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Use of multiple media for the cultivation of mycobacteria from specimens other than sputum

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An investigation was carried out on the efficacy of using multiple media in the isolation of *Mycobacterium tuberculosis* from specimens other than sputum, during the period 1980-1984. Of a total of 3807 specimens examined, 818 were urine, 1428 cerebrospinal fluid (CSF), 567 lymph glands, 94 pus samples, 224 operation specimens, 91 gastric aspirates, 108 ascitic fluid and 477 were other types of specimens. Each specimen was inoculated onto one set of media consisting of two slopes each of 'Lowenstein-Jensen' medium (LJ), LJ medium containing 0.5 per cent sodium pyruvate (LJP) and 7H11 oleic acid albumin medim (7H11) and two bottles of selective Kirchner's liquid medium (KL). In all, 550 (14%) were positive by culture in any one of the four media used. Considering the different media individually, KL had the highest efficiency yielding 339 (62%) of the total 550 positive cultures. Also, KL detected 162 positives which were not positive by any of the other media. This was followed by LJ with 328 (60%) positives. LJ and KL when considered together detected 93 per cent of the positives and LJ, LJP and KL increased the positivity to 99 per cent. Thus LJ and KL seems to be the best combination for the isolation of mycobacteria from specimens other than sputum.

In paucibacillary extra-pulmonary tuberculosis, the procedures used for processing sputum specimens could often be detrimental to the viability of tubercle bacilli[1]. Also, specimens collected during surgery usually cannot be repeated and hence, intensive efforts have to be made to isolate the few organisms present in these specimens.

Over the years, several studies have been undertaken to improve the procedures for processing specimens other than sputum, in order to reduce their deleterious effect on the viability of tubercle bacilli[2-4] and increase the isolation rates. Mitchison *et al* [5] suggested treatment with acid for a standard short period and inoculation on selective medium. We undertook an investigation using multiple media on 3807 specimens from extrapulmonary sources during the period 1980-84, and the findings are reported here.

Material & Methods

Specimens : Over 95 per cent of the specimens reported in this study were from patients admitted to various chemotherapeutic studies at the Tuberculosis Research Centre, Madras and the

remaining specimens were referred from other hospitals for bacteriological confirmation.

Of the total of 3807 specimens examined over a 5 yr period, 818 (21%) were urines, 1428 (38%) cerebrospinal fluids (CSF), 567 (15%) lymph glands, 94 (2%) pus specimens, 224 (6%) operation specimens, 91 (2%) gastric aspirates, 108 (3%) ascitic fluids and 477 (13%) were other types of specimens (miscellaneous; Table I). The CSF specimens and lymph gland biopsy specimens were obtained from children with meningitis and lymphadenitis respectively, and specimens excised during surgery (bone and, tissue) were from patients with tuberculosis of the spine and Pott's paraplegia.

Culture media : The culture media used were Lowenstein-Jensen (LJ) medium, LJ medium containing 0.5 per cent sodium pyruvate (LJP), 7H11 oleic acid albumin medium with malachite green and Kirchner's liquid (KL) medium[6]. For the KL medium, 10 per cent horse serum was used in the place of calf serum. The 7H11 and KL media were made selective by the addition of polymyxin B sulfate 200 000 units/l, carbenicillin 100 mg/l, trimethoprim 10 mg/l and amphotericin B 10 mg/l. Each specimen was inoculated onto one set of media consisting of two slopes each of LJ, LJP and 7H11, and two bottles of KL, and examined at weekly intervals. If microcolonies were seen in KL medium or if no growth was seen by 6 wk, KL was subcultured onto LJ slopes which were then incubated until growth occurred or until 8 wk.

Treatment of specimens : Urine : An early morning midstream urine sample was collected and allowed to stand at 4°C. After 4 h, a major proportion of the supernatant was decanted without disturbing the lower portion of the sample

Table I. Bacteriological results by specimen type

Type of specimen	Total specimens	Smear positive	culture positive	Contaminated	NTM
Urine	818	–	32 (4)	31 (4)	35 (4)
Cerebrospinal fluid	1428	72 (5)	156 (11)	67 (5)	35 (2)
Lymph glands	567	107 (19)	176 (31)	32 (6)	14 (2)
PUS	94	30 (32)	42 (45)	5 (5)	4 (4)
Operation specimens	224	62 (28)	104 (46)	14 (6)	4 (2)
Gastric aspirate	91	7 (8)	13 (14)	7 (8)	8 (9)
Ascitic fluid	108	2 (2)	6 (6)	4 (4)	4 (4)
Miscellaneous	477	16 (3)	21 (4)	6 (1)	20 (4)
Total	3807	296 (10)*	550 (14)	166 (4)	124 (3)

Figures in parentheses are percentages. NTM, non-tuberculous mycobacteria
*Based on specimens excluding urine

containing the sediment, if any. After mixing thoroughly, the lower portion of the urine sample was transferred to a universal container and centrifuged at 3000 rpm for 15 min. The supernatant was discarded and the deposit was re-suspended in 0.5–1 ml of sterile water. An equal volume of 5 per cent (v/v) H_2S0_4 was added to the suspension; 15 min later about 15 ml of sterile water was added, the contents centrifuged, and the deposit suspended in 0.2 ml of sterile water. One loopful of each of the material obtained thus was inoculated onto LJ, LJP and 7H11 media; the remaining material was inoculated onto KL medium. Urine samples were not subjected to smear examination.

Cerebrospinal fluid (CSF) and ascitic fluid: Smear : Using a 5 mm loop, a smear was made by placing three successive drops on the same spot on the slide, each one after the previous one was dry.

Culture by direct inoculation : One set of all the four media was directly inoculated before processing. One loopful of CSF was inoculated onto each of the 3 solid media and 0.2 ml was added to KL using sterile pipettes.

Inoculation after decontamination : After direct inoculation, the remaining CSF was centrifuged at 3000 rpm for 15 min. To the deposit, 1 ml of sterile distilled water was added followed by 1 ml of 5 per cent H_2SO_4. The bottles were shaken well and kept for 15 min. 5 ml of sterile distilled water was added to this and the contents centrifuged at 3000 rpm for 15 min. To the deposit 0.2 ml of sterile distilled water was ad&d and one loopful each was inoculated onto the 3 solid media; the remaining inoculum was transferred to KL medium.

Lymph gland and operation specimens : Specimens were cut into small pieces and approximately 1 g was ground in a Griffith's tissue grinder with 5 ml of sterile distilled water; if the specimen was too small the whole specimen was ground. The homogenate was then transferred to a sterile universal container, leaving any coarse pieces behind, and then processed as for CSF by the H_2SO_4 method.

Pus specimens : If two swabs were received, smear was made with one and the other used for culture, If only one swab was received, it was used only for culture. For culture, the swab was immersed in a tube containing 4 per cent H_2SO_4, for 10 min. It was then transferred to a tube containing 1 per cent NaOH for 1 min. The swab was then inoculated onto all 3 solid media and the swab together with a small portion of the stick was left fully in KL medium.

Gastric aspirate : If the specimen was mucoid it was processed as for sputum[4]. If it was fluid, the specimen was first centrifuged, the supernatant was discarded and the deposit was treated as for sputum.

Culture reporting : The specimen was reported positive for *Mycobacterium tuberculosis* or non-tuberculous mycobacteria (NTM) if growth was found in any one of the four media used, negative if there was no growth of mycobacteria on any of the media up to 8 wk, and contaminated only if all the four media were contaminated.

Results & Discussion

Smear examination was carried out on all specimens other than urine. Of the 2989 specimens, 296 (10%) were smear positive. Of the 3807 specimens examined by culture, 550 (14%) were positive. In all, 166 (4%) specimens were contaminated, and NTM were isolated from 124 (3%) of the specimens; the remaining specimens (78%) were negative (Table I).

Considering the different types of specimens, pus specimens, operation specimens and lymph nodes were most often found positive by direct smear (32, 28 and 19% respectively) and culture (45. 46 and 31% respectively).

The contamination rate was 1 per cent for miscellaneous specimens; the rates were broadly similar for the other types of specimens, ranging from 4 to 8 per cent.

The comparison of different media was based only on specimens which were reported to be culture positive. Of the media tested, when considered alone, KL had the highest efficiency yielding 339 (62%) of the total 550 positive cultures (Table II). This was followed by LJ slopes with 328 (60%) positives. LJ slopes containing sodium pyruvate yielded lesser number of positive cultures (50%). Selective 7H11 slopes, which had yielded variable results with earlier workers[3,4], showed the lowest positivity (30%).

KL detected 162 positives which were not positive by any of the other media. In contrast, LJ and LJP yielded only 50 and 23 such positives respectively, and 7H11 yielded only 4 such positives.

Table II. Specimens yielding positive culture on various combinations of media

Culture media	Type. of specimen with no. and % of +ve culture						
	Urine	CSF	Lymph gland	Pus	Operation specimens	Miscellaneous (Incl. Asp., A.F)	All
LJ	11 (34)	66 (42)	135 (77)	30 (71)	66 (63)	20 (50)	328 (60)
LJP	8 (25)	51 (33)	114 (65)	29 (69)	56 (54)	17 (42)	275 (50)
7H11	6 (19)	25 (16)	73 (41)	19 (45)	32 (31)	8 (20)	163 (30)
KL	26 (81)	112 (72)	98 (56)	21 (50)	56 (54)	26 (65)	339 (62)
LJ, LJP	11 (34)	82 (53)	148 (84)	37 (88)	77 (74)	23 (58)	378 (69)
LJ, KL	32 (100)	144 (92)	168 (95)	38 (90)	94 (90)	39 (98)	515 (94)
LJP, KL	30 (94)	138 (88)	154 (88)	38 (90)	89 (86)	36 (90)	485 (88)
LJ, LJP, 7H11	11(34)	87 (56)	149 (85)	38 (90)	80 (77)	23 (58)	388 (71)
LJ, LJP, KL	32 (100)	154 (99)	175 (99)	42 (100)	103 (99)	40 (100)	546 (99)
All	32 (100)	156 (100)	176 (100)	42 (100)	104 (100)	40 (100)	550 (100)

Asp, gastric aspirate; AF, ascitic fluid. Figures in parentheses are percentages

The high yield from KL medium, which is the only liquid medium among the 4 tested, may be because in a liquid medium the tubercle bacilli inoculated multiply into large numbers and micro-colonies are more easily visible than in a solid medium. Also, Mitchison *et a1* [6] have stated that the higher yield of positive cultures is likely to result from the addition of larger volumes of specimens to the fluid medium than would be possible with solid medium. He also observed that large inocula of tissues or pus often inhibit the growth of tubercle bacilli on solid media and that inocula from fluid specimens are difficult to add to slopes of solid medium and that bacilli out of contact with the slope might not have optimal nutrition. However, inclusion of egg containing solid medium is necessary to obtain growth suitable for identification and sensitivity testing.

Considering the type of specimen and the media one by one, in urine and CSF specimens, KL yielded high proportion of positive cultures. The positivity rates with KL were 81 and 72 per cent for urine and CSF respectively, compared with 34 and 42 per cent with LJ, the differences being statistically significant ($P<0.01$ and $P < 0.001$ respectively). On the contrary, in lymph glands, pus and operation specimens, LJ yielded higher proportion of positivity than KL, the difference with lymph node being statistically significant ($P < 0.001$). The proportion of positive cultures with LJP was less than that with LJ in all types of specimens, the differences being significant with CSF ($P=0.04$) and lymph glands ($P<0.01$).

Considering the media two by two, LJ and KL media gave 93 per cent positives, while LJP with KL was almost as good (88%); when LJ, LJP and KL were-considered together, 546 (99%) of the 550 positives were detected.

Our findings show that LJ and KL media may be recommended for the isolation of tubercle bacilli from specimens other than sputum.

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