

Effect of environmental temperature on the isolation of *Mycobacterium tuberculosis* from sputum samples stored in cetylpyridinium chloride

Isolation of *Mycobacterium tuberculosis* from sputa of suspected tuberculosis patients is essential for treatment initiation and drug resistance studies. In case the sputa cannot be processed within 72 h, they can be stored at room temperature with the addition of cetylpyridinium chloride (CPC) for longer periods (up to two weeks) without compromising on the quality of mycobacteria isolation^{1,2}. It has been recommended that CPC containing sputum should be stored at temperatures above 20°C as CPC crystallizes at low temperatures and may become inactive as a preservative^{3,4}. However, in the present study, the sputa collected in CPC were stored in the designated microscopy centres (DMCs) of Rayagada district, Odisha, and exposed to seasonal variations in temperature ranging from 10°C or lower in winter months to 40°C in summer months. The aim of the study was to observe the effect of environmental temperature on CPC-stored sputa for recovery of *M. tuberculosis* in solid culture.

This study was subcomponent of a cross-sectional study undertaken in collaboration with state and district TB Department, Government of Odisha from June 2011 to May 2013, for assessment of anti-TB drug resistance in Rayagada district⁵. The district is located between 19°0' and 19°58'N, 82°5' and 84°2'E in the southern part of Odisha. The minimum temperature of the district reaches as low as 10°C or lower in winter months (December–February), and maximum temperature reaches up to 40°C in summer months (April and May). The minimum and maximum temperature of the district in other months like March and June–November remains approximately between 20°C and 37°C. For the present study, smear-positive sputum samples were collected from pulmonary TB patients attending 18 out of 20 DMCs of Rayagada district⁶. The DMCs were supplied with freshly prepared 1% CPC in 2% sodium chloride solution and replaced fortnightly with fresh ones. Sputum samples were collected in sterile 50 ml falcon tubes to which equal volumes of CPC were added, tightly capped, sealed, labelled and maintained at room temperature. At weekly intervals sputum

samples were transported to the Regional Medical Research Centre (RMRC), Bhubaneswar laboratory, about 500 km from Rayagada district by overnight bus service. The temperature in Bhubaneswar is similar to that of Rayagada district; however, the minimum temperature during winter months remains around 15–18°C. The sputum samples were processed immediately and not stored at the RMRC laboratory. For culture processing, the specimens were centrifuged at 3000 g for 15 min; supernatant discarded and sediment was resuspended in 40–45 ml sterile distilled water. Centrifuged again at the same speed and time, supernatant discarded and sediment was resuspended in 1–2 ml of sterile distilled water and a loopful was inoculated in Lowenstein–Jensen (LJ) medium. The LJ slants were incubated at 37°C in a walk-in-incubator and examined for growth of *M. tuberculosis* complex once per week up to eight weeks. Any suspected growth on LJ medium was confirmed by Ziehl–Neelsen staining for acid fast bacilli, and positive growth on LJ medium was identified by colony morphology, growth rate and biochemical tests⁷, including niacin production, catalase activity at 68°C and susceptibility to *p*-nitrobenzoic acid (500 mg/ml). The transit time was calculated as the time taken from the date of sample collection to the inoculation date. Written informed consent was obtained from all the patients. The Ethical Committee of RMRC, Bhubaneswar approved the study protocol.

Out of the 564 sputum samples collected in CPC and processed within 14 days (median delay of 7 days) from the date of collection, 500 (88.7%) yielded *M. tuberculosis*, 48 (8.5%) yielded no growth, 13 (2.3%) had contaminants and 3 (0.5%) were nontuberculous mycobacteria. While comparing culture positivity with season-wise collection, it was observed that 94% sputum specimens yielded a positive culture in winter compared to that of 90.5% in summer and 84.9% in other months (Table 1). The rate of culture positivity between winter months was significantly higher ($P < 0.02$) than other months. It is evident from Table 1 that in the monsoon months, 10.9% specimens failed to pro-

duce visible colonies to that of 7.9% in summer and 4.3% in winter months. The rate of contamination observed between the seasons like winter, summer and other months was 1.7%, 1.6% and 3.1% respectively (Table 1). About 30.9% sputum specimens could not be processed within the duration of two weeks as transportation took a longer time. In these sputum samples, culture isolation was 77.4%, 77.8% and 74.3% in winter, summer and other months respectively.

The primary requirement for solid culture-based drug susceptibility testing necessitates a sputum sample free from contamination by other organisms, excluding mycobacterium and its confluent growth in LJ medium. Earlier studies showed that sputum specimens stored up to two weeks in CPC yielded 88.7% of culture positivity⁶. While segregating specimens based on exposure to various seasonal temperatures, it was observed that specimens collected and stored at low temperatures produced significantly higher number of cultures. In winter months the minimum temperature of Rayagada district goes down to 10°C and even lower in DMCs in the interior parts of the district. Though it is recommended to maintain CPC-containing sputum specimens above 20°C to avoid crystallization, due to delay in transport, the specimens were exposed to low temperatures in winter months. However, the isolation of mycobacteria was not affected in winter compared to other seasons. Contrary to high or low temperature exposure in summer or winter months, isolation of *M. tuberculosis* was affected in other months. The high contamination rate of 3.1% observed during the other months may be due to high humidity during these months, as these are monsoon months suitable for the growth of fungi. The effect of temperature variation with culture positivity was not observed in sputum specimens processed beyond two weeks (median 22.5 days, range 15–69 days) of storing in CPC, as the overall culture positivity reduced from 88.7% to 76%. It was observed from this study that sputum specimens stored in CPC may withstand low temperatures of 10–20°C, contrary to the belief that low temperature in this range is detrimental

SCIENTIFIC CORRESPONDENCE

Table 1. Isolation of *Mycobacterium tuberculosis* from cetylpyridinium chloride stored sputum during various seasons

Season	Within two weeks (N = 564)				Beyond two weeks (N = 252)			
	Cul +ve number (%)	Cul -ve number (%)	Conta number (%)	NTM number (%)	Cul +ve number (%)	Cul -ve number (%)	Conta number (%)	NTM number (%)
Winter	109 (94.0)	5 (4.3)	2 (1.7)	0 (0)	41 (77.4)	10 (18.9)	2 (3.7)	0 (0)
Summer	172 (90.5)	15 (7.9)	3 (1.6)	0	49 (77.8)	13 (20.6)	1 (1.6)	0 (0)
Other	219 (84.9)	28 (10.9)	8 (3.1)	3 (1.1)	101 (74.3)	26 (19.1)	5 (3.7)	4 (2.9)
Total	500 (88.7)	48 (8.5)	13 (2.3)	3 (0.5)	191 (75.8)	49 (19.4)	8 (3.2)	4 (1.6)

Winter months, December–February; Summer months, April and May; Other months March and June–November. Cul +ve, Culture positive; Cul -ve, Culture negative; Conta, Contamination; NTM, Nontuberculous mycobacteria.

to the recovery of *M. tuberculosis*. Specimen collection from inaccessible areas is certainly a problem and transport delays are common leading to exposure of samples to environmental temperatures. Laboratory-based control studies on the effect of low temperature on CPC-containing sputum specimens may be made, to know the exact temperature up to which it can be used. However, the present observations may be helpful for laboratories interested in using CPC as a transport medium in areas with similar low temperature variations. The study was not planned to find the impact of temperature on CPC-stored sputum specimens, and the monthly temperatures based on which the results are interpreted are only indicative of maximum and minimum temperatures during a month.

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Perspectives for geoscience in India

Geoscience deals with the earth system, specifically the geosphere, on which humans and other biota live and depend for water, food and energy requirements. The understanding of dynamic processes within the earth's crust, mantle and core, and their linkages with geophysical observations and processes at the land surface is complex but significant. These processes vary greatly in space from local, regional to global scale and from a few seconds to millions of years in time. Our knowledge about the composition of the earth, and its dynamic state has advanced manifold in the last few decades, however, there are several scientific challenges to meet. This knowledge is critical in the context of water, mineral and energy resources, natural hazards and environment.

The evolution of the Indian subcontinent is linked to the fragmentation and

dispersal of the Gondwanaland Super-Continent¹. The Gondwanaland started fragmenting about 133 million years ago. The study of fragmentation processes is one of the most challenging areas. India has recently set up a permanent station at the Larsmann Hills on the east coast of Antarctica. One of the key reasons for choosing this site is to provide opportunities to study the fragmentation process on Antarctica and the east coast of India. We need to probe geology below the ice, through remote sensing, geophysical methods and scientific drilling.

The journey of India from the Southern to the northern hemisphere began about 83 million years ago. About 65 million years ago, Reunion hotspot activity led to widespread volcanism over the Indian land mass and created the present day Deccan Traps. Recent deep drilling (up to 1.5 km) in the Koyna–Warna area,

penetrating the Deccan basalt all the way into the underlying granitic basement, has revealed the thickness of the Deccan Traps as ~1200 m in the Koyna region. It has also provided evidence for the penneplaned nature of the basement. Both these features were not known previously^{2,3}. It is interesting to note that no infra-trappean sediments were found at Koyna drilling sites. Drill cores from bore holes, about 10 km in length have been recovered, and petrological and geochemical studies are in progress to understand endogenic processes that gave rise to the Deccan Traps. The study is expected to provide hitherto unknown facts about the Deccan Traps and basement granitic rock formations.

India separated from Antarctica during the early Cretaceous⁴ and moved at a speed of 18–20 mm/yr, while today the plates are moving around 8 mm/yr. How