Indian J Med Res 102, October 1995, pp 152-155

Vancomycin for controlling contamination of selective Kirchner's liquid medium in the culture of gastric lavage for tubercle bacilli

Sara Mathew, C.N. Paramasivan, Manjula Datta & R. Prabhakar

Tuberculosis Research Centre, Madras

Accepted August 31, 1995

Gastric lavage (GL) was collected for culture of tubercle bacilli from children too young to expectorate sputum. The selective Kirchner's liquid medium (SKLM), routinely used as one of the media for culture of all extrapulmonary specimens, was found to often get contaminated when cultured with GL. We have shown that vancomycin at a concentration of 10 mg/l successfully reduced the contamination from about 60 to 20 per cent, and enhanced the Isolation rate of tubercle bacilli from 3 to 6 per cent. Decontamination of the liquid culture before subculture on solid medium also helped to reduce the contamination rate. Vancomycin was found to be an effective selective drug for use In the Kirchner's liquid medium for culture of tubercle bacilli.

Key words Childhood tuberculosis-enhanced culture positivity-gastric lavage culture-Kirchner's liquid medium-vancomycin

Specimens from extrapulmonary forms of tuberculosis are cultured for tubercle bacilli in multiple media including the selective Kirchner's liquid medium (SKLM) because they are generally paucibacillary[1]. The advantages of the liquid medium are that larger inocula can be used and the growth emerges relatively faster. This in turn improves the yield of positive cultures. SKLM is made selective by the addition of four drugs, *viz,* polymyxin, amphotericin B, carbenicillin and trimethoprim with the aim of preventing the growth of Gram positive and Gram negative bacteria and fungi[2]. In this way, pathological specimens can be cultured after relatively milder decontamination which helps to retain the viability of the few tubercle bacilli normally present in such specimens. This also assumes importance in situations like tuberculosis in children where bacteriological evidence is hard to obtain since infants and children cannot expectorate sputum. In such cases, gastric lavage (GL) cultures, recommended as a suitable alternative for sputum, have shown encouraging results[3-5]. However, in our experience with gastric lavage, despite decontamination with 4 per cent NaOH, about 60 per cent of the SKLM cultures were contaminated, most often with aerobic spore bearers (ASB) . Apparently the drugs used in SKLM were inadequate to contain ASB in GL collected in tropical conditions.

Vancomycin has bactericidal action on many Gram positive bacteria including *Streptococcus, Staphylococcus, Clostridium* and *Bacillus* spp., and it is ineffective against *Mycobacterium tuberculosis,* and fungi[6]. Preliminary experiments (not tabulated) had shown that this drug at a concentration of 10 mg/l was able to completely inhibit growth of ASB without affecting the growth of *M. tuberculosis.*

The aim of this investigation, therefore, was to study the usefulness of vancomycin in SKLM in reducing contamination in GL culture. The adverse

effect of the drug, if any, on the growth of tubercle bacilli especially when present in small numbers as in these specimens, was also studied.

Material & Methods

A total of 221 GL samples were collected from children who were being investigated for developing a methodology for detection of childhood tuberculosis by active case finding methods. The lavage was collected from the children early in the morning before intake of food by trained health workers at the respective village centres and transported on ice to the laboratory about 50 km away, by noon, the same day. Of the 221 samples, 195 were processed without delay on receipt and 26 after overnight storage in the refrigerator. As only very few positives were expected among the GL samples, 26 sputum samples which had scanty AFB by smear, were included as positive controls.

Media : The media used were, *(i)* SKLM *i.e.,* Kirchner's liquid medium made selective with polymyxin 200,000 units/l, amphotericin B 10 mg/l, carbenicillin 100 mg/l and trimethoprim 10 mg/l (PACT) as described earlier[1]; *(ii)* SKLM with PACT and vancomycin 10 mg/l (SKLMV); and *(iii)* Lowenstein-Jensen (LJ) medium as used in this Centre[7].

Procedure : Sputum and GL samples were processed by the modified Petroff's method[7]. From the deposit obtained after processing, a pair of LJ medium slopes were inoculated first. The remainder of the deposit was equally divided between a bottle each of SKLM and SKLMV in random order and incubated at 37ºC. The bottles were randomised and examined weekly noting turbidity and pH changes as indicated by the colour change of the medium. At the end of six weeks (or earlier if they were suspected to be positive or contaminated with fungus), the SKLM and SKLMV cultures were subcultured on LJ medium after decontamination with 4 per cent NaOH as done routinely and also from the centrifuged deposits prior to decontamination (direct culture) in order to check whether positives were adversely affected by the decontamination procedure. Thus each sample had two liquid cultures, each of which had two pairs of subcultures. All slopes were randomised and examined weekly for 8 wk by an independent reader recording the growth of turbercle bacilli, non tuberculous mycobacteria (NTM) and contamination.

The Chi square and 'McNemar's tests were applied in comparing the results obtained with the two media.

Results

The results of culture of 221 GL and 26 smear positive sputum samples based on decontaminated subcultures are presented in Table I. It showed that 135 (61.1%) of the GL and 13 (50%) of the sputum samples were contaminated in the SKLM subculture, compared to only 47 (21.3%) and 3 (11.5%), respectively, in the subculture from SKLMV. Thus a definite culture result was available with SKLMV in 174 (78.7%) of the GL samples (5 positives, 45 NTM and 124 negatives) as compared to only 86 (38.9%) with the SKLM. Analysis of the relative rates of contamination in the two media showed that out of 148 subcultures contaminated in SKLM, 115 were not contaminated in SKLMV. On the other hand, of the 50 contaminated in SKLMV, only 17 were not contaminated in SKLM. This difference was statistically highly significant showing that the addition of vancomycin to SKLM greatly reduced the contamination (McNemar's test $P < 0.00001$).

In all, 18 positive cultures were isolated from the 221 GL and 26 sputum samples (Table II). Of the 15 samples positive in SKLMV, 8 were contaminated in the SKLM . Only 8 were positive by SKLM, one of which was contaminated in SKLMV. Further, none

Table 1. Results of culture of gastric lavage and sputum in SKLM and SKLMV

Growth on subculture	Gastric lavage		Sputum	
	SKLM	SKLMV	SKLM	SKLMV
Positive	3 (1.4)	5 (2.3)	5 (19.2)	10(38.5)
NTM	20 (9.0)	45 (20.4)	1 (3.8)	4 (15.4)
Negative	63 (28.5)	124 (56.1)	7 (26.9)	9 (34.6)
Cont.	135 (61.1)	47 (21.3)	13 (50.0)	3 (11.5)

Figures in parentheses are percentage values
GL : 221; Sputum : 26.
NTM, non-tuberculous mycobacteria; Cont, contaminated; SKLM, selective Kirchner's liquid medium; SKLMV, SKLM with vancomycin

of the GL samples were positive on the primary LJ slopes.

In order to see whether vancomycin had an adverse effect on the isolation of *M. tuberculosis,* the culture results of 82 samples (70 GL and 12 sputa) not contaminated in either media, were analysed (Table III). Seven samples were positive with each medium, 5 of them being common and 2 each being positive with one of the two media only. The isolation rate of NTM was also similar, 17 from SKLM and 21 from SKLMV. This showed that vancomycin did not affect the growth of *M. tuberculosis* or NTM even when they were present in small numbers.

The effect of decontamination of the liquid culture prior to subculture was analysed using the results obtained with SKLMV (Table IV). Contamination occurred in 77 direct and 50 decontaminated subculture and the number of positives isolated were 14 and 15, respectively. The analysis of the relative rates of contamination showed that it was significantly less when subcultured after decontamination ($P < 0.001$), showing that the step of decontamination was beneficial. Further, there was no difference in the numbers of positives isolated before and after decontamination.

Table II. Relative rates of isolation of positives from SKLM and SKLMV

SKLMV	SKLM			Total
	Positive	Negative	Contaminated	
Positive	5	2	8	15
Negative	2	0	0	2
Contaminated	1	0	0	1
Total	**8**	**2**	**8**	**18**

Table III. Rates of isolation of positives from SKLM and SKLMV from uncontaminated samples*

SKLMV	SKLM			Total
	Positive	NTM	Negative	
Positive	5	0	2	1
NTM	0	9	12	21
Negative	2	8	44	54
Total	7	17	58	82

* 70 GL and 12 sputum samples

Discussion

This study showed that vancomycin at a concentration of 10 mg/l was able to reduce the rate of contamination from 61 to 21 per cent in SKLM culture of GL. Consequently, definite culture results were available for nearly 79 per cent of the samples. Such an outcome is important since even a negative result would be valuable to the clinician in the management of patients.

The relative rate of contamination in SKLMV was significantly lower than in the SKLM ($P < 0.00001$). Although a contamination rate of 20 per cent is unacceptable when compared to < 10 per cent contamination in primary culture on LJ medium, it is an inherent problem with this type of specimen. Analysis not tabulated, showed that 90 per cent of GL deposits had some ASB remaining after decontamination as evident from growth seen on Mueller Hinton agar inoculated with it. These multiply rapidly in the highly favourable liquid medium which is incubated for up to six weeks to facilitate growth of the tubercle bacilli. This was seen in the analyses (not tabulated here), which showed that there was change in pH of the medium and turbidity earlier and more often in the SKLM (8 1%) than in the SKLMV (54%). A high proportion of cultures showing a marked pH change *(i.e.,* colour change to white or deep pink) was contaminated on subculture (31 of 38 from SKLM and 7 of 16 in SKLMV) as compared to those showing no pH change (8 of 29 from SKLM and 9 of 70 from SKLMV). Until documented evidence of pH changes were obtained in this study, it was not known whether the changes observed in the SKLM were due to growth of contaminants or due to the nature of the GL deposits which were mostly

Table IV. Comparative results of subcultures set up before and after decontamination of SKLMV cultures

Direct subculture	Decontaminated subculture				Total
	Positive	NTM	Negative	Contaminated	
Positive	12	1	1	0	14
NTM	0	34	3	1	38
Negative	2	8	96	12	118
Contaminated	1	6	33	37	77
Total	15	49	133	50	247

turbid and often copious in volume, compared to the clear deposits of other extrapulmonary samples. Now it can be safely assumed that liquid cultures showing extreme changes in pH as evidenced by colour change warrant immediate subculturing to contain contamination.

Following the reduction in contamination by the presence of vancomycin in SKLMV, twice as many of the positives present were retrieved on culture. The analysis of results also showed that vancomycin had no detrimental effect on the isolation of *M. tuberculosis* or NTM even when present in small numbers. The importance of using a liquid medium such as the SKLMV for primary isolation of mycobacteria from paucibacillary samples of GL became apparent as none of the primary cultures of GL on LJ slope were positive for growth of tubercle bacilli.

SKLM cultures are routinely decontaminated by the Petroff 's method[7] to ensure pure growth on subculture. Comparison of subcultures made before and after decontamination showed that this step was beneficial in reducing the contamination significantly. Further, it did not adversely affect the yield of positives. Therefore decontamination of the liquid culture is both useful and acceptable for highly contaminated material such as the GL. Also, if the specimens are transported in a medium containing vancomycin it may help arrest multiplication of ASB and other Gram positive contaminants, permitting the use of milder decontamination procedures to increase the yield of positive cultures. Since vancomycin has no action on mycobacteria, addition of this drug may have wider application in the field of mycobacteriology besides being a valuable adjunct in the primary culture of tubercle bacilli from pathological specimens and in the transport of specimens. Though the addition of vancomycin would increase the cost of the culture, it is justified by the increase in the number of positives obtained from paucibacillary specimens especially from children with tuberculosis.

Acknowledgment

The authors are grateful to Smt. S. Sudhamathi, Shriyuts K.J. Ilampuranan, and G.Kubendiran for their valuable technical assistance.

References

1. Paramasivan CN, Vanaja Kumar, Alexander C, Venkatesan P, Somasundram PR, Prabhakar R. Use of multiple media for the cultivation of mycobacteria from specimens other than sputum. *Indian J Med Res* 1987; *86* : 290-4.
2. Mitchison DA, Allen BW, Carrol L, Dickinson JM, Aber VR. A selective oleic acid albumin agar medium for tubercle bacilli. *J Med Microbiol* 1972; *5 :* 165-75.
3. Darzins E. *Bacteriology of tuberculosis,* Minneapolis: University of Minnesto Press, 1958: 175
4. Starke JR, Jacobs RF, Jereb J. Resurgence of tuberculosis in children. *J Pediatr* 1992; *120 :* 839-55.
5. Abadco DL, Steiner P. Gastric lavage is better than bronchoalveolar lavage for isolation of *Mycobacterium tuberculosis* in childhood pulmonary tuberculosis. *Paediatr Infect Dis J* 1992; *11 :* 735-8.
6. Barber M, Garro LP. *Antibiotic and chemotherapy,* Edinburgh: E & S Livingstone Ltd. 1963: 169.
7. Tuberculosis Chemotherapy Centre, Madras. A concurrent comparison of isoniazid plus PAS with three regimens of isoniazid alone in the domicilliary treatment of pulmonary tuberculosis in south India. *Bull WHO* 1960 : *23* : 535-85

Reprint requests : The Director, Tuberculosis Research, Centre, Chetput, Madras 600031