube were inefficient in the standard decontamination method (1%NaOH) to kill or suppress the growth of identified bacterial and fungal contaminants.

The concentration of PANTA supposed to be added as recommended by the manufacturer per ml of MGIT tube were (Polymixin B, 46unit/ml; Amphotericin B, 5ug/ml; Nalidixic acid, 18ug/ml; Trimethoprim, 5ug/ml; and Azlocillin, 5ug/ml) (Becton Dickinson 2013). The concentration used in this study has a relatively slight difference (Polymixin B, 50unit/ml; Amphotericin B, 5ug/ml; Nalidixic acid, 20ug/ml; Trimethoprim, 5ug/ml; Azlocillin, 10ug/ml) than with a study conducted in Switzerland by Realini *et al.* (2000).

Most of the contaminants isolated in this study were gram positive and fungi. This implies the concentration of antibiotics assumed to be effective against these microorganisms (Trimethoprim and Nalidixic acid, respectively), might not be effective. In the other hand, antibiotics added for gram negative bacteria (Polymixin B) could effectively suppress their growth. Differences on the three drugs could be a reason for high contamination recorded by CONS. Consequently, other study showed that increasing the PANTA concentration might significantly reduce the contamination rate (Peres *et al.* 2011).

In general, the final concentration of 1.5% NaOH with NALC as a component of decontamination method was found to be equivalent to the standard method in culture recovery rate with the standard. Whereas reduction of the contamination rate is the most important advantage of this decontamination method for the reason that deprived of affecting the smear positive culture positivity and smear negative culture negativity outcomes. Additional benefits of increasing the concentration of sodium hydroxide to the level of evaluation in this study were found to increase the overall performance of MGIT liquid culture system.

Conclusion and Recommendations

Results presented in this study demonstrated that a final concentration of 1.5% sodium hydroxide with

NALC decontamination method have acceptably improved the culture contamination rate without affecting the recovery of mycobacterial species, smear positivity versus culture positivity rate and smear negativity versus culture positivity rates. In addition, the increase of the final concentration of NaOH by 0.5% has no effect on the recovery of *Mycobacterial species*. Consequently, the use of a final concentration of 1.5% NaOH with NALC method are suitable and aids in reducing culture contamination rate for decontaminating clinical specimens referred for tuberculosis culture diagnosis particularly in settings where culture contamination is a high quality problem. It also might be very important for clinical specimens usually collected from follow up patients referred for culture diagnosis in Ethiopia. With the intension of supplementing the current study, further studies are recommended to assess the factors associated with increased culture contamination rate, and to examine antimicrobial susceptibility of those more prominent contaminants associated with MGIT liquid culture system are recommended.

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