ORIGINAL ARTICLE

DIFFERENCES IN CULTURAL YIELD OF MYCOBACTERIUM TUBERCULOSIS ON MEDIA PREPARED USING COMMERCIAL AND HOUSEHOLD EGGS

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Background: Mycobacterial culture is considered as the gold standard for TB diagnosis. It is performed on egg-based media using commercially available eggs to grow *Mycobacteria* from clinical samples. These eggs are known to contain high concentration of antibiotics, including fluoroquinolones, given to chicken to prevent early mortality. This study was performed to compare Mycobacterial growth on media prepared from commercial and antibiotic free household eggs. **Methods:** Sputum samples from negative (No bacilli in 100 oil immersion field), scanty (1–9 AFB in 100 fields), 1+ (10–99 bacilli per field), 2+ (1–10 bacilli per field) and 3+ (>10 bacilli per field) were inoculated dually on Ogawa medium prepared from commercial and household eggs. Tubes were inspected every fourth day for the appearance of colonies till 60 days. Data tabulations and statistical analysis (F test for variation and unpaired Student's *t* test) were performed on Microsoft Excel®. **Results:** One microscopically negative sample showed growth on media prepared from household eggs, while all were negative on that prepared from commercial eggs. There were significant differences in time to culture positivity for samples graded 1+ (p=0.02), 2+ (p=0.002) and 3+ (p=0.0003). **Conclusion:** Commercial eggs containing antibiotics can be a source of false negativity in cultures especially in microscopically negative samples. This can be of special concern in HIV patients who have high smear negativity. It is therefore important to either develop provision of antibiotic free eggs for media preparation or to develop and validate other laboratory investigations for smear negative TB patients.

Keywords: Tuberculosis, Mycobacterium tuberculosis, HIV, ZN Smear, Diagnosis

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INTRODUCTION

Tuberculosis is a slowly progressive illness, which mainly affects developing countries.[1] A number of diagnostic techniques are now used for tuberculosis,[2,3] among which culture is the definitive diagnostic procedure, performed after smear microscopy of presumptive patients of tuberculosis.[4] It also helps in differentiation of *Mycobacterium tuberculosis* from other atypical *Mycobacteria* and is considered as the gold-standard for TB diagnosis.[5] Culture can now be performed on radioactive BACTEC medium, however, solid medium called Lowenstein Jansen agar is still used widely.[2] This medium is prepared using fowl eggs to provide complex nutrients to growing bacteria.[5] The growth of *Mycobacteria* is slow and colonies take around 4–6 weeks to appear.

It has widely been reported that locally bred, commercially available domestic fowl eggs contain many antibiotics, which are present in their feed to control early mortality from infections.[6,7] Among these, especially fluoroquinolones are of major concern. These are broad spectrum antibiotics which are important in treating a number of bacterial infections. They are also included in second line regimen for TB and are commonly used in patients allergic to first line drugs or those with drug resistant tuberculosis.[8]

Since many *Mycobacteria* are sensitive to fluoroquinolones[8], there is a possibility that fluoroquinolones present in commercially available eggs can slow or completely inhibit *Mycobacterial* growth in routine cultures.

To study the differences in growth of Mycobacteria in commercially available and household eggs, growth comparisons were performed between cultures grown from the same patient samples on two different sets of culture media prepared from commercially available and household fowl eggs, which were fed on material free of antibiotics.

MATERIAL AND METHODS

This was a laboratory based comparative study. Two sets each of Modified Ogawa media were prepared from commercial and household eggs. Samples from negative (No bacilli in 100 oil immersion field), scanty (1–9 AFB in 100 oil immersion fields), 1+ (10–99 bacilli per oil immersion field), 2+ (1–10 bacilli per oil immersion field) and 3+ (>10 bacilli per oil immersion

field) graded samples based upon microscopy, were inoculated in pairs, in such a way that one sample was inoculated on two slopes, one prepared from commercial eggs, while the other from household eggs. After inoculation, media were incubated at 37 °C and read every fourth day for the appearance of colonies till 60 days.

All tabulations and statistical analyses were performed on Microsoft Excel®.[9] After tabulations, F tests were performed to confirm that variance is comparable, after which Student's Unpaired T test were used to compare samples with equal variance.

RESULTS

Altogether 108 slopes were prepared for bacterial inoculation, 54 from commercial and 54 from household eggs and each sputum sample was inoculated dually one into each of the pair. Contamination rate remained 3.7% and 4 pairs of slopes were discarded due to contamination in one of them.

Out of ten microscopically negative samples, one of the negative samples showed positive growth on media prepared from domestic eggs, though it remained negative even at the end of experiment (60 days)

Media inoculated with microscopically scanty samples (n=8) became positive on 35.43±6.29 days on experimental group, while the controls (n=8) took 39.33±6.89 days. Two tailed student's unpaired t test showed that the difference was statistically insignificant. (p=0.3). One sample failed to grow on control media, which was excluded for calculations of mean values. One of the cultures became contaminated, so the pair was discarded and excluded from analysis.

Media inoculated with 1+ samples (n=14) took 27±6.14 days to grow on experimental media, while 33.23±6.41 days to grow on control media (n=14). The difference was shown to be statistically significant by two tailed student's t test. (p=0.02). One of the cultures became contaminated, so the pair was discarded and excluded from analysis.

For samples graded 2+ microscopically (n=14), samples in the experimental media took 21.66±3.6 days, while those in control media (n=14) took 28.33±5 days. The difference was shown to be statistically significant by two tailed student's t test. (p=0.002). Pairs containing two contaminated cultures were discarded and excluded from the samples.

3+ samples also showed significant difference in both the groups. The samples in control group (n=8) took 17±1.85 days, while experimental group (n=8) took 24±3.7 days (p=0.0003). The results are summarized in table-I and comparisons are shown in figure-1.

Table-1: Differences in time (days) taken by Mycobacterial cultures grown on household and commercial eggs

	Number of Days		
Microscopic Grading	Household	Commercial	p value
Scanty	35.43±6.29	39.33±6.89	0.3
1+	27.07±6.14	33.23±6.41	0.02
2+	21.66±3.6	28.33±5.51	0.002
3+	17±1.85	24±3.7	0.003

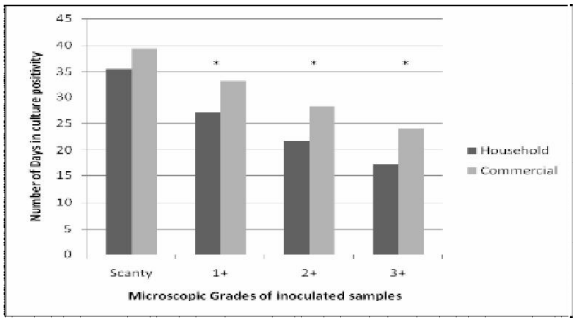

Figure-1: Comparison of time (days) taken by Mycobacterial cultures grown on household and commercial eggs.

DISCUSSION

Our results showed that the use of antibiotic free eggs can have significant effects on Mycobacterial growth and detection. One of the most important findings of the experiment was growth of Mycobacteria in one of the experimental groups while all the cultures prepared from commercial eggs did not show any growth inoculated with sputum which was microscopically negative. Though we used ten tubes for each group a rate of missed diagnosis in 10% of the commonly used culture media is quite alarming. Since culture is considered the gold standard for TB diagnostics[5], variation in results due to different eggs used can result in a number of missed cases, which can result in spread of the disease in the society.[10] This can be of greater significance in HIV positive patients, where smear negativity is comparatively high.[11]

Cultures grown from sputum specimens graded scanty on microscopy did not show statistically significant difference, however there was a mean difference of four days to positivity in the two groups.

Cultures obtained from samples graded 1+, 2+ and 3+ on microscopy showed increasingly statistically significant differences (p=.01, p=0 .002, p=0.003) in growth times, however there was no difference in detection rate (Figure-I). This shows that media prepared from commercially available eggs is mostly capable of slowing down the growth but it does not inhibit the growth altogether.

It can be argued however, that the differences in the results are not due to antibiotics, but difference in the types and amount of nutrients present in the two types of eggs, but its confirmation will require the

same testing of antibiotic free commercial eggs, which are not available in the market.

The results show that antibiotic free eggs provide a better and faster yield as compared to commercially available eggs, which are the most commonly, used resource for preparing egg based media for growing Mycobacteria. A missed diagnosis of up to 10% smear negative samples on the media prepared from commercial eggs also highlights the fact that either effort should be made for the provision of antibiotic free eggs for diagnosis of TB in smear negative cases or alternative techniques should be considered for definitive diagnosis of TB in smear negative cases.

CONCLUSION

Commercial eggs containing antibiotics can be a source of false negativity in cultures especially in microscopically negative samples. This can be of special concern in HIV patients who have high smear negativity. It is therefore important to either develop provision of antibiotic free eggs for media preparation or to develop and validate other laboratory investigations for smear negative TB patients.

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AUTHOR'S CONTRIBUTION

MYN: Conceived the idea, performed statistical analysis, planned the project and wrote the final draft.
ZA: Did operation planning, performed the lab work.
GK: Wrote the initial draft, contributed to the lab work. SS: Provided expert lab supervision, logistic support, reviewed the final draft. MM: Provided expert clinical supervision, logistic support, reviewed the final draft

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