

Effective Use of Penicillin to Improve Culture Yield for Mycobacterium Tuberculosis

REHAN ANWAR¹, ASIM FIDA², MADIHA MUMTAZ³, LUQMAN ANWAR⁴, AYESHA JAMIL⁵

¹Associate Professor Medicine, Sialkot Medical College, Sialkot

²MBBS, MRCCGP (INT) General Medicine, NMC Specialty Hospital Al Ain UAE

³Assistant Professor Microbiology, Khawaja Safdar Medical College, Sialkot

⁴FCPS Neurology, Senior Registrar, Mayo Hospital Lahore

⁵Associate Professor of Dermatology, Azra Naheed Medical College, Lahore/ Superior University

Corresponding author: Rehan Anwar, Email: Drrehan85_hfh@hotmail.com, Cell: +92 336 446 2026

ABSTRACT

Background: The aim of this study to evaluate the Mycobacterium tuberculosis (MTC) sputum culture contamination and recovery between Lowenstein-Jensen medium (LJ) contains Penicillin+LJ and free Penicillin LJ.

Study design: This was a cross sectional study conducted at Khawaja Safdar Medical College, Sialkot /Allama Iqbal Memorial Hospital Sialkot for the duration of six months from September 2022 to February 2023.

Methods: A total number of the participants was 140. The male and female gender were both included who suffered pulmonary tuberculosis (PTB). The sample n=140 were analyzed for culture and contamination. The sputum samples were taken in the early morning in the falcon tube and digested, decontaminated, and homogenized by Modified Petroff method. The specimen of sputum was processed on addition of Penicillin+ LJ media, and incubation was carried out at 37°C. After eight weeks, cultures were examined. The data were analyzed by SPSS 21 software.

Results: The male 64.2% show greater number of cases as compared to female 36%. The suspected cases of PTB was compared with Penicillin+ LJ media and LJ media alone. 21.4% of the samples were positive mycobacterium (MTB), 79% were contaminated on LJ media while 93% were positive (MTB), and 7.1% of the samples were contaminated on LJ media without penicillin. This difference was statistically significant ($p < 0.005^{**}$). The grade 2+/3+ show 50% recovery by the treatment of penicillin+LJ on positive sample and 47% contaminated sample $P < 0.001^{**}$ while graded scanty/+1 show recovery was 38%; $p = 0.001^{**}$ with penicillin+LJ then 37% contaminated sample; $p = 0.002^{**}$. The difference in recovery between treatment with penicillin and without penicillin LJ was statistically significant; $p < 0.005$.

Conclusion: The contamination rate was reduced by 47% by penicillin+LJ media. Penicillin offered great alternatives for decreasing contamination in LJ alone. Penicillin should be further used as a medium supplement to enhance recovery for LJ media.

Keywords: Culture media, Mycobacterium, Penicillin

INTRODUCTION

Mycobacterium tuberculosis, as well as *M. bovis* and *M. africanum*, occasionally produce the specific chronic infectious disease known as tuberculosis (TB). Granuloma development in the diseased tissue is one of its distinguishing features. Usually, inhaling this organism causes it to enter the body via the lymphatic system, airways, the bloodstream, or duct extension to other organs.^{1,2} Any part of the body can contract TB, a potentially fatal and contagious disease, but the lungs are most frequently affected. Both non-tuberculous mycobacteria and the Mycobacterium tuberculosis organism can cause tuberculosis (TB). TB has overtaken HIV as the top cause of death worldwide.³ World Health Organization (WHO) estimates that a global socioeconomic upheaval caused by the epidemic is unprecedented. It is likely to have a global impact on TB control, especially in terms of case detection and short-term TB mortality. The increase of 6.3 million new TB cases and 20% increase in TB-related mortality during that time. The number of new human TB diagnoses and reports had significantly decreased prior to COVID-19, falling from 7.1 million in 2019 to 5.8 million in 2020. After an 18% drop, the numbers increased to 2012 levels, which is a far cry from the estimated 10 million TB cases in 2020. In China, for example, a significant decline in case notifications was connected to COVID-19 interventions: the case reporting rate was 20% lower during the 11 weeks of the COVID-19 lockdown and shortly afterwards than it was during the same period in 2019.^{4,5} With an estimated 1.8 million fatalities each year, mostly in the underdeveloped world, TB is still one of the deadliest infectious diseases. At every step of the collecting and processing of the specimens, decontamination measures have been used.⁶ The liquid-culture mycobacterium growth indicator tube (MGIT) contains a fast-acting, sensitive culture media with low contamination potential.⁷ But in resource-constrained settings (RLS), its high cost is prohibitive. PANTA is used in the liquid culture method to decreased contamination.⁸ Mycobacteria are isolated, grown, and cultivated using Lowenstein Jensen (LJ) Medium, which also serves as the foundation for mycobacteria-specific selective, differential,

and enriched media. However, bacterial and fungal contamination seriously reduces the efficacy of culture. By lowering the fraction of interpretable results, contamination limits the diagnostic use of the culture system. The use of penicillin may help to partially eliminate this risk. When added to LJ media, penicillin is effective at concentrations between 10 and 125 units per ml of the medium.⁹ Different characteristics make contaminated cultures identifiable. Because of this, cultures of tubercle bacilli should be thrown away.¹⁰

METHODOLOGY

This was a cross sectional study conducted at Khawaja Safdar Medical College, Sialkot Allama Iqbal Memorial Hospital Sialkot for the duration of six months from September 2022 to February 2023. A total number of the participants was 140. The male and female were both included who suffered TB caused by Mycobacterium tuberculosis. According to inclusion criteria: all consenting PTB suspects, including new and previously treated, sputum producers, and participants of any age. Both LJ with penicillin and LJ media alone were used for all suspected cases of tuberculosis. The early morning sputum samples were taken and placed in a falcon tube that was labeled with the lab serial number. The McNemar's test was used to digest, decontaminate, and homogenise the sputum. The tubes were incubated for an additional 8 weeks after the tube tops were snugly put on after a week at 37°C and cultures were checked 48–72 hours later for obvious contamination. When *M. tuberculosis* and other slow-growing Mycobacteria were detected in the culture, it took 3–4 weeks before the culture showed signs of growth. Culture was graded. TB positive participants had sputum specimen positive and as a TB negative if there was no growth in the sputum. SPSS 21 was used to analyze the data.

RESULTS

The demographic data show total number of the participants N=140.

A total number of the participants was 140 which include both male 90 (64.2%) and female 50 (36%) to show male to female ratio

of 2:1. The maximum age of TB participants was the age (20-30) years was 61%, (31-40) years was 18%, and >50 years was 7.1%. The mean age of study participants was 31 years, ranging from 15 to above 50 years. The married participants of maximum cases of TB was 57.1% then single participants 29%. The minimum cases was diagnosed TB 18% in higher education level as compared to primary 50% and secondary level 39.2%. The maximum TB was diagnosed in labour worker 64.2% then merchant 11% and students 25% were seen in Table 1.

Table 1: Demographic variables

Variables	Total no of participants N=140(%)
Gender	
Male	90 (64.2%)
Female	50 (36%)
Age	
20-30	85 (61%)
31-40	25 (18%)
41-50	20 (14%)
>50	10 (7.1%)
Marital status	
Single	40 (29%)
Double	80 (57.1%)
Others	20 (14%)
Educational status	
Primary school	70 (50%)
Secondary school	55 (39.2%)
Higher education	25 (18%)
Occupation	
Labour	90 (64.2%)
student	35 (25%)
Merchant	15 (11%)

Mean ± SEM: ANOVA SPSS 21 Test* p<0.0; **p<0.0; ***p<0.00.

Table 2: Clinical profile study of participants among selected of health facilities

Variables	Total no of participants N=140(%)	P=value
Previous history of TB		
YES	30 (21.4%)	0.234
NO	110 (79%)	0.008
Daily activity before first vist		
Full day work	100 (71.4%)	0.006
house/bed bound	40 (29%)	0.223
Smoking status		
YES	20 (14%)	0.111
NO	120 (86%)	0.0006
Alcoholic status		
YES	15 (11%)	0.055
NO	125 (89.2%)	0.007

Mean ± SEM: ANOVA SPSS 21 Test* p<0.0; **p<0.0; ***p<0.00.

In this study, 79% participants of TB was no previous history; p=0.008 and 21.4% of previous history of TB ;p=0.234. The participants of house bound was 29% cases of TB due to current illness; p=0.223 then full day worker 71.4%; p=0.006. TB cases in smoker participants was 14%; p=0.111 then nonsmoker 86%; p=0.0006. TB cases in alcoholic user participants was 11%; p=0.055 then non-alcoholic user was 89.2%; p=0.007 were seen in Table 2.

Table 3: To evaluate in penicillin treated LJ as compared to alone LJ

	M.tuberculosis (+ve samples)		Contaminated samples	
	No= (%)	p=value	N0= (%)	p=value
penicillin+LJ	130 (93%)	0.0001***	10 (7.1%)	0.001**
Alone LJ	30 (21.4%)	0.001**	110 (79%)	0.0001***

Mean ± SEM: ANOVA SPSS 21 Test* p<0.0; **p<0.0; ***p<0.00.

Table 4: M.tuberculosis and contaminated and recovery regarding microscopy grading

Microscope grade	Antibiotics	M.tuberculosis +ve tubes N= (%)		Conatminated cultures N=(%)	
		N= 80 (%)	P=value	N= 60 (%)	P=valu e
No AFB	Penicillin	10 (12.5%)	0.001**	10(13.3%)	0.003**
Scanty/+1	Penicillin	30(38%)	0.001**	22(37%)	0.002**
2+/3+	Penicillin	40(50%)	0.001**	28 (47%)	0.001**

Mean ± SEM: ANOVA SPSS 21 Test* p<0.0; **p<0.0; ***p<0.00.

We compared yield, contamination rate and recovery by using McNemar's test for samples. The contaminated samples rates were 7.1% in penicillin + LJ; show significantly reduced p=0.001**, and M.tuberculosis positive smples rates were 93% in penicillin+LJ media tube; show significantly higher p=0.0001*** as compared to

LJ group free penicillin (anibiotics) in M.tuberculosis 21.4%; p=0.001** and contaminated sample was 79% ;p=0.0001*** were seen in Table 3.

Among specimens graded 2+/3+ by microscopy, recovery was 50% in penicillin+ LJ; p=0.001** while 47% contaminated sample treated with penicillin+LJ; p=0.001**. The specimens graded scanty/+1 show recovery was 38%; p=0.001** with penicillin+LJ then 37% contaminated sample; p=0.002**. The difference in recovery between treatment with penicillin and without penicillin LJ was statistically significant; p<0.005 were seen Table 4.

Table 5: Contaminants isolate from alone LJ tubes

Gram reaction	organisms	Detected
Positive	Staphylococcus	6 (4.2%)
	Streptococcus spp.	5 (4%)
	Fungi	4 (3%)
	Cellulomonas spp.	5 (4%)
	Total	20 (14%)
Negative	Acromonas spp	3 (2.1%)
	Seratia spp	4 (3%)
	Entrobacter spp	3 (2.1%)
	Total	10 (7.1%)

Mean ± SEM: ANOVA SPSS 21 Test* p<0.0; **p<0.0; ***p<0.00.

DISCUSSION

A primary cause of death globally, tuberculosis (TB), one of the earliest known human diseases, is brought on by microorganisms from the Mycobacterium tuberculosis complex.^{11, 12} The growth and contamination rate were compared with penicillin of LJ and aine LJ media. The necessity to preserve the bulk of tubercle mycobacteria present in the sample while also killing contaminating bacteria was considered to be balanced at a contamination rate of 6-9%.¹³ According to our interpretation to found that for the decrease of TB morbidity and mortality to be achieved, it is essential to comprehend the causes linked to patient delays. Numerous factors contribute to the delayed treatment of tuberculosis patients, studies from various nations have shown. According to the study, having a feminine gender was a distinct risk factor for developing an intolerable complete delay in TB therapy compared to their male counterparts. This may be because women's financial and cultural status may limit their prospects and impose further restrictions on their ability to seek healthcare and meet their needs. Limited decision-making capacity, involvement in domestic work, unemployment, and facility located too far from the study region may all be contributing factors.¹⁴ In our study to found that, There were a total of 140 individuals with pulmonary tuberculosis, both new suspicions and previously treated patients. The likelihood of developing TB was higher among the males (n=90, 64.2%) than the females (n=36, 50%). According to the evidence, men are more likely to have serious TB conditions such cavity lesions and other TB-related conditions. In the meantime, it has also been suggested that men are more susceptible to M. tuberculosis due to probable effects of sexual hormones and variations in immune responses between men and women. In addition, sample size bias, behavioural, and socioeconomic factors may be significant. In terms of educational standing, not attending formal schooling was listed as a contributing factor to the development of an unsatisfactory total delay in TB treatment. This might be as a result of the 18% of students who attend higher education institutions having more knowledge about tuberculosis, being more conscious of the disease, and having positive treatment seeking behaviors reinforced, all of which improve the likelihood that they will seek care early. We were agreed from the previous study.^{15, 16, 17} We assessed which penicillin antibiotics were most successful at lowering contamination in LJ. Our analyses of the results show that adding penicillin+LJ to specimens that were sputum microscopically categorised as 2+/3+ boosted yield for those samples by 50% and decreased contamination by 47%. These improvements were seen in the decontamination rates, positive culture rates, and yield for the decontaminated medium. Although media containing penicillin also showed lower contamination rates, yield and recovery improvements were not as noticeable. Selectatab medications

contain penicillin, which has a specific effect against bacterial spp. Additionally, it is one of the few antibiotics with the ability to treat an isolated major contaminant. We were agreed with the previous study.^{18, 19,20} According to our findings, the contamination rates without penicillin LJ were around 79%, $P < 0.001^{**}$ higher due to samples took several days to arrive at the lab. The patients who had been coughing for more than two weeks without experiencing any other TB symptoms were enrolled.²¹ These findings highlight the need for better decontamination techniques in labs that use LJ culture for TB diagnosis and susceptibility testing. Other methods of lowering contamination rates have been reported, with varying degrees of success. A recent investigation on the use of oral rinse solutions (chlorhexidine and nystatin) demonstrated a lower contamination rate in samples taken from individuals with suspected TB, but this was also shown to influence *M. tuberculosis*'s ability to recover. It is thought that by doing this, it will eventually help to eradicate tuberculosis from the earth.^{22, 23}

CONCLUSION

The contamination rate was reduced by 47% by penicillin+LJ media. Penicillin offered great alternatives for decreasing contamination in LJ alone. Penicillin should be further used as a medium supplement to enhance recovery for LJ media.

REFERENCES

- Sia, J. K., & Rengarajan, J. (2019). Immunology of Mycobacterium tuberculosis infections. *Microbiology spectrum*, 7(4), 7-4.
- Chandra, P., Grigsby, S. J., & Philips, J. A. (2022). Immune evasion and provocation by Mycobacterium tuberculosis. *Nature Reviews Microbiology*, 20(12), 750-766.
- Bateson, A., Ortiz Canseco, J., McHugh, T. D., Witney, A. A., Feuerriegel, S., Merker, M., ... & Timm, J. (2022). Ancient and recent differences in the intrinsic susceptibility of Mycobacterium tuberculosis complex to pretomanid. *Journal of Antimicrobial Chemotherapy*, 77(6), 1685-1693.
- Huang, F., & Zhao, Y. (2022). Global control of tuberculosis: current status and future prospects. *Zoonoses*.
- Chaisson, R. E., Frick, M., & Nahid, P. (2022). The scientific response to TB—the other deadly global health emergency. *The International Journal of Tuberculosis and Lung Disease*, 26(3), 186.
- Du, F., Xing, A., Li, Z., Pan, L., Jia, H., Du, B., ... & Zhang, Z. (2022). Rapid Detection of Mycobacterium tuberculosis in Pleural Fluid Using Resuscitation-Promoting Factor-Based Thin Layer Agar Culture Method. *Frontiers in Microbiology*, 13, 91.
- Nguyen, H. V., de Haas, P., Nguyen, H. B., Nguyen, N. V., Cobelens, F. G., Mirskhulava, V., ... & Tiemersma, E. W. (2022). Discordant results of Xpert MTB/Rif assay and BACTEC MGIT 960 liquid culture to detect Mycobacterium tuberculosis in community screening in Vietnam. *BMC Infectious Diseases*, 22(1), 1-9.
- Kumar, G., Bhalla, M., Singh, N., Verma, A. K., & Dewan, R. K. (2022). Recovery rates of mycobacterium from suspected extra-pulmonary tuberculosis patients using liquid culture at a tertiary referral centre of India. *Indian Journal of Tuberculosis*.
- Jamali, S., & Richa, S. V. Evaluation of modified petroff's method and manual MGIT culture system to facilitate detection of acid-fast bacilli in smear-negative presumptive cases of pulmonary tuberculosis *Indian J Comm Health*. 2019; 31 (2): 231-235. Source of Funding: Nil Conflict of Interest: None declared.
- Tchatchouang, S., Nzouankeu, A., Donkeng, V., Eyangoh, S., Ngando, L., Penlap, V., ... & Njouom, R. (2019). Prevalence of opportunistic pathogens *Pneumocystis jirovecii* and *tubercle bacilli* in HIV-infected patients with respiratory infections in Yaounde, Cameroon. *AIDS Research and Human Retroviruses*, 35(5), 428-429.
- Ranjitha, J., Rajan, A., & Shankar, V. (2020). Features of the biochemistry of *Mycobacterium smegmatis*, as a possible model for *Mycobacterium tuberculosis*. *Journal of Infection and Public Health*, 13(9), 1255-1264.
- Miggiano, R., Rizzi, M., & Ferraris, D. M. (2020). Mycobacterium tuberculosis pathogenesis, infection prevention and treatment. *Pathogens*, 9(5), 385.
- Saber, S., & Hossain, M. M. (2022). Socio-Demographic Determinants of Adult Pulmonary Tuberculosis Patients: A Hospital Based Study at Dhanmondi, Dhaka, Bangladesh. *European Journal of Medical and Health Sciences*, 4(1), 103-107.
- Sentis, A., Vasconcelos, P., Machado, R. S., Caylà, J. A., Guxens, M., Peixoto, V., ... & Carvalho, C. (2020). Failure to complete treatment for latent tuberculosis infection in Portugal, 2013–2017: geographic-, sociodemographic-, and medical-associated factors. *European Journal of Clinical Microbiology & Infectious Diseases*, 39(4), 647-656.
- Aggarwal, A. N. (2019). Quality of life with tuberculosis. *Journal of clinical tuberculosis and other mycobacterial diseases*, 17, 100121.
- Awoke, N., Dulo, B., & Wudneh, F. (2019). Total delay in treatment of tuberculosis and associated factors among new pulmonary TB patients in selected health facilities of Gedeo zone, southern Ethiopia, 2017/18. *Interdisciplinary perspectives on infectious diseases*, 2019.
- Wobudeya, E., Bonnet, M., Walters, E. G., Nabeta, P., Song, R., Murithi, W., ... & Marcy, O. (2022). Diagnostic advances in childhood tuberculosis—Improving specimen collection and yield of microbiological diagnosis for intrathoracic tuberculosis. *Pathogens*, 11(4), 389.
- Husain, A. A., Nayak, A. R., Jain, R. K., Daginawala, H. F., Tumane, R., Jawade, A., ... & Kashyap, R. S. (2022). Tuberculosis in Mine Workers: Advances in Current Diagnostic Landscape. In *Medical Geology in Mining: Health Hazards Due to Metal Toxicity* (pp. 49-81). Cham: Springer International Publishing.
- Khan, M. A., Rajendram, R., Al-Harbi, A., Al-Ghamdi, M., Masuadi, E., Obaidi, M., & Al-Jahdali, H. (2021). The diagnostic yield and safety of sputum induction in suspected pulmonary tuberculosis: The experience of a single tertiary care center in Saudi Arabia. *The International Journal of Mycobacteriology*, 10(4), 388-392.
- Ali, G. A., Goravey, W., Howady, F. S., Ali, M., Alshurafa, A., Abdalhad, A. M., ... & Omrani, A. S. (2022). The Role of Post-Bronchoscopy Sputum Examination in Screening for Active Tuberculosis. *Tropical Medicine and Infectious Disease*, 8(1), 13.
- Asandem, D. A., Asante-Poku, A., Asare, P., Aboagye, S. Y., Stephen, O. W., Danso, E., ... & Yeboah-Manu, D. (2018). OMNIgene SPUTUM: a good transport and decontaminating reagent for tuberculosis testing. *Int J Mycobacteriol*, 7(3), 222-227.
- Muzanyi, G., Peace, A., Wamuntu, B., Joseph, A., & Nassali, J. (2021). The threat of persistent bacteria and fungi contamination in tuberculosis sputum cultures. *African Health Sciences*, 21(2), 628-632.
- Kabore, A., Tranchot-Diallo, J., Sanou, A., Hien, H., Daneau, G., Gomgnimbou, M. K., ... & Sangaré, L. (2019). Why oral antiseptic mouth rinsing before sputum collection cannot reduce contamination rate of mycobacterial culture in Burkina-Faso. *African Health Sciences*, 19(1), 1321-1328.