

Effective Use of Penicillin to Improve Culture Yield for *Mycobacterium tuberculosis*

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ABSTRACT

Objectives: To compare LJ media and LJ media with penicillin for the growth of *Mycobacterium tuberculosis* and contamination, in pulmonary tuberculosis (PTB) suspected patients.

Methods: A total of 300 PTB suspected cases at National Tuberculosis center (NTC) for analyzed for culture and contamination. Early morning sputum samples were collected in sterile leak-proof falcon tube. Digestion, decontamination and homogenization of sputum were done using NALC-NaOH (Modified Petroff method). The sputum sample was processed on LJ media and penicillin added LJ media and incubated at 37°C. Cultures were examined after 8 weeks.

Results: All the PTB suspected cases were compared in LJ media and LJ media with penicillin, 29.7% (89) were positive, 21% (63) were contaminated on LJ media whereas 41% (123) were positive, 3.7% (11) were contaminated on penicillin added LJ media. Also, 25 (8%) were 1⁺ grading, 14 (4.7%) were 2⁺ grading, whereas 81 (27%) and 45 (15%) were 3⁺ grading LJ + Penicillin and LJ media respectively.

Conclusion: Contamination is reduced with the addition of penicillin to LJ media and isolation of total positive cultures of *Mycobacterium tuberculosis* enhanced.

Key words: Culture, Penicillin, Contamination, Pulmonary tuberculosis (PTB), LJ media

INTRODUCTION

Tuberculosis (TB) is a specific chronic infectious disease caused by *Mycobacterium tuberculosis* and occasionally by *M. bovis* and *M. africanum*. It is characterized by formation of granuloma in the infected tissue. This organism usually enters the body by inhalation through lungs. They spread from initial location in the lungs to other body parts via blood stream, the lymphatics system, via the air ways or duct extension to other organs (Park 2005). TB is potentially fatal & contagious disease that can infect any part of the body but most importantly the lungs. TB is caused by any of *Mycobacterium tuberculosis* complex (MTC) organism as well as Non-tuberculous Mycobacteria (NTM) (NTP 2014). TB now ranks alongside HIV as a leading cause of death worldwide. It is estimated by World Health Organization (WHO) that between 2000 and 2020, nearly one billion people will be newly infected, 200 million will get sick and 35 million will die from TB if

global control is not strengthened. Nepal is currently considered an immediate TB burden country with 45% of total TB being infected (NTP 2014).

Culture still relies on relatively cumbersome and lengthy process starting with collection of clinical specimens and their transport to the laboratory, decontamination of the clinical specimen, likely to be contaminated by commensal flora, inoculation and incubation of appropriate media for growth detection and mycobacterium identification (Asmar and Drancourt 2015). Lowenstein Jensen (LJ) Medium is used for the isolation and cultivation of mycobacteria and as bases for selective, differential and enriched media for mycobacteria. However, the effectiveness of culture is greatly undermined by contamination with bacteria and fungi. Contamination reduces the proportion of interpretable results there by limiting the diagnostic value of culture system. This hazard might be partly eliminated by use of penicillin, since

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the principle contaminants are penicillin sensitive whereas tubercle bacilli itself is relatively resistant. Penicillin is effective when incorporated into LJ media at concentration ranging from 10- 125 units per ml of the medium (Abbott 1951). Penicillin inhibits most of the Gram-positive bacteria (Hardy Diagnostic 2014). Low-level concentrations of penicillin (50.0 units/ml) and nalidixic acid (35.0 mg/ml) are included in the LJ Medium to inhibit Gram-positive as well as some Gram-negative bacterial contaminants. (Hi Media 2014).

Contaminated cultures are recognizable from various characteristics. Tubercle bacilli will not grow under these conditions and cultures should be discarded. If the contamination is present only in a part of the slant and the medium maintains its characteristics, the cultures can be retained until 8 weeks (NTP 2014). This study aims to compare the growth and contamination rate on penicillin treated LJ media & LJ media alone and will help to reduce the level of contamination to yield better growth colonies for identification.

MATERIALS AND METHODS

Study design

A comparative cross-sectional study was conducted between February to September 2015 at National Tuberculosis Center, Thimi, Bhaktapur, Nepal. A total of 300 samples were taken, consenting new and previously treated patients suspected of PTB, able to produce sputum, of any age and gender visiting NTC, were included in the study. All TB suspected were inoculated on both LJ with penicillin and LJ media.

Sample collection

The two consecutive early morning sputum samples were collected in sterile leak-proof, wide mouthed, screw-capped, transparent 50 ml single use plastic falcon tube labeled with laboratory serial number. The patients were given clear instruction about the quality and quantity of the samples and method of collection. The patients were suggested to cough deeply from the chest and spit out 3-5ml sputum in the given tube. The saliva, nasal secretions and specimen less than 3ml in volume were avoided. Similarly, sputum containing food particles residues and other extraneous matter were also rejected (STAC 2011).

Macroscopy

The sputum sample was examined macroscopically and characterized as purulent, mucopurulent, mucoid, salivary, mucosalivary or bloody.

Sputum processing

Digestion, decontamination and homogenization of sputum was done using NALC-NaOH (Modified Petroff's method) and concentrated by centrifugation at 3000×g for 15 minutes at 4°C (STAC 2011).

Primary culture of *Mycobacteria*

The sputum sample was further processed for culture on penicillin added LJ media and LJ media alone, in accordance to STAC 2011.

Inoculation and incubation

The centrifuged sediment sputum sample of 0.2-0.4 ml (2-4 drops) was inoculated to each of two slopes of LJ medium and LJ + Penicillin medium, each. The inoculum was spread evenly over the whole surface of each medium and the caps of the inoculated medium tubes were loosened at least for 1 week to ensure even distribution of the inoculums and the tubes were laid on the slanting bed with the slants facing upward. The inoculated slants were incubated at 37°C. After a week, the caps of the tubes were tightened securely and further incubated in upright position at 37°C for 8 weeks. All inoculations were done under BSC level II facility.

Culture examination and reporting

The cultures were examined at 48-72 hours after inoculation to detect gross contaminants. The culture was observed at one week for rapid growers and 3-4 weeks for positive cultures of *M. tuberculosis* as well as other slow growing *Mycobacteria*. If the colonies were not appeared at the 4th week, weekly observation was done till 8 weeks before discarding and reporting as negative. The grading of culture was done. The culture isolates were confirmed as *M. tuberculosis* by biochemical tests and interpreted. A patient was considered as a "TB positive subject" if the sputum specimen had a positive culture and as a "TB negative subject" if the sputum showed no growth (STAC 2011).

RESULTS

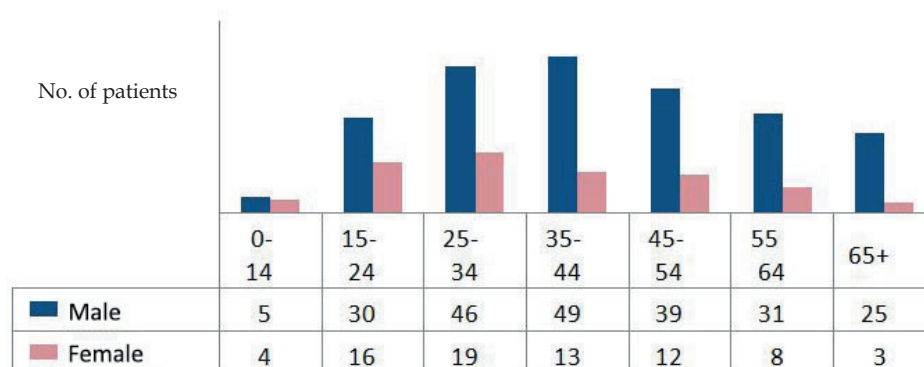


Figure 1: Demographic characteristics of study population

Among 300 PTB patients 225(75%) were male and 75(25%) were female. In this study, the maximum number of TB cases found between the productive age

(15-54) was 224(74.7%), followed by above 55 years was 67(22.3%) and below 15 was 9(3%).

Table 2: Comparative isolation of *M. tuberculosis* on LJ & LJ + penicillin media

Growth/ Media	Positive growth	Negative growth	Contamination
LJ media	29.70%	49.30%	21%
LJ+P media	41%	55.30%	3.70%

* LJ + P media = LJ + Penicillin media Among 300 TB patients, 29.7% (89) were positive, 49.3% (148) were negative, 21% (63) were contaminated on LJ media

whereas 41% (123) were positive, 55.3% (166) were negative and 3.7% (11) were contaminated on penicillin added LJ media

Table 3: Variation of growth on LJ + penicillin & LJ media

Colony count	Growth on LJ + penicillin N (%)	Growth on LJ N (%)
1+	25 (8.3%)	25 (8.3%)
2+	14 (4.7%)	14 (4.7%)
3+	81 (27%)	45 (15%)
Exact No.	3 (1%)	5 (1.7%)
Negative	166 (55.3%)	148 (49.3%)
Contamination	11 (3.7%)	63 (21%)

Out of 300 PTB patients, 25(8%) were 1⁺ grading, 14(4.7%) were 2⁺ grading, whereas 81(27%) and 45(15%) were 3⁺ grading and 3(1%) and 5(1.7%) had exact number on penicillin treated LJ media and LJ media respectively. Similarly, 166(55.3%) and 148(49.3%) were negative and 11(3.7%) and 63(21%) were contaminated LJ + penicillin and LJ media respectively.

DISCUSSION

In this study, comparative study on rate of contamination and growth pattern on LJ media and penicillin added LJ media was done. A contamination rate of 3-5 % is considered a good balance between

need to kill contaminating bacteria and the need to keep the majority of tubercle mycobacteria present in the sample.

A total number of 300 PTB patients from previously treated and new suspects were included. Males, 75% (n=225) were likely to suffer from TB than females 25% (n=75) which is higher with earlier findings by National Tuberculosis Program (64%) during the fiscal year 2012/13 but consistent with the other findings by Khati (2012) 71.65%. This finding is similar to other countries by Kamal et al. (2009) in Bangladesh 79%, Mubarak and Mohammad (2012) in UAE (79%), Feng et al. (2012) in

Taiwan, 77.3% and Range et al. (2012) 69.23%. Evidences show that males are more prone to get severe form of TB like cavity lesion and so forth. Meanwhile, the possible impact of sexual hormones and the differences between men and women in immunological reactions have also been proposed as factors causing men to be more susceptible to *M. tuberculosis* (Neyrolles 2009). Besides that, bias in sample size, behavioral and socio-economic factors may play important role (Sangare et al. 2010).

Out of 300 PTB cases, the isolation rate of *M. tuberculosis* was 41% (n=123) on LJ media with Penicillin and 29.7% (N=89) on LJ media alone. This finding is consistent to other findings by Lamsal (2012) 31% culture positive in Kathmandu, but not consistent with Affolabi et al. 2011(10.9%) in France, Kamal et al. 2009 (44%) in Bangladesh, Abd-El Aal et al. 2014 (54.5%) in Egypt, Kelfie (2014) 51% in Ethiopia, 33.7% in Zambia. According to Kassaza et al. 2014, TB positive culture rate was 12.4% and 9.8% in penicillin treated LJ media and LJ media, respectively. Though the present result was much higher than of Kassaza et al. (2014), but on internal examination positivity rate on this study was 11.3% (N=34) higher in penicillin treated LJ media than LJ media alone. Culture identification is still the gold standard for diagnosis of pulmonary tuberculosis despite the fact *M. tuberculosis* is a slow growing organism and culture may take up to 4-8 weeks to provide a positive result (Castro et al. 2015). Penicillin containing media also demonstrated higher rates of *M. tuberculosis* isolation.

The culture contamination rate was 3.7% (N=11) on penicillin added LJ media and 21% (N=63) on LJ media. Contamination is greater than the recommended threshold of 5% on LJ media alone, while contamination rate on LJ + Penicillin was within threshold for the laboratories that receive freshly collected samples and 5-10% in cases on transportation of the samples. The contamination rate for LJ alone was approximately 31% and 9% for penicillin containing LJ media (Kassaza et al. 2014). In this study, contamination rate is higher than reports by Thakuri 2013 (12%) in Kathmandu for LJ media. Contamination rate was reported 14.9% by Zambian National Laboratory (Muyuyeta et al. 2009), 9% by Nagarajan et al. (2012) in India, 14.2% by 9.3% by Chihota et al. (2010) in South Africa, on LJ media alone. The contamination in this study might be due to delay in transportation of the sputum sample. A

contamination rate of 0-1% may indicate too strong decontamination process. However, according to WHO guidelines the contamination rate 5-10% is acceptable in case of delay in transportation. As there was no provision on use of oral rinse solutions such as chlorohexidine and nystatin, penicillin is effective when incorporated into LJ medium, concentration ranging from 10125 units/ml (prior to inspissation) definitely reduce contamination, but the limitations of this method of using penicillin have yet been adequately tested. The high rate of contamination on LJ media could partly be due to the fact that this method used highly nutritious medium that can easily supports growth of other bacteria or may be due to enrollment of patients with cough for more than 2 weeks and other TB symptoms. Although, LJ contains malachite green, which has antibiotic properties, several other groups have reported similarly high contamination rates (Abott 1951; Kassaza et al. 2014).

CONCLUSION

Contamination was reduced by 17.3% with the addition of penicillin to LJ media. The isolation of total positive cultures was also enhanced by 11.3%. Thus, addition of penicillin on LJ medium ought to be better media for isolation of *M. tuberculosis*, as the cost of adding penicillin is low and effective in suppressing the contaminating bacteria and improving culture yield. This suggest that, LJ + penicillin is efficient than LJ media alone, as it saves time, cost and effort.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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