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Lowenstein-Jensen Selective Medium for Reducing Contamination in *Mycobacterium tuberculosis* Culture

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We compared *Mycobacterium tuberculosis* sputum culture recovery and contamination rates between Lowenstein-Jensen medium (LJ) containing the following decontaminants and LJ alone: (i) PANTA ($n = 299$), (ii) Selectatab-MB ($n = 299$), and (iii) penicillin G ($n = 234$). The contamination rate for LJ alone was approximately 31%, versus 5.0% for PANTA-containing, 2% for Selectatab-containing, and 9% for penicillin-containing media ($P < 0.001$). *M. tuberculosis* isolation rates were 9.8%, 17%, 18%, and 12% for standard LJ, PANTA, Selectatab, and penicillin cultures, respectively.

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a high-burden disease, with over 95% of TB cases occurring in developing countries (1). Culture remains the gold standard for detecting TB and drug sensitivity testing (2). However, the effectiveness of culture systems is greatly undermined by contamination with bacteria and fungi (3). Contamination reduces the proportion of interpretable results, thereby limiting the diagnostic value of culture systems. Contaminated cultures must be repeated, at additional cost to public health systems, which delays or ultimately prevents TB diagnosis.

Decontamination techniques have been implemented at all stages of specimen collection and processing. Antimicrobial and antifungal oral rinse solutions can be used prior to sputum expectoration (4), and decontamination using cetrimide, oxalic acid, and sulfuric acid can be used during sputum processing (5, 6). A variety of decontamination methods have been developed for various *M. tuberculosis* culture media. The liquid-culture mycobacterium growth indicator tube (MGIT) contains a rapid and sensitive culture medium with low contamination rates. However, its high cost is prohibitive for resource-limited settings (RLS). The Bactec 960 liquid culture system uses PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) to reduce contamination (7). A combination of vancomycin, amphotericin B, and nalidixic acid (VAN) has also been shown to minimize contamination in the Middlebrook 7H11 agar formulation (7).

Although Lowenstein-Jensen medium (LJ) remains the most common medium used in RLS (8), few studies have assessed the efficacy of antibiotics on LJ. We assessed the performance of PANTA (Becton, Dickinson, Franklin Lakes, NJ, USA), mycobacterium Selectatab (Selectatab-MB; Mast, Merseyside, United Kingdom), and penicillin G (Sigma-Aldrich, Gillingham, United Kingdom) in reducing contamination and improving the yield and recovery of *M. tuberculosis* in LJ. We also characterized a set of contaminants isolated from antibiotic-free LJ.

Sputum samples were collected as part of routine clinical care from patients with presumptive TB attending the outpatient department of Mbarara Regional Referral Hospital (MRRH) and the Immune Suppressed Syndrome (ISS) (HIV) clinic, Mbarara, Uganda, between October 2011 and January 2012. Sputum smears were prepared for microscopic examination using the auramine-LED fluorescence technique, and results were reported according to World Health Organization (WHO) guidelines (9, 10).

All laboratory procedures were performed at the laboratory of the Epicentre Research Centre in Mbarara, Uganda, which is monitored by the Institute of Tropical Medicine of Antwerp, Antwerp, Belgium. We first processed the sputum for culture using the N-acetyl-L-cysteine-sodium hydroxide method (11) and inoculated 200 μl of sediment into plain LJ or antibiotic-containing LJ tubes. Two distinct groups of specimens were evaluated by matched comparison of decontamination techniques. The first group of 299 sputum specimens was analyzed in triplicate with plain LJ, LJ with PANTA, and LJ with Selectatab-MB. A second group of 234 sputum specimens was analyzed in duplicate to compare plain LJ against LJ containing penicillin G. All media were prepared according to manufacturer guidelines. Penicillin-treated LJ was prepared to make a final concentration of 100 IU/ml before coagulating. The costs of PANTA, Selectatab-MB, and penicillin per test were approximately $0.80, $0.40, and $0.14, respectively.

Tubes were incubated at 37°C for a maximum of 8 weeks. Cultures showing no growth after 8 weeks of incubation were reported as negative. Liquefied or discolored (dark green) LJ media or LJ slants with colonies of non-acid-fast bacteria were considered contaminated. Internal quality control for microscopy and LJ was performed as previously described (12). For a subset of 20 contaminated LJ tubes, culture liquid was inoculated on blood agar and incubated at 37°C for 48 h. After growth was detected, an isolated colony was picked for further characterization using Gram staining and API Gallery (bioMérieux, Durham, NC, USA).

Data were double entered into Epidata (version 3.1; Epidata Association, Odense, Denmark) and analyzed using Stata SE version 12 software (StataCorp, College Station, TX, USA). We compared contamination rate, yield (proportion of *M. tuberculosis* culture-positive specimens among smear-positive specimens),

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TABLE 1 General *M. tuberculosis* yield and contamination ratio in PANTA-, Selectatab-MB-, and penicillin-treated LJ versus antibiotic-free LJ

Antibiotic and medium	*M. tuberculosis*-positive samples		Contaminated samples	
	No. (%)	*P*	No. (%)	*P*
PANTA		0.38		<0.0001
LJ-PANTA ($n = 299$)	52 (17.4)		15 (5.0)	
Plain LJ ($n = 299$)	33 (11.0)		96 (32.0)	
Selectatab-MB		0.38		<0.0001
LJ–Selectatab-MB ($n = 299$)	55 (18.4)		7 (2.3)	
Plain LJ ($n = 299$)	33 (11.0)		96 (32.1)	
Penicillin		0.34		<0.0001
LJ-penicillin ($n = 234$)	29 (12.4)		21 (8.9)	
Plain LJ ($n = 234$)	23 (9.8)		72 (30.8)	

TABLE 2 Contamination and *Mycobacterium tuberculosis* and recovery according to microscopy grading

Microscopy grade[a]	Antibiotic	No. (%)	
		Contaminated cultures	*M. tuberculosis*-positive tubes
No AFB ($n = 253$)	PANTA	10 (4.0)	12 (4.7)
	Selectatab-MB	5 (2.0)	12 (4.7)
	None	78 (30.8)	6 (2.4)
Scanty/+1 ($n = 12$)	PANTA	1 (8.3)	10 (83.3)
	Selectatab-MB	1 (8.3)	10 (83.3)
	None	4 (33.3)	7 (58.3)
2+/3+ ($n = 34$)	PANTA	4 (11.8)	30 (88.8)
	Selectatab-MB	1 (2.9)	33 (97.1)
	None	14 (41.2)	20 (58.8)

[a] Microscopy grading was done according to the WHO/IUATLD scale (1). AFB, acid-fast bacilli.

and recovery (proportion of *M. tuberculosis* culture-positive specimens by sputum smear result) between plain LJ and antibiotic-containing LJ using McNemar's test for paired samples.

Contamination rates were 32.1% in plain LJ, 5.0% in LJ-PANTA, and 2.3% in LJ–Selectatab-MB among 299 matched specimens (Table 1). While both decontaminant-containing cultures had significantly lower contamination rates than plain LJ ($P < 0.001$), the difference in rates between the two antibiotic-containing media was not significantly different ($P = 0.10$). Among the 234 specimens cultured in both penicillin-treated medium and plain LJ, contamination rates were 8.9% and 30.7%, respectively ($P < 0.001$).

TB-positive-culture rates were 11.0% (33/299), 17.4% (52/299), and 18.4% (55/299) for plain LJ, LJ-PANTA, and LJ–Selectatab-MB, respectively, with a significantly higher proportion in the decontaminated samples than in plain LJ. In group 2, positive-culture rates were 12.4% (29/234) and 9.8% (23/234) in penicillin-treated and plain LJ, respectively ($P = 0.34$) (Table 1).

Recovery differed by culture medium type. Among specimens graded 2+/3+ (corresponding to more than 10 acid-fast bacilli per field in at least 50 fields) by microscopy, recovery was 58.8% (20/34) in plain LJ, 88.2% (30/34) with PANTA (McNemar's exact $P = 0.01$), and 97.1% (33/34) with Selectatab-MB (McNemar's exact $P < 0.001$) (Table 2). Overall, recovery was 58.7% (27/46) in plain LJ, which increased to 87% (40/46) with PANTA (McNemar's exact $P < 0.001$) and to 93.5% (43/46) with Selectatab-MB (McNemar's exact $P < 0.0001$). The difference in recovery between the two treated-medium strategies was not statistically significant (McNemar's exact $P = 0.25$). The recovery rate in LJ with penicillin was 78.6%.

We evaluated the effectiveness of selected antibiotics in reducing contamination in LJ. Notably, contamination rates decreased from approximately 30% with plain LJ to ≤5% for LJ with the addition of PANTA or Selectatab-MB (Table 3). Notwithstanding the improved decontamination rates, positive culture rates increased from 11% to 17 to 18% for the decontaminated media, and yield increased from 56% to 89 to 97% for specimens that were graded 2/3+ by sputum microscopy. While penicillin G-containing media also demonstrated lower rates of contamination, yield and recovery improvements were not seen to the same degree. Selectatab-MB contains ticarcillin, a penicillin which acts on Gram-negative bacteria, particularly *Pseudomonas aeruginosa*. It is also one of the few antibiotics capable of treating *Stenotrophomonas maltophilia*, one of the major contaminants isolated in this study, and this is probably why Selectatab-MB performed better overall. In summary, the addition of PANTA and Selectatab-MB to standard LJ appears to reduce contamination rates without reducing the growth or recovery of TB in culture.

Our contamination rates with plain LJ were approximately 31%, greater than the recommended threshold of 5% for laboratories that receive freshly collected sputum samples or 5 to 10% for settings where samples take several days to reach the laboratory (9). This may be due to enrollment of patients with cough for more than 2 weeks and no other TB symptoms. Although LJ contains malachite green, which has antibiotic properties, several other groups have reported similarly high contamination rates (13). For example, in a recent study carried out at the Zambian National Reference Laboratory, an LJ contamination rate of 14.9% was reported (14). These data reinforce the need for improved decontamination methods for laboratories that rely on LJ culture for TB diagnosis and susceptibility testing.

Other strategies to reduce contamination rates have been reported, with varying results. In our setting, we have tested mineral water rinsing prior to sputum production but with limited effects (Y. Boum II, unpublished data). A recent study on the use

TABLE 3 Characterization of contaminants isolated from antibiotic-free LJ tubes ($n = 20$)

Gram reaction	Organism	No. (%) detected
Positive	*Streptococcus* spp.	2 (16.7)
	Staphylococcus spp.	6 (49.9)
	Cellulomonas spp.	2 (16.7)
	Fungi	2 (16.7)
	Total	12 (60)
Negative	*Stenotrophomonas* spp.	3 (37.5)
	Aeromonas spp.	2 (25)
	Serratia spp.	1 (12.5)
	Brevibacterium spp.	1 (12.5)
	Enterobacter spp.	1 (12.5)
	Total	8 (40)

of oral rinse solutions (chlorhexidine and nystatin) showed a lower contamination rate in samples collected from patients with presumptive TB, but this was also shown to affect the recovery of *M. tuberculosis* (4).

Conclusion. Both Selectatab-MB and PANTA offer excellent solutions for reducing contamination in LJ. Due to its low cost, Selectatab-MB should be further explored for use as a medium additive to improve recovery rates and decrease contamination for LJ-based culture media in resource-limited settings.

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