

## RETROSPECTIVE STUDY EVALUATING THE EFFICACY OF LJ MEDIUM ALONG WITH PENICILLIN TO IMPROVE CULTURE YIELD OF MYCOBACTERIUM TUBERCULOSIS IN PATIENTS

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**Abstract:** *This study evaluated the efficacy of penicillin in improving the culture yield of M. Tuberculosis in cases of pulmonary tuberculosis. A retrospective study was conducted in the Department of Pathology, MMDC, Multan. A total of 350 suspected pulmonary tuberculosis patients were selected for the study. An early morning sputum specimen was collected. NaOH citrate and N-acetyl-L-cysteine were used for digesting, decontaminating, and homogenizing the sputum. The samples were processed on Lowenstein Jensen media and then Lowenstein Jensen media with penicillin. The cultures were then examined after 2 months. On LJ media, 100 (28.5%) suspected cases were positive, and 80 (22.8%) cases were contaminated. On LJ media with penicillin, 145 (41.4%) cases were positive, and 15 (4.3%) were contaminated. In addition, 30 (8.5%) were 1+ grading, 15 (4.3%) were 2+ grading on both media, 95 (27.1%) were 3+ grading on LJ media, and 50 (14.2%) were 3+ grading on LJ media with penicillin. Contamination in Mycobacterium tuberculosis is reduced by adding penicillin to the LJ media, and isolation-positive cultures are enhanced.*

**Keywords:** Mycobacterium Tuberculosis, Pulmonary Tuberculosis, Penicillin, Lowenstein Jensen Media

### Introduction

Tuberculosis is a bacterial infection mostly affecting the lung caused by *M. tuberculosis*, *M. bovis*, and *M. africanum* (Suárez et al., 2019). It can also affect other parts of the body other than the lungs. The bacteria can usually enter through the respiratory system. It travels through the bloodstream and airways to other organs and the lymphatic system. Tuberculosis is a fatal disease and one of the major causes of death globally. According to WHO, one billion people have been affected by TB in the last twenty years, and 35 million deaths have been caused by it (Glaziou et al., 2018). In Pakistan, an average of 500,000 tuberculosis cases were reported in the last year (Tahseen et al., 2020).

Culture is a lengthy and complicated process involving specimen collection, transport, decontamination, the likelihood of contamination, inoculation and incubation of specific growth detection media, and identification of mycobacterium. Lowenstein Jensen media is a growth medium for isolation and cultivation and as a base for media for

mycobacterium (THANGAVELU et al., 2021). However, contamination by commensal flora is a likely disadvantage of the culture, limiting the results' authenticity. The addition of penicillin solves this problem as the main components of culture are penicillin sensitive.

This study evaluated the efficacy of penicillin in improving the culture yield of Mycobacterium Tuberculosis in cases of pulmonary tuberculosis.

### Methodology

A retrospective study was conducted in the Department of Pathology, MMDC, Multan. A total of 350 suspected pulmonary tuberculosis patients were selected for the study. All patients provided their consent to become a part of the study. The ethical board of the hospital approved the study design. All the patients were inoculated on both Lowenstein Jensen media alone and Lowenstein Jensen media with penicillin. An early morning 3-5ml sputum samples were collected consecutively for both

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media after instructing the patients to cough deeply and spit. NAOH citrate and N-acetyl- L-cysteine were used for digesting, decontaminating, and homogenizing the sputum. The samples were processed on Lowenstein Jensen media and then Lowenstein Jensen media with penicillin. Centrifuged 2-4 drops of sputum samples were inoculated and incubated at 37°C for 8 weeks in the upright position. Cultures were examined 2-3 days post-inoculation to check gross contamination. At 1 week, it was examined for rapid growers, and at 3-4 weeks, it was observed for a positive culture of Mycobacterium tuberculosis and mycobacteria. If no significant results were obtained in 4<sup>th</sup> week, culture was observed every week till 8 weeks and then reported as negative. Biochemical tests confirmed the positive cultures.

## Results

A total of 350 suspected TB patients were included. Among these, 265 (75.7%) were male, and 85 (24.3%) were female.

Adding penicillin to the LJ media showed significantly better results than LJ media alone. Among 350 patients, 100 (28.5%) were positive on LJ media, and 170 (48.6%) were positive on LJ media with penicillin. In addition, 145 (41.4%) patients were negative on LJ media, and 190 (54.3%) were negative on LJ media with penicillin (Table I).

In addition, 30 (8.5%) patients were 1<sup>+</sup> grading, 15 (4.3%) were 2<sup>+</sup> grading on both media, 95 (27.1%) were 3<sup>+</sup> grading on LJ media, and 50 (14.2%) were 3<sup>+</sup> grading on LJ media with penicillin (Table II).

**Table I: Comparative isolation findings of LJ media alone and LJ media with penicillin**

	Positive growth	Negative growth	Contamination
LJ media	100 (28.5%)	170 (48.6%)	80 (22.8%)
LJ media with penicillin	145 (41.4%)	190 (54.3%)	15 (4.3%)

**Table II: Comparative growth in both medium**

	LJ media with penicillin	LJ media
1 <sup>+</sup>	30 (8.5%)	30 (8.5%)
2 <sup>+</sup>	15 (4.3%)	15 (4.3%)
3 <sup>+</sup>	50 (14.2%)	95 (27.1%)
Exact number	4 (1.14%)	5 (1.43%)
Negative	190 (54.3%)	170 (48.6%)
Contamination	15 (4.3%)	80 (22.8%)

## Discussion

This study compared the growth and inoculation of Mycobacterium tuberculosis in Lowenstein Jensen media alone and Lowenstein Jensen media with the addition of penicillin. The media with penicillin showed a low contamination rate of 4.3% which qualifies as a balanced sample.

Among 350 subjects, 265 (75.7%) were male, and 85 (24.3%) were female, showing that the prevalence of tuberculosis was higher in males than in females. These findings are consistent with other studies (Hertz and Schneider, 2019; Selmane and L'Hadj, 2020; Yen et al., 2018). Among 350 patients, 100 (28.5%) were positive on LJ media, and 170 (48.6%) were positive on LJ media with penicillin. Other studies reported similar results (Kassaza et al., 2014; Ma et al., 2020). There was a significant difference between contamination in both mediums. 22.8% of patients showed contamination in their samples when inoculated under LJ media alone, while only 4.3% of contamination was observed with the addition of penicillin. Contamination in LJ media was higher than the normal 5% threshold in laboratories. However,

these results were consistent with other studies (Rasool et al., 2019; Tharmalingam et al., 2019; Umar et al., 2019). To Kassaza et al., 31% of contamination was reported in LJ media, and 9% was observed in penicillin media (Kassaza et al., 2014). A 9.3% contamination was also observed in Chihota et al. when adding penicillin (Chihota et al., 2010).

## Conclusion

Contamination in Mycobacterium tuberculosis is reduced by adding penicillin to the LJ media and isolation-positive cultures are enhanced.

## Conflict of interest

The authors declared an absence of conflict of interest.

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