

Use of vancomycin in the culture of *Mycobacterium tuberculosis* from gastric lavage

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Background & objectives: Earlier studies from the Tuberculosis Research Centre, Chennai, on culture of *Mycobacterium tuberculosis* from gastric lavage (GL) specimens in selective Kirchner's medium (SK) resulted in a loss of 60 per cent culture results due to contamination with aerobic spore bearers (ASB). Addition of vancomycin to SK (SKV) effectively reduced the contamination rate to 20 per cent. The objective of the present study was to further reduce the contamination by collecting the specimens in bottles containing vancomycin, thus providing continuous exposure of the sample to the drug, which is bactericidal to ASB.

Methods: One thousand GL specimens collected from children in vancomycin containing bottles were decontaminated and cultured in SK medium, with and without vancomycin, subcultured on Lowenstein Jensen (W) medium and the culture results compared.

Results: The contamination of cultures in SK and SKV was 15 and 4 per cent respectively when the specimens were collected in bottles containing vancomycin compared to 60 and 20 per cent contamination reported in the earlier studies.

Interpretation & conclusion: The reduced contamination in SK and SKV is most likely due to the collection of sample in vancomycin containing bottles. Although a concurrent comparison of samples processed in vancomycin free conditions would have been ideal, it could not be done due to practical difficulties. The study thus confirms the value of vancomycin as a major deterrent for contamination due to aerobic spores and better results can be obtained if vancomycin is used in sample collection bottles, transport media and liquid culture media used in mycobacteriology laboratories particularly in humid and tropical environment.

Key words Culture from gastric lavage - *Mycobacterium tuberculosis* - selective liquid medium - vancomycin

Culture in liquid medium of *Mycobacterium tuberculosis* from gastric lavage (GL) obtained from children, has a high risk of contamination in tropical setting. We had found that it was often due to aerobic spores and had demonstrated that this Contamination could be brought down from 60 to 20 per cent by incorporating vancomycin 10 mg/l in the

liquid medium'. However, even this level of contamination is unacceptable considering that the samples are paucibacillary in nature and collected from children with considerable difficulty. We believed that the contamination was caused by spore formation occurring in the sample before the decontamination procedure. It was therefore decided

to use vancomycin in the sterile collection bottles to prevent the formation of spores against which normal decontamination procedures are ineffective. The findings of this investigation are presented here.

Material & Methods

Samples: We cultured 1000 GL samples collected in vancomycin containing bottles, from children with any clinical symptoms suggestive of tuberculosis (TB), in the course of a study on childhood TB. Approximately 5 ml of GL were collected early in the morning on two occasions where possible, from children too young to expectorate sputum. The sterile specimen bottles contained 50 µg vancomycin (0.25 ml of a 200 mg/l aqueous solution) to ensure approximately 10 mg/l of the drug in 5 ml of the sample.

Media: (i) Lowenstein Jensen (LJ) medium² for the primary culture and for subculture from the liquid culture; (ii) Kircher's liquid medium made selective by the addition of polymyxin B 20,000 units/l, amphotericin B 10 mg/l, carbenicillin 100 mg/l and trimethoprim 10 mg/l (PACT) and referred to as SK³; and (iii) SK, further fortified by the addition of vancomycin 10 mg/l (SKV)¹ (vancomycin hydrochloride from Sigma Chemical Co., USA. Cat. No. V2002).

Culture procedure: The culture method was as described earlier¹. Briefly, the samples were decontaminated by the modified Petroff's method⁴ and a loopful of the deposit was inoculated onto each of st pair of LJ slopes and the rest divided equally between a bottle each of SK and SKV media. All bottles were incubated and examined weekly, for growth. The LJ slopes were read up to 8 wk and the liquid media up to 6 wk. The latter were decontaminated and subcultured on LJ medium and again incubated for a further period of 8 wk. Growth of *M. tuberculosis* was identified based on niacin production, catalase activity and sensitivity to para nitrobenzoic acid^{5,6}. Any growth of non-tuberculous mycobacteria (NTM) encountered, was subjected to the above mentioned minimum identification tests to differentiate them from *M. tuberculosis*.

Statistical analysis: The agreement between the two methods was analysed by McNamar's test.

Results & Discussion

Among the 1000 samples cultured, there was loss of results due to contamination in 253 subcultures from the SK medium compared to 40 from the SKV medium, a difference which was statistically significant ($P < 0.0001$). The number of positives isolated was more from the SKV medium, i.e., 23 as against 19 from the SK medium. With the reduction of contamination, more casual isolates of NTM were found in the SKV medium (107) than in the SK medium (79). However, neither of these differences were statistically significant (Table).

Analyses not tabulated showed that only 33 of the primary LJ medium cultures from the same samples got contaminated, and yet they yielded fewer positives (20) compared to the liquid medium. Moreover, the growth on the primary cultures consisted of less than 5 colonies; 11 of 20 had yielded only a single colony each. As expected, the subcultures gave heavier growth; 16 of 19 from SK and 19 of 23 from SKV media had >20 colonies to as much as confluent growth.

A liquid medium is included among the multiple media used for primary culture of extra pulmonary samples in order to increase the yield of positives: but they have a greater risk of contamination, Mitchison et al^{3,7} advocated a combination of four drugs to be added to Kirchner's or Middlebrook's

Table. Comparison of growth on LJ subcultures

Growth on subculture	SK	SKV
M.Tb	19 (1.9)	23 (2.3)
Cont.	153 (15.3)	40 (4.0)
NTM	79 (7.9)	107 (10.7)
Total tested	1000	1000

M.Tb, *M. tuberculosis*; Cont, contamination other than NTM; NTM, non-tuberculous mycobacteria; LJ, Lowenstein Jensen; SK, selective Kirchner's medium; SKV, selective Kirchner's medium with Vancomycin. Figures in parentheses are percentages

liquid media and Middlebrook's 7H11 agar medium to make them selective for tubercle bacilli; the drugs recommended were polymyxin and trimethoprim to control Gram negative contaminants, carbenicillin for Gram positive organisms and amphotericin B for fungi, any of which may be present in the specimens or enter from the environment during collection. In a preliminary study we had observed that, decontaminated sputum and GL deposits had aerobic spore bearers that survived the action of the aforesaid decontaminating agents. The deposits from the liquid culture, also showed large numbers of spores indicating that the bacilli survived the action of antibiotics in the medium by becoming spores. For this reason, the selective Kirchner's liquid medium, which is most satisfactory for samples collected from other non pulmonary sites, showed 60 per cent contamination when GL was cultured in it. It was clear that the selective antibiotics in the medium needed modification if it was to yield results for GL cultures¹.

Vancomycin is bactericidal to many Gram positive organisms. It is particularly effective against most members of the bacillus species and has no action on *M. tuberculosis*⁸. Though the effective concentration is less than 5 mg/l, we used twice that concentration in the SK medium to compensate for binding by the serum proteins. The modified selective Kirchner's medium with vancomycin (SKV) had, in our earlier study¹, brought down contamination to 20 per cent. In order to reduce it further, the vegetative forms of the bacteria had to be eliminated before they could form spores and escape the action of the antibiotics. This was achieved in this present study, by collecting the samples of GL in bottles with vancomycin.

The results obtained, justified the use of vancomycin in the collection bottles and again in the medium. The contamination in the SK medium without vancomycin was 15.3 percent showing that collecting samples in vancomycin bottles was a beneficial step. When the same samples were cultured in SKV medium, contamination became 4.0 per cent which was a very significant improvement over the SK medium. The study clearly showed that continuous maintenance in a vancomycin containing

environment could effectively control spore bearer contamination in the GL cultures.

Although vancomycin is a relatively expensive drug at Rs. 5 per mg, its use in the specimen bottle and again in the medium is a small price to pay to save a precious specimen from contamination. In this study the number of positives was too small to fully determine the merit of the liquid medium. However, even a negative culture result is valuable as it is a definite result unlike a contaminated culture.

Vancomycin may be superfluous for samples collected from normally sterile sites but it can be used in place of carbenicillin which has been reported to have an inhibitory effect on *M. tuberculosis*⁹.

The study has thus confirmed the value of vancomycin as a major deterrent for aerobic spore bearer contamination wherever it may be encountered. It can be used in specimen collection bottles, transport media, and in the major liquid culture media used in mycobacteriology work in the humid and tropical environment.

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