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A comparision of blood and egg based media for the rapid isolation and drug susceptibility testing of Mycobacterium Tuberculosis

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ABSTRACT

This study determine the comparison of growth pattern and time required for isolation and drug susceptibility testing of Mycobacterium tuberculosis on blood agar (BA) and Lowenstein-Jensen (LJ) media.Mycobacterium tuberculosis was isolated from 250 sputum. The sputum samples were proceed for Mycobacterium tuberculosis by Ziehl-Neelsen method, culture on LJ and BA media followed by biochemical tests. Drug susceptibility test was performed on LJ and BA against 10 Mycobacterium tuberculosis isolates.Among 55·2% smear positive, growth of Mycobacterium tuberculosis was 71·7% on LJ and 82·6% on 10% blood agar media.Similarly, smear negative specimens, growth of Mycobacterium tuberculosis was 12·5% on LJ and 10·7% on blood agar. Statistically, there was no significant difference for isolation of Mycobacterium tuberculosis on both LJ and BA media (P=1·39). Growth of Mycobacterium tuberculosis (3+) was 34% on BA than 30·8% on LJ media. Time taken for Mycobacterium tuberculosis on blood agar (2-3 weeks) than for LJ (4-5 weeks).Drug susceptible test, 50% (5/10) were resistance to one or more drugs containing LJ and BA media. Blood agar slants may be a good substitute of LJ medium for rapid detection and drug susceptible test of Mycobacterium tuberculosis

Keywords: Blood agar culture system, Mycobacterium tuberculosis, Drug susceptible test, Lowenstein-Jensen media

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INRODUCTION

Tuberculosis is still one of the main public health problems in most developing countries. It remains the most relevant infectious disease worldwide, and its etiological agent, Mycobacterium tuberculosis (Mtb), infects one-third of the world population.[1] Global estimation indicates that approximately 8 million new cases of active tuberculosis are notified and 2 million people die of tuberculosis every year [2]. Incidences are arising in most parts of the world, especially in developing countries where the epidemic of human immunodeficiency virus (HIV) has had the effect of increasing the number of tuberculosis cases. The prevalence and continual rise of drug-resistant TB (MDR-TB & XDR-TB) stands to derail the progress made in TB control over the last decade. Further, a National TB prevalence survey revealed that 1 in 10 TB patients harbored MDR-TB [3].

Specific media, such as egg-based media (e.g. Lowenstein -Jensen medium), agar-based media (e.g. Middle brook media) and liquid media (e.g. Middle brook and BACTEC broths) are recommended for culturing Mycobacterium. Such requirements pose logistic, economic and time taken problems, especially in resource-limited areas where bacteriological culture facilities are few and the prevalence of mycobacterial infections, notably tuberculosis, is high. Among these highly expensive, more time taken and automated system, a very simple, less time taken and easily available standard blood agar (BA) method is also used for the isolation of Mycobacterium tuberculosis incubated only for 3weeks or more [4] and 1 to 2 weeks instead of egg-based medium [5]. Similarly, drug susceptibility of Mycobacterium tuberculosis obtained on blood agar in two weeks as compared to three weeks on 7H10 Middlebrook agar [6].

Present study compare blood agar and LJ media for rapid isolation and drug susceptibility of Mycobacterium tuberculosis Blood agar method requires only an incubator, basic equipment in clinical laboratory and aseptically collected mammalian blood and NaOH. The time to detect and susceptibility testing of M. tuberculosis to isoniazid (INH), rifampin (RIF), Ethambutol (EMB) and streptomycin (SM) from smear sputum specimens is shorter with blood agar slants as compared to LJ medium.

METHODS

Study population and sample collection: The study was conducted at National Tuberculosis Centre (NTC), Thimi, Bhaktapur, from July 2010 to March 2011. A total of 250 clinically suspected patients were enrolled in the study. Sputum samples were collected following standard protocol (WHO, 1998). Briefly as:

- Day 1: Sample 1- suspect provided an “on the spot” sample.
- Day 2: Sample 2- suspect brings an early morning sputum sample.
- Day 3: Sample 3- suspect provided another “on the spot” sample.

The sputum was collected in a wide mouthed, transparent, plastic, sterile, leak-proof, screw capped container. About 4 ml mucopurulent sputum was collected.

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Processing of the samples: The sputum samples were proceeds for AFB smear then cultured on 10% blood agar and LJ media. The isolated organism was then confirmed by different biochemical tests. Finally, the culture positive isolates were tested for anti-TB drug susceptibility testing by Proportional Method as standard protocol

Statistical analysis: Statistical analysis was done using SPSS 16.0 (SPSS, Inc., Chicago, IL. USA). The Pearson's chi-square test were used to analyze the differences in the given data. A p-value <0.05 was considered significant.

RESULTS

Sex-wise distribution of total Suspected PTB Patients (N=250)

Among 250 suspected pulmonary tuberculosis patients, 64.4% (n=161) were male and 35.6% (n=89) were female. (Fig. 1)

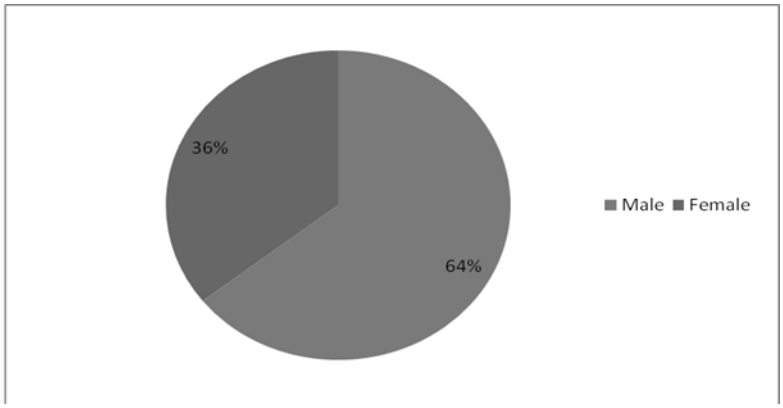

Fig 1 Sex-wise Distribution of Total Suspected Patients

Age-wise distribution of total Suspected PTB Patients

Within the age group range 8 to 76 years, the highest number of patients (28.4%) was in the age group 21-30 followed by age group 31-40 (20.8%) (Fig. 2)

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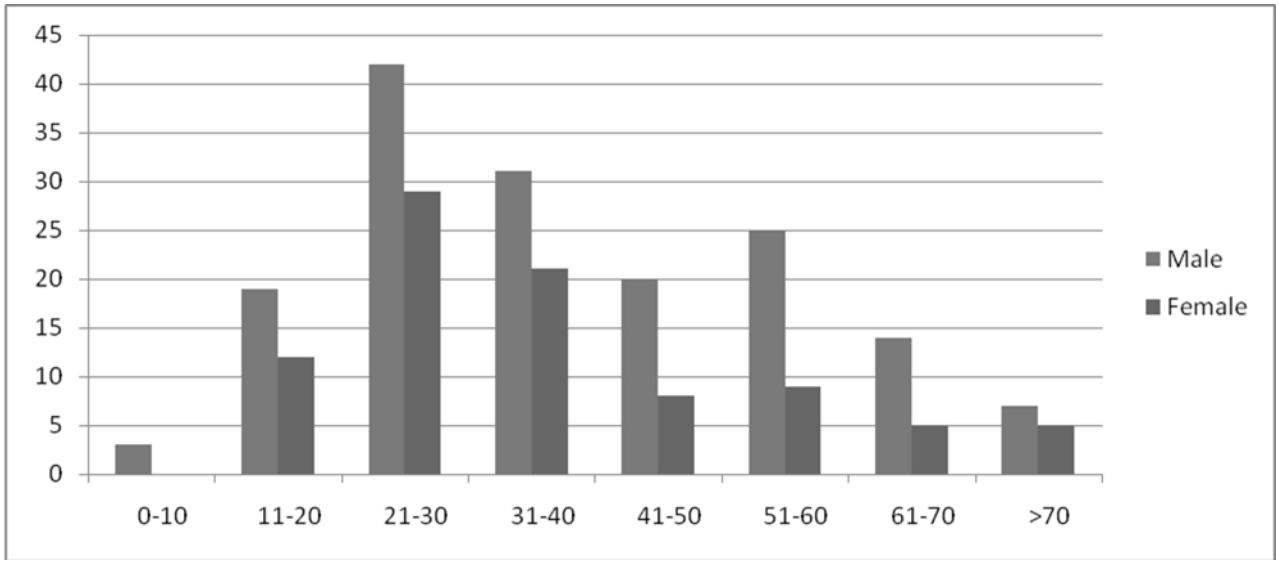

Fig 2 Age-wise Distribution of Total Suspected PTB Patients

AFB smear microscopy

Among total 250 sputum samples, 55·2% (138/250) were smear positive. Among 138 positive, 10.14% (14/138) were observed as < 10 AFB, 35.5% (49/138) as 1^+, 21.73% (30/138) as 2^+ and 32.6% (45/138) as 3^+ (Fig 3).

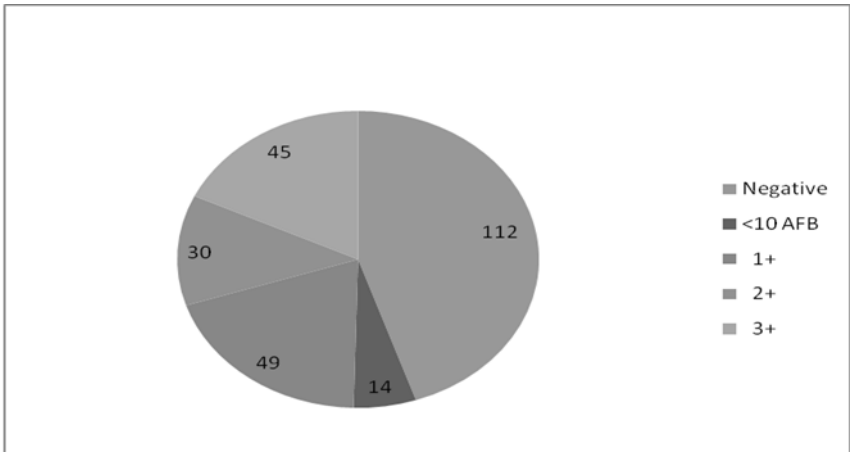

Fig 3 AFB Smear Result of Total Sputum Samples

Comparative Growth Pattern on LJ and BA

Among 250 sputum samples, growth of M.tuberculosis was observed on LJ and BA media from 45·2% (113/250) and 50·4% (126/250) respectively. Contamination rate was found same (2%) on both media. (Fig 4)

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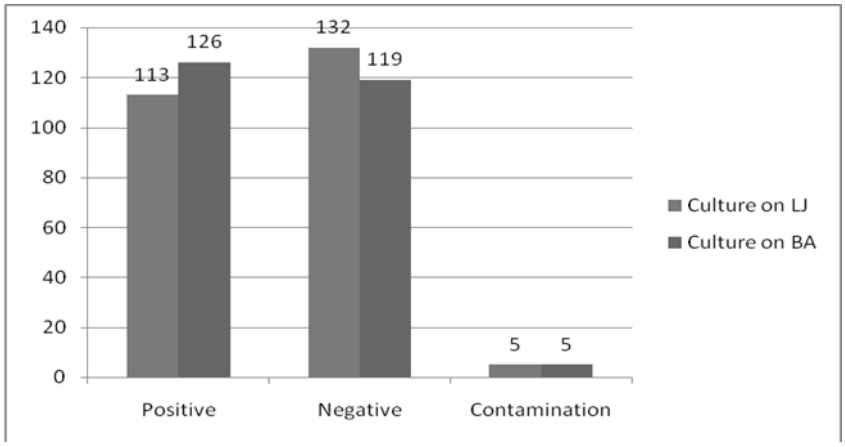

Fig 4 Comparative Result of Growth pattern on LJ and BA

Colonies count on both media (LJ/BA)

Among culture positive samples, more visible colonies (3+ growths) were in blood agar (90/126) than in LJ media (77/113). (Fig 5)

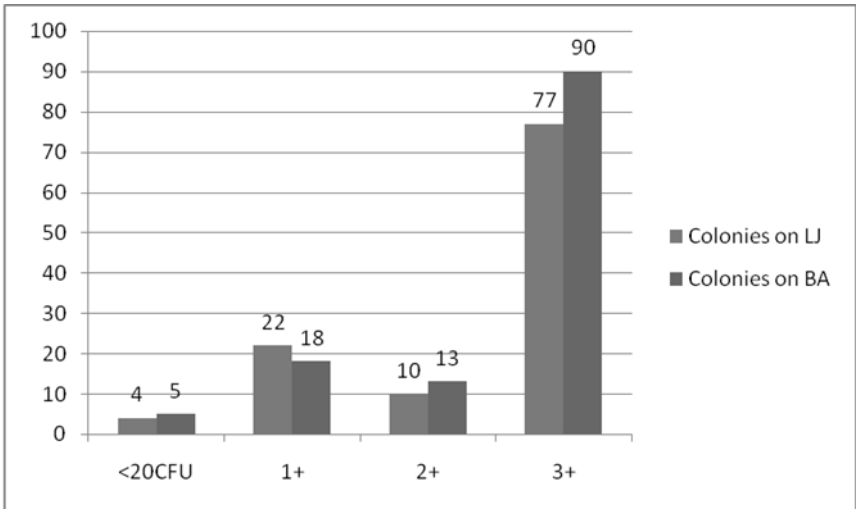

Fig 5 Comparative Result of Colonial Count On LJ and BA media

Time for visible colonies on LJ and BA

28.4% sample showed visible colonies on BA in 3rd weeks than 12% sample on LJ in 6th weeks. (Fig.6)

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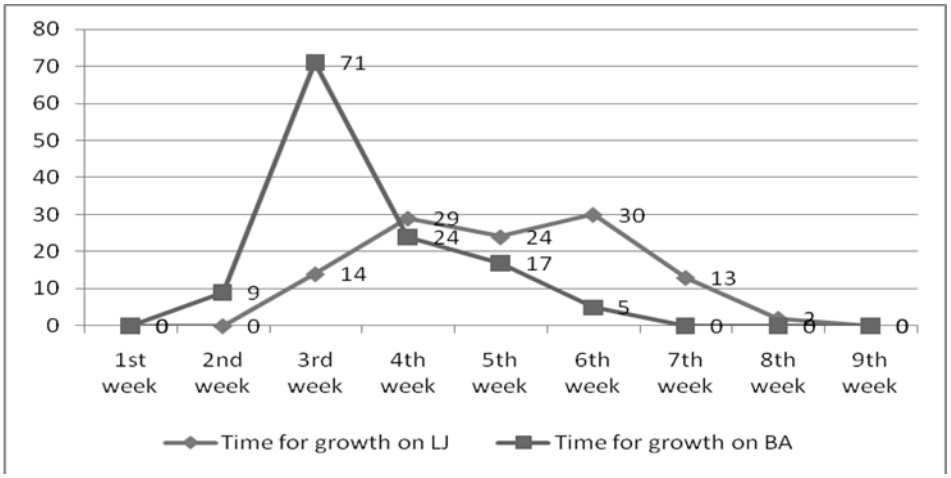

Fig 6 Time Required Forming Visible Colonies on LJ/BA media

Drug Susceptibility Testing

Among 10 isolates, 3 were resistant to all first line drugs, 5 were susceptible to all drugs on both media containing drugs. Only two were found resistant against two drugs. Drug susceptibility pattern was found exactly same on both LJ and BA media.

Table 1: Drug Susceptibility Patterns of Culture Positive Isolates (n=10)

Drugs	Proportion Method			
	On LJ		On BA	
	Resistant	Sensitive	Resistant	Sensitive
INH	5	5	5	5
RFP	4	6	4	6
SM	4	6	4	6
EMB	4	6	3	7

DISCUSSION

The number of male patients were found higher than female. This finding is statistically significant indicating male are more susceptible to TB as compare to the female. This finding was concordant with similar studies in other countries. In Cameroon, 65.76% of male and 34.25% of the female TB cases among 111 cases [9]; in Thailand 77% male and 23% of the female cases among 1441 cases [10]. Tuberculosis Control Programme, Nepal reported 66.77% male and 33.23% female of TB cases among 14,384 newly diagnosed TB cases during 2002/2003.47% males and 3.05% of female TB cases in histopathological specimens at Tribhuvan University Teaching Hospital [11]. 75.69% were male and 24.30% were female [12]. Possible factors to explain the gender differences observed includes women are less exposed to infection than men, the biological difference such as an increased susceptibility in male and infected women may progress more frequently to disease and die more rapidly, leaving a cohort with a low prevalence of infection.

Among the smear positive samples more growth of M.tuberculosis was on the LJ than BA media as well as among the smear negative samples. LJ media has more sensitivity and specificity than BA regarding the smear result. Among the 1121 samples of pulmonary sources 16.2% and 4.8% of pulmonary tuberculosis isolated from the AFB staining and culture [12]. Similar result was obtained during the study of PCR to detect Mycobacterium tuberculosis in sputum specimens, against Ziehl-Neelsen (Z-N) stain and culture as a standard method.

The growth of M.tuberculosis was more on BA media than on LJ media though the difference is not statistically significant. 94.2 % isolates of M.tuberculosis were obtained on the blood medium as compared to 90.1% on the Lowenstein-Jensen medium, a difference of 4.1%, which is not significant [13]. The contamination rate was slightly less on the blood medium than on the Lowenstein-Jensen.Determined the sensitivity of blood agar 98·9% as compared 92·6% sensitivity of the BACTEC medium [14]. More colonies of Mycobacterium tuberculosis were on the BA than LJ media.[15]

The colonies development of M.tuberculosis was faster on BA than LJ, time required to develop macroscopic colonies of M.tuberculosis on blood agar was 13.6±5.2 days and on LJ it was 20.4±5.1 days on LJ medium. [6] Also found time required to form visible colonies of M. tuberculosis was 19±5 days (range: 3–45) using blood-agar and 26±6 days (range: 7–39) using reference automated mycobacterial culture.

BA media compared with LJ medium as a gold standard for susceptible of M.tuberculosis towards all first line drugs was found similar pattern on both media.The first reported susceptibility of M.tuberculosis on blood agar and found shorter (2 weeks) time than using 7H10 agar (3 weeks). [6] The use blood agar as alternatives to Middlebrook 7H11 agar for testing the susceptibility of Mycobacterium tuberculosis to first-and second-line drugs by the E-test method. [6]

CONCLUSION

Blood agar slants may be a good substitute of LJ media for isolation and drug susceptibility testing of M.tuberculosis from sputum and save about 1/3 time for isolation of M.tuberculosis.

REFERENCES

[1] World Health Organization. Global tuberculosis control: epidemiology, planning, financing: WHO report. Geneva 2009.
[2] Davies, Girling DJ and Grange JM. Tuberculosis and its problems in developing countries. In; Oxford Textbook of Medicine 1996; 638-661.
[3] Wang, Zhen XA, Gao YS, and Du CM. Zhonghua Jie He Hu Xi Za Zhi 2006; 29:527–530.
[4] Arvand M, Mielke ME, Weinke T, Regnath T, Hahn H. Infection 1998; 56: 254.
[5] Drancourt M, Carrieri P, Ge´vaudan MJ, Raoult D. J Clin Microbiol 2003; 41: 1710–1711.

[6] Coban AY, Bilgin K, Uzun M. J Clin Microbiol 2005; 43:1930-1.
[7] Cheesbrough M. District Laboratory practice in tropical countries part II. LPE, Cambridge University Press, India 2002; 71-76: 207-212.
[8] Fujuki A. Bacteriology examination to stop TB. JICA 2001.
[9] Kuban C, Bercion R, Noeske J, Cunin P, Nkamsse P and Ngo Niobe S. The International Journal of Tuberculosis and lung Disease 2002; 4(4):356-360.
[10] Raintawan P, Punnotok J, Chaisuksuwan R and Prasugarit V. The International Journal of Tuberculosis and Lung Disease 1997; 1(4): 299-301.
[11] Shrestha, B Nehar A and Breyer U. Journal of the Nepal Medical Association TB special 1996; 117: 36-40.
[12] Rijal KR, Ghimire P, Bam DS and Rijal B. An epidemiological study of anti-tuberculosis drug resistance pattern in the pulmonary tuberculosis patients visiting National Tuberculosis Centre. A Dissertation submitted to the Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal, 2004.
[13] Tarshis MS. Am J Clin Pathol 1996; 23: 661-670.
[14] Solanki A,Sharma R,Gaur J, Mathur ML. Rapid culture of Mycobacter tuberculosis on blood agar in resource limited setting. Danish Medical Bulletin 2009; 56(4).