COMPARATIVE STUDY OF GROWH OF *Mycobacterium tuberculosis* ON MODIFIED OGAWA MEDIA, MODIFIED LOWENSTEIN JENSEN MEDIA AND 2% BUFFERED LOWENSTEIN JENSEN (BLJ) MEDIA

A

Dissertation

Submitted to the Central Department of Microbiology

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In Partial Fulfillment of the Requirement for the Award of the Degree of

Masters of Science in Microbiology

(Environment and Public Health)

By

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RECOMMENDATION

This is to certify that Mr. Sajeen Bahadur Amatya has completed this dissertation work entitled "Comparative study of growth of *Mycobacterium tuberculosis* on Modified Lowenstein-Jensen media, Modified Ogawa media and 2% Buffered Lowenstein-Jensen (BLJ) media" as a partial fulfillment of Master of Science Degree in Microbiology under our supervision. To our knowledge this work has not been submitted for any other degree.

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On recommendation of Mr. Binod Lekhak and Dr. Bhabana Shrestha, this dissertation work by Mr. Sajeen Bahadur Amatya entitled "Comparative study of growth of *Mycobacterium tuberculosis* on Modified Lowenstein-Jensen media, Modified Ogawa media and 2% Buffered Lowenstein-Jensen (BLJ) media" has been approved for the examination and is submitted to the Tribhuvan University in partial fulfillment of Master of Science Degree in Microbiology.

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ABSTRACT

This prospective hospital based study was conducted from December 2005 to September 2006 at German-Nepal Tuberculosis Project- National Reference Laboratory (GENETUP-NRL), Kalimati, Kathmandu. In the present study, a total of 324 sputum samples from suspected TB patients were collected. In all the samples 220 were smear positive and 104 were smear negative samples as determined microscopically by standard fluorochrome method.

In all the samples 177 were collected from patients suspected of pulmonary tuberculosis. All of the 324 sputum samples were divided into two equal halves and transferred in two calibrated centrifuge tubes. All of the samples were decontaminated by 4% NaOH (Petroff's method) and Nekal-BX methods separately. Each decontaminated sample was cultured on three different culture media; viz Modified Lowenstein-Jensen (MLJ), Modified Ogawa (MOG) and newly proposed 2% Buffered Lowenstein-Jensen (BLJ) media.

In all the smears positive samples 94.091% samples was culture positive in one or all culture media used. In all smear negative samples 9.62% samples were culture positive at least in one culture media. Among smear positive samples decontaminated by 4% NaOH, 91.36%, 81.36% and 92.27% samples were culture positive on MOG, MLJ and BLJ medium, respectively. Similarly, in all smear positive samples decontaminated by Nekal, 92.72% were culture positive in MOG, 87.73% in MLJ and 92.72% in BLJ.

In all the smear negative samples decontaminated by 4% NaOH, 3.85%, 2.88% and 3.85% samples were culture positive on MOG, MLJ and BLJ medium. Similarly, among smear negative samples decontaminated by Nekal, 4.81% were culture positive in MOG, 5.77% in MLJ and 3.85% in BLJ.

The MOG yielded more positive result (92.05%) followed by MLJ (84.32%) ($P<0.01$) and BLJ (92.50%). Among the decontamination techniques the Nekal method yielded better result (87.27%) than 4% NaOH (81.3%) for the recovery of bacteria on MLJ ($P<0.05$). No significant difference obtained among samples decontaminated by 4% NaOH (91.36%) and Nekal (92.73%) with respect to recovery of bacteria on MOG ($P>0.05$).

Similarly, no significant difference obtained among samples decontaminated by 4% NaOH (92.27%) and Nekal (92.73%) for the recovery of bacteria on BLJ ($P>0.05$). In all the smear negative samples decontaminated by Nekal, highest culture positive result was observed on MLJ (5.77%) followed by BLJ (5.77%).

While no significant difference was observed in the case of Nekal or 4% NaOH decontamination method with respect to recovery of bacteria on MOG and BLJ.

The result indicated that the BLJ medium is suitable for the recovery of bacteria followed by MOG and MLJ. However, the addition of 2% monopotassium phosphate buffer in MLJ yielded significant increase in culture positive result. Similarly, the Nekal method was suitable decontamination method than 4% NaOH, for the better recovery of bacteria on MLJ. Bacteriological examination of sputum is essential for the diagnosis of pulmonary tuberculosis. The detection of acid-fast bacilli (AFB) in smears of sputum sample by Ziehl-Neelsen staining provides fastest evidence of the presence of *Mycobacteria*. However, the definitive diagnosis of Tuberculosis demands sputum positive culture for the *M. tuberculosis* which depends on the suitable decontamination method of the sputum sample and selection of suitable culture media.

Key words: *Mycobacterium tuberculosis*, Decontamination and Homogenization, Petroff's 4% NaOH method, Nekal-BX, Modified Lowenstein-Jensen media (MLJ), Modified Ogawa media (MOG), 2% Buffered Lowenstein-Jensen media (BLJ).

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ABBREVATIONS

AFB	Acid fast bacilli
AIDS	Acquired Immuno Deficiency Syndrome
ATS-CDC	American Thoracic Society and Center for Disease Control
BCG	Calmette-Guerin Bacilli
BLJ	2% Buffered Lowenstein-Jensen media
CPC	Cetylpyridinium Chloride-Sodium Chloride
DNA	Deoxyriboxy Nucleic Acid
DOTS	Directly Observed Treatment Short-course
ELISA	Enzyme-Linked Immunosorbent Assays
GENETUP-NRL	German Nepal Tuberculosis Project-National Reference Laboratory
HBC	High Burden countries
HIV	Human Immunodeficiency Virus
INH	Isoniazid
IUATLD	International Union against Tuberculosis and Lung Disease
LAMP	Loop Mediated Isothermal Amplification
LJ	Lowenstein-Jensen media
LTBI	Latent tuberculosis infection
kDa	Kilo Dalton
MDG	Millennium Development Goal
MDR	Multidrug resistant
MGIT	Mycobacterial Growth Indicator Tube
MLJ	Modified Lowenstein-Jensen media
MOG	Modified Ogawa media
MOTT	Mycobacterum other than tuberculosis
MTC	Mycobacterium tuberculosis complex
NAA	Nucleic Acid Amplification
NALC-NaOH	N-Aceyl-L-Cysteine-Sodium Hydroxide
NTC	National Tuberculosis Centre
NTP	National Tuberculosis Project

OADC	Oleic acid-albumin-dextrose-catalase
PCHRD	Philipine council for health research and development
PCR	Polymerase chain reaction
PNB	p-Nitrobenzoic
RMP	Rifampicin
PPD	Purified protein derivative
RNA	Riboxy Nucleic Acid
SAARC	South Asian Association of Regional Countries
SDA	Strand displacement amplification
TB	Tuberculosis
TMA	Transcription-mediated amplification
UV	Ultra Violet rays
VF	Visual fields
WHO	World health oragnization
ZN	Ziehl-Neelsen stain
Z-TSP	Zephiran-Trisodium Thiosulphate

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