Use of culture methods for recovery of atypical mycobacteria from stools of AIDS patients

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Abstract

Objective: To establish recovery rates of atypical mycobateria from stools of suspected AIDS patients using culture media.
Design: Laboratory evaluation of recovery rates, contamination rates, optimum exposure time and optimum concentration of alkali used for decontamination.
Setting: The study was conducted in Harare, Zimbabwe at two medical institutions: Beatrice Road Infectious Diseases Hospital (BRIDH) (a tuberculosis referral hospital) and Mashambanzou Care Unit (MCU) (a home-based care centre).
Subjects: A total of 386 stool specimens from suspected AIDS patients from the two health institutions plus 81 stool specimens from clinically healthy patients were collected. The number of patients from MCU was 144 (49 females, 95 males) and 242 from BRIDH (119 males, 123 females).
Main Outcome Measure: The main goals were to determine optimum exposure time and optimum concentration of alkali used in decontamination and to identify the culture medium with the best recovery rates of atypical mycobacteria.
Results: Optimum recovery of atypical mycobacteria was achieved on Peizer TB medium after treating stool specimens with 4% sodium hydroxide for 35 minutes. In addition, the use of Kirchner's medium improved isolation rates, although with a slight increase in contamination at levels of 2.9%.
Conclusion: A stool specimen can be used to recover atypical mycobacteria in suspected AIDS patients. Recovery is achieved using Peizer TB medium at a concentration of 4%. Varying the exposure time of the stool specimen to the decontaminating alkali and incorporating antifungal agents and antibiotics into the medium, improves recovery of atypical mycobacteria.

Cent Afr J Med 2003;49(3/4):31-7

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Introduction

Atypical mycobacteria occasionally cause disease in animals and man.[1] They cause four main types of opportunistic mycobacterial disease. These include skin lesions, localised lymphadenitis, tuberculosis-like pulmonary lesions and disseminated disease.[2]

Infection with Mycobacterium-avium complex (MAC) is extremely rare in immunocompetent individuals; by 1980, only 24 cases had been reported in the medical literature.[3] Beginning in 1982, when the infection was recognised in Acquired Immunodeficiency Syndrome (AIDS) patients, the number of cases increased dramatically.[4] The AIDS epidemic has therefore, contributed to the recognition of other species of mycobacteria in human infection such as *M. haemophilum*[5] and *M. genavense* in developed countries[6] and *M. kansasii* and *M. scrofulaceum* in Human Immunnodeficiency Virus (HIV) positive individuals in sub-Saharan Africa.[7]

Studies have shown that those individuals with disseminated and gastro-intestinal MAC infection, often suffer from abdominal pain, diarrhoea, wasting, night sweats, fever or chills.[8,9]

Most stool specimens submitted for culture of atypical mycobacteria contain fungal and bacterial contaminants. Antifungal and antibacterial agents can be incorporated into the culture medium to reduce these contaminants.[1] Isoniazid-containing Lowenstein-Jensen (L-J) medium was found to increase the primary isolation of mycobacteria in one study.[10]

The media for cultivation of mycobacteria from clinical specimens can either be solid or liquid.[11] The solid media used in the present study namely L-J and Peizer TB media, were selected due to their potential to allow characteristic, reproducible colonial morphology to develop from small inocula.[12]

Decontamination is one technique used to promote recovery of atypical mycobacteria when processing specimens from non-sterile sites. The chemical agent used, selectively destroys organisms other than mycobacteria which are resistant. Egg-based media are more suited for decontamination methods than agar-based ones when using strong acids or alkalis.[13]

The time the specimen is exposed to the chemicals can be varied to obtain one that allows maximum recovery of atypical mycobacteria while keeping contaminants at low levels.

There are a variety of decontamination methods available in the literature. The N-acetyl-L-cysteine sodium hydroxide method used for sputum is regarded as the "gold standard" .[14] Since then little practical improvement has been made in the methods of digestion and concentration to destroy contaminating organisms in the population of tubercle bacilli.

Rationale.

Methods of recovering mycobacteria from stools seem to lag behind in improvement, compared to recovery from gastric lavage, intestinal biopsies, bronchial washings, blood and aspirates of the bone marrow, probably due to high contamination rates. The recovery of atypical mycobacteria in gastro-intestinal infection has increased in significance due to the increase in patients with AIDS.[9] There has been little recent practical improvement made in methods of decontamination and concentration of the bacilli.[14]

The aim of this study, therefore, was to develop decontamination methods, which improve recovery of atypical mycobacteria from the stool specimens of AIDS patients. This would allow determination of the proportion of suspected AIDS patients with atypical mycobacteria in their stools.

Materials and Methods

Specimen Collection.

A total of 386 suspected AIDS patients were recruited from Beatrice Road Infectious Disease Hospital (BRIDH) (242 patients) and Mashambanzou Care Unit (MCU) (144 patients). The patients selected were those showing clinical signs and symptoms suggestive of AIDS and fulfilled the 1987 Revised Case Definition for AIDS[15] which compares favourably with the 1993 Revised Classification System16 with the exception of the former's lack of emphasis on CD4+ T-lymphocyte count and exclusion of invasive cervical cancer. The HIV positive patients had, among other conditions, undiagnosed abdominal pain, diarrhoea, wasting, night sweats and fever among other symptoms and signs.[9] The patients were selected with the assistance of a qualified clinical matron. A collection form accompanied each specimen. An additional 81 stool specimens submitted to a diagnostic laboratory from clinically healthy workers showing none of the conditions used in the selection criteria for HIV positive patients, served as controls. The stools obtained were either formed, semi-solid or liquid. A single stool specimen was obtained from each patient. The Medical Superintendent of BRIDH and the Sister-in-Charge of MCU approved the study. Informed written consent was obtained from each patient.

Specimen Processing.

The processing of the stool specimen was done in the safety cabinet of the Department of Medical Microbiology Laboratory, University of Zimbabwe. All specimens were decontaminated by a modified sodium hydroxide method.

The sample from a patient was subdivided into 18 aliquots. Each aliquot was placed in a separate Vac-U-glass tube with a rubber stopper. Six tubes contained 2ml of 1% sodium hydroxide. Another batch of six tubes contained 2ml of 2% sodium hydroxide and the last batch of six tubes had each 2ml of 4% sodium hydroxide. Each tube was thoroughly shaken for a few seconds to mix the alkali and the stool specimen. A tube from each concentration was allowed to stand for time intervals of 20, 25, 30, 35, 40 and 45 minutes at room temperature. The time included the few seconds taken for shaking the mixture. After each interval, each mixture was neutralised with sterile distilled water or phosphate buffer added to reach

within 1cm of the top of the tube. The tube was inverted to mix the solutions and stop the decontaminating process.

The tubes were centrifuged at 3 000g for 15 minutes. The supernatant was discarded, leaving the sediment. Smears were prepared from the sediments of the specimen for Ziehl-Neelsen (ZN) acid-fast staining technique to detect any acid-fast bacilli.

The control specimens were treated with 4% NaOH for 35 minutes. This time the concentration had shown good recovery of mycobacteria after a series of pre-trials.

Preparation of Media.

Lowenstein-Jensen Medium: This medium was prepared according to routine instructions without modifications.

Peizer TB Medium: The medium base was prepared according to the manufacturer's instructions. After sterilising and cooling to 45 to 50°C, the contents of eight whole eggs were added and mixed thoroughly.

Kirchner's TB Medium: The medium was mixed according to the manufacturer's instructions before dispensing as 9ml volumes into McCartney bottles. The bottles were sterilised at 121°C for 10 minutes before use. One millilitre inactivated sheep serum and selective agents shown in Table II were added. From the decontaminated stool sediments processed with 4% sodium hydroxide (NaOH) for 35 minutes, a loopful was added directly to the Kirchner's liquid medium. The bottles were then incubated aerobically at 37°C until growth appeared. The growth that appeared was used to prepare smears for ZN staining and presumptive identification based on growth characteristics done. Subculture was then done on Peizer TB medium after growth appeared.

Inoculation.

Initial inoculation of part of the sediment was done on Lowenstein-Jensen medium (120 specimens) and Peizer TB medium (120 specimens) with no antifungal or antibacterial agents incorporated. The specimens had been decontaminated with 4% NaOH and exposed for 30 and 35 minutes. Inoculation was done by applying a loopful of the sediment on the solid media in petri dishes. The plates were incubated at 37°C in plastic bags and read two days after inoculation to note growth of any contaminants. The plates were examined for any contaminants almost daily thereafter.

If characteristic mycobacterial colonies grew before overgrowth of contaminants and were ZN positive, the plate was regarded as positive and subcultured onto fresh media. The contaminants that grew were identified. Subsequent media preparation was then done incorporating carbenicillin at 50mg/ml, Amphotericin B at 10mg/ml and nystatin at 10mg/ml.

The selective agents were dissolved in 9ml of acetone in a vial and added to media cooled to below 40°C after autoclaving. Inoculations using sediments previously used on plain media were repeated on media containing selective agents. Plates were kept for six weeks before being discarded as negative.[14] If distinct colonies typical of mycobacteria appeared, they were confirmed using ZN staining and subcultured so that the time of their appearance and colonial morphology could be noted. Subsequent biochemical identification using the niacin, nitrate reduction, catalase and urease tests among others, was done using the subcultures. These were done as indicated in the literature[14] although for the niacin test, an incubation period of 40 minutes was used, instead of 15 to 30 minutes, before use of the test strip to achieve stronger reaction. This made it unnecessary to repeat the test on negative cultures.

Presumptive Identification.

Besides ZN staining and colonial morphology, presumptive identification was done by examining for pigment production after exposing the isolates to light for at least 24 hours.

Safety Considerations.

The high-risk specimens were covered in double layered khakhi paper and boxed with hazard warning labels. Specimen processing was done in a biosafety laminar flow Class 2 cabinet (Heranes, Hanan Germany).

Results

Patients involved in the study.

The total number of patients who submitted stool specimens from the two institutions was 386 as shown in Table I.

Table I: Distribution of HIV positive patients recruited.

	Number	Mean Age (in years)	Age Range (in years)
Patients from BRIDH			
Males	119	35.1	20-67
Female	123	30.3	20-61
Patients from MCU			
Males	49	36.0	12-49
Female	95	30.6	13-44
Total	**38.6**	**32.6**	**12-67**

Culture and ZN smear results.

The number of Ziehl Neelsen (ZN) smear positive specimens examined for acid fast bacilli (AFB) were recorded. Enumeration of AFBs seen was regarded as rare (+) for one to two bacilli seen per smear, few (++) for three to nine bacilli per smear and numerous (+++) for 10 or more bacilli seen. Twenty seven ZN smear positive results were found to be in the rare category, 42 were few and 68 were numerous, giving a total of 137. The smear positive specimens represented 35.5% of all specimens collected. Smear positive/culture negative and smear positive/culture positive sediments are summarised as shown in Table II.

Table II: Smear / culture results.

Isolates	Smear negative/ Culture positive	Smear positive/ Culture positive
M. tuberculosis	23	37
Atypical mycobacteria	10	27
Total	**33**	**64**

There were more *M. tuberculosis* isolates (57.8%) than atypical mycobacteria isolates (42.2%) obtained. All smear positive and culture positive specimens constituted 16.6% of all the 386 specimens submitted for culture. Some specimens, however, turned out to be culture positive despite being smear negative by ZN staining.

The overall number of culture positive isolates (smear negative and smear positive) was 97. This was 24.9% of all specimens processed. Any association between smear status and type of mycobacteria isolated from culture was determined using the Chi-squared test. There was no association between isolation of *M. tuberculosis* and atypical mycobacteria from smear positive and smear negative sediments (p=0.2536). Of the total number of specimens obtained therefore, *M. tuberculosis* constituted 19.2% and atypical mycobacteria constituted 9.6%.

Isolates obtained from patients.

A number of biochemical tests, growth rate and pigmentation characteristics were used to identify the atypical mycobacteria, up to complex level. Based on pigmentation, 30 non-chromogens, three photochromogens and four scotochromogens were obtained. Table III shows the distribution of isolates identified biochemically.

Table III: Isolates of atypical mycobacteria identified by biochemical testing.

Species	No. of Isolates
MAIC	19
M. kansasii	3
M. malmonse	4
M. xenopi	4
Inconclusive	7
Total	**37**

The highest number of isolates obtained was from the MAI complex that contributed to a large number of non-chromogenic isolates. Seven isolates could not be identified with certainty beyond the fact that they were non chromogenic but not *M. tuberculosis*.

The patients from whom the isolates were obtained (females n = 25, males n = 12) had an average age of 34.1 years. Twenty seven (73%) of the specimens from which isolates were obtained came from BRIDH. The modal range of length of illness was one to five years with 18 patients. Those with a length of illness of less than one year were 16 whereas those with a length of illness of between six and 10 years were three.

Of the patients from whom the isolates were obtained, 33 (89.2%) submitted diarrhoeal stools and all 37 complained of gastro-intestinal infections at some stage during their illness. A number of patients recruited were on TB drugs i.e. 255 (66.1%). Among the patients from whose specimens isolates were obtained, 16 were actually on TB drugs, namely ethambutol, rifampicin, pyrazinamide, isoniazid and / or streptomycin. The other nine were on some other drug regimen for illness other than TB.

The specimens from 181 clinically healthy control patients, showing none of the clinical conditions contained in the 1993 Revised Classification for HIV infection, yielded three isolates (1.7%) of which one belonged to the *M. terrae* complex and two were MAIC.

Decontamination results.

Contamination after exposing a specimen for 20 minutes was so high that subsequent treatment was done starting at 25 minutes. One isolate treated with 4% sodium hydroxide was obtained after 20 minutes exposure.

Preliminary specimen processing on both plain media and that containing selective agents showed that treatment with 1% sodium hydroxide resulted in quite high levels of contamination rates (>45 inoculations contaminated for every 50 done). It was then decided to treat the stool specimens at two different concentrations of sodium hydroxide of 2% and 4%. Table IV shows the number of isolates obtained at different combinations of concentrations and times of exposure to sodium hydroxide.

Table IV: Number of isolates obtained at different NaOH concentrations and duration of exposure times.

Time in minutes	2%	4%
25	4	2
30	12	10
35	11	15
40	11	8
45	5	3

The time of exposure that resulted in the highest total number of isolates was 35 minutes. At a concentration of 2% however, the number of isolates was slightly higher at 30 minutes exposure than at 35 and 40 minutes. A short time of exposure of 25 minutes and that of 45 minutes were not optimal for high recovery of isolates at both concentrations. The total number of isolates obtained at each concentration was not significantly different (p=0.1453).

The combination of time and concentration that gave the highest number of isolates was 4% for 35 minutes. The total number of isolates obtained in this case was 81 (excluding one isolate obtained at 4% NaOH after exposing the specimen for 20 minutes). This included isolates that were obtained from specimens submitted by a single patient. The isolates excluded those that turned out to be *M. tuberculosis*. There were no instances where more than one type of isolate was obtained from a specimen submitted by one patient.

Recovery with respect to the type of media and level of contamination.

The level of contamination on plain LJ media and Peizer TB media was evaluated using 120 inoculations on each media. The percentages of the inoculations that resulted in contamination were recorded and are shown in Table V.

Table V: Percentage contaminated inoculations on plain media.

	LJ medium		Peizer TB medium	
Time of exposure (min)	30	35	30	35
Conc. of Na OH				
2%	80.0	60.0	63.3	56.7
4%	73.3	53.3	46.7	50.0

The x^2 tests (p=0.9458 and p=0.7182) performed on the original results (not percentages) in Table V, failed to establish any association between time of exposure and concentration of sodium hydroxide. There was a higher percentage of contaminated inoculations on LJ medium than Peizer TB medium at both concentrations of sodium hydroxide and at both times of exposure. The results were then compared with those obtained using media in which selective agents had been incorporated and whose results are shown in Table VI.

Table VI: Percentage contaminated inoculations on L-J and Peizer TB media with selective agents.

Time of exposure	25	30	35	40	45
(min)/Conc. of NaOH					
(on L-J)					
2%	73.1	67.6	60.6	48.4	47.9
4%	69.2	57.8	52.6	46.9	42.2
(on Peizer TB)					
2%	63.2	61.4	50.8	39.6	30.0
4%^	53.9	50.3	39.1	28.0	25.4

The results in Table VI show that contamination was higher in LJ media than in Peizer TB media with selective agents at the two NaOH concentrations and at all times of exposure to alkali. This was the case on nonselective media. Another observation was that use of selective agents in media resulted in a lower percentage of inoculations that were contaminated compared to those done on plain media.

No attempts were made to isolate mycobacteria from nonselective media. The main purpose of this media was to allow comparison of levels of contamination.

Of the 386 specimens inoculated on Kirchner's medium, and cultured on Peizer TB medium, 60 (15.5%) were culture positive for mycobacteria. Twelve specimens which had been grown on LJ or Peizer TB medium and had been culture negative, grew on the latter medium after incubation in Kirchner's medium. Kirchner's medium increased isolation by 12.4%. Isolates of mycobacteria recovered from LJ (n=21) were less compared to those from Peizer TB medium (n=39).

Although Kirchner's medium improved recovery, it resulted in high inoculations that were contaminated on Peizer TB medium compared to direct inoculation. Direct inoculation at 4%, 35 minutes exposure was 39.1% (Table VI) compared to 42.0% after incubation in Kirchner's medium.

Common contaminants encountered.

Identification of the contaminants present in the stool samples was quite problematic, especially at 2% sodium hydroxide concentration. Gram stain of the colonies that grew on the media revealed plenty of gram negative bacilli that were differentiated on the basis of lactose fermentation on MacConkey agar. Many, however, appeared thin, gram negative bacilli and were found to be *Campylobacter* species. Many clumps of gram positive cocci were found to be *Staphylococcus* species. There were also some gram positive bacilli of *Clostridium* species. The most common contaminant on media containing selective agents was a non-lactose fermenter that produced beta haemolysis on blood agar and was found to be a pseudomonad species. The other one was a lactose fermenter that produced mucoid colonies on both LJ and Peizer TB media. This was a *Klebsiella* species.

Besides bacterial contamination there were also both endogenous and exogenous fungal contamination of the media. There was one problematic yeast, which formed wrinkled colonies that spread quickly, especially on L-J media. Some gram stained smears revealed budding yeast cells of *Candida* species.

Discussion

Outcome of results with regards to patients and specimen processing.

The number of specimens that turned out to be culture positive for atypical mycobacteria (4.9%) was lower compared to other studies where up to 53% of patients with AIDS may have disseminated infection.[8] However, this was higher than those recovered from control patients (1.7%). The proportion of patients whose specimens were smear positive but culture negative was higher at 4% NaOH compared to those at 2% NaOH. Increased concentration of the decontaminant reduced the number of viable bacilli in the specimen and showed the harmful effects of this suppressive agent. It is, therefore, possible to regard some patients as being negative for atypical mycobacteria based on culture results alone if the concentration of decontaminant used to process their stools is high.

The low number of culture positive results suggests the critical nature of the balance required between the time the specimen is exposed to a decontaminant and the concentration of that decontaminant. The high levels of contamination (Tables IV to VI) which did not allow slow growers to appear contributed to the low number. If specimens were taken from patients several times and for at least a month, this may have increased the yield.

Most patients in the study (especially from BRIDH) were on antituberculous therapy that included rifampicin. This therapy could have eliminated some of the mycobacteria in vivo and reduced their recovery from stools.

The length of illness, unexpectedly did not result in an increase in the number of positive cultures. A small

proportion of patients recruited had had illness of a gastro-intestinal nature for more than six years. The proportion of isolates that could have been obtained from this group could not be easily ascertained. It is believed, however, that few patients with AIDS and MAC bacteremia live for more than seven months after MAC infection.[17]

Most patients from whom isolates were culture positive provided diarrhoeal stool specimens. This suggested that intestinal infection with MAIC in patients with AIDS manifests as diarrhoea.[18]

Higher *M. tuberculosis* culture positive results than those of atypical mycobacteria (Table III) were obtained. This was expected as most patients recruited in this study had been diagnosed and were being treated for tuberculosis. Some had pulmonary TB whereas others had disseminated TB. M. *tuberculosis* produced quite distinct colonies compared to those of atypical mycobacteria.

The patients recruited for this study, mainly from BRIDH, were selected based on presumptive identification. Less than 4% of those from MCU were not lab-confirmed HIV positives. The rest of the patients from this unit were confirmed, although permission to access their medical records showing their HIV status was not granted. There is a possibility that some patients included might not have been suffering from AIDS and hence were less likely to be suffering from infection by atypical mycobacteria. Patients could have been tested to confirm their HIV status and their CD4 T cell counts determined. A CD4 lymphocyte number less than 200 cells/mL of blood is known to be associated with MAIC infection.[19]

Choice of specimen.

Use of a stool specimen presented a number of drawbacks especially due to high contamination levels (Tables V and VI). This was expected, as the patients from whom the specimens were collected were immunosuppressed. It was probably better to use a specimen from a sterile site as recommended by previous researchers.[17] Blood was risky to handle since AIDS patients were involved and no blood handling facilities were available for use. Culture remains quite useful as serological methods can fail if an antibody response is not mounted, as is often the case in AIDS patients.[20]

Assessment of media used.

Use of a combination of liquid medium and a minimum of two solid media to culture stool enhanced recovery of mycobacteria by 20%. This finding is comparable to those obtained by other reseachers.[21] Cultures in this study and from previous ones,[21] had high contamination. The researchers recommended the use of MB-Check and BACTEC systems in addition to conventional culture methods. These gave an earlier detection of mycobacteria than conventional culture methods.

Peizer TB medium allowed less contamination and was more stable than LJ medium. This was probably due to its simple formulation that did not have glycerol and asparagine. These could have supported glycerophilic organisms and those that require a nitrogen source respectively. Peizer TB medium was more useful for isolating slow growing atypical mycobacteria and *M. tuberculosis.*

The increase in positive cultures after using Kirchner's medium suggests that it allowed even a small inoculum of viable bacilli to multiply during incubation. The concentrations of selection agents used reduced contamination levels. Different concentrations of each selective agent could have been used. In other studies,[22] reduced concentration of penicillin was used with some success. The use of antimicrobials remains of great value in reducing contamination rates in cultures inoculated with faeces and urines.[23]

The use of Kirchner's medium in combination with LJ medium was not evaluated in this study. It could have resulted in an increase in yielding positive cultures as was obtained by other reseachers.[23]

Assessment of decontamination procedure used.

Varying the time of exposure and concentration of sodium hydroxide had an effect on both recovery levels of isolates and contamination levels. No association between concentration and time of exposure could be established using the Chi-squared test on both solid media. Other factors could have affected contamination levels such as number of living micro-organisms in the original specimen.

A low number of isolates was obtained after exposing the specimen to sodium hydroxide for a short time. This did not imply that atypical mycobacteria were absent in the specimen. The media was overgrown by contaminants before the mycobacteria had grown. Some specimens from the same patient were culture positive after a longer time of exposure. The reverse was the case with the concentration of sodium hydroxide. Lower concentration overally produced slightly more isolates than at higher concentration (Table IV). This might have been due to toxic effects of 4% treatment that reduced the number of viable bacilli in the specimen. This was also affected by the length of time the specimen was exposed to sodium hydroxide. At 25 minutes, a smaller number of positive cultures were obtained than at 30 or 35 minutes because some positive cultures could have been missed after being overgrown by fast growing contaminants (<2 days) compared to the atypical mycobacteria (≥ 4 days). The disadvantages of using plates instead of slopes were high exogenous fungal contamination and failure of media to solidify after heating in some instances. There was also increased risk of infection from *M. tuberculosis* to the experimenter.

Conclusion

Stool specimens from suspected AIDS patients could be used to isolate atypical mycobacteria. This was possible despite some patients being on antituberculous therapy, which is believed to reduce mycobacterial counts. The combination of sodium hydroxide as a decontaminant and culture media incorporating selective antimicrobial agents

had limited success in isolating mycobacteria. This was probably due to high levels of contamination of the stool specimens from immunosuppressed patients. This might contribute to underestimation of the actual number of patients infected with atypical mycobacteria in a population. Peizer TB medium was shown to be a better solid medium than LJ medium with regards to both recovery of isolates and prevention of overgrowth by contaminating flora. Among the atypical mycobacteria isolated MAIC was the most commonly isolated.

Acknowledgements

The authors would like to give sincere thanks to staff at Mashambanzou Care Unit and Beatrice Road Infectious Disease Hospital for assistance in specimen collection. Further thanks go to the Department of Medical Microbiology technical staff.

References

1. Grange JM Mycobacterial diseases. Edward Arnold Ltd;1980.
2. Collins CH, Grange JM, Noble WC, Yates MD. *Mycobacterium marinum* with infections in man. *J Hyg* 1985;94:135-49.
3. Horsburgh CR, Mason UG III, Farhi DC, Iseman MD. Disseminated infection with *Mycobacterium avium intracellulare*. *Medicine* (Baltimore) 1985; 64:36-48.
4. Horsburgh CR. *Mycobacterium avium* complex infection in the acquired immunodeficiency syndrome. *N Engl J Med* 1992;324:1332-8.
5. Kiehn TE. *Mycobacterium haemophilum* : a new opportunistic pathogen. *Clin Microbiol Newsletter* 1992;14:81.
6. Coyle MB, Carlson LC Wallis CK. Laboratory aspects of "*Mycobacterium genavense*", a proposed e species isolated from AIDS patients. *J Clin Microbiol* 1992;30:3026.
7. Corbett EL, Blumberg L, Churchyard GJ, Moloi N, Mallory K, Clayton T. Non tuberculous mycobacteria: defining disease in prospective cohort of South African miners. *Am J Resp Crit Care Med*1999;160(1):15-21.
8. Hawkins CC, Gold JWM, Whimbey E, Kiehn TE, Brannon P, Cammarata R *et al. Mycobacterium avium* complex infections in patients with the acquired immunodeficiency syndrome. *Ann Int Med* 1986;105:184-8.
9. Gray JR, Rabeneck L. Atypical mycobacterial infection of the gastrointestinal tract in AIDS patients. *Am J Gastroenterol* 1989;84:1521-4.
10. Miorner H, Olsson B. Improved isolation of mycobacteria other than *Mycobacterium tuberculosis* on Isoniazid-containing Lowenstein-Jensen medium. *Euro J Clin Microbiol Infect Dis* 1988;7:47-9.
11. Baron EJ, Peterson LR, Finegold SM. Baily and Scott's Diagnostic Microbiology. 9th ed. 1994;590-633.
12. Mitchison DA, Allen BW, Manickavasagar D. Selective Kirchner's medium in the culture of specimens other than sputum for mycobacteria. *J Clin Pathol* 1983;36:1357-61.
13. Jenkins PA, Pattyn SR, Portaels F. Diagnostic bacteriology in the biology of Mycobacteria. Vol. I. Ratledge C, Stanford J, editors. 1982;442-70.
14. Kubica GP, Dye WE, Cohen ML Middlebrook G. Sputum digestion and concentration with N-acetyl-L-cysteine sodium hydroxide for culture of mycobacteria. *Am Rev Resp Dis* 1963;87:775.
15. Centers for Disease Control. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. *MMWE* 1987;36:3s-15s.
16. Centers for Disease Control and Prevention. 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS among adolescents and adults. *MMWR* 1992;41:1-17.
17. Young LJ, Inderlied CB, Berlin OGW Gottlieb MS. Mycobacteria infections in AIDS patients, with an emphasis on the *Mycobacterium avium* complex. *Rev Infect Dis* 1986;8:1024.
18. Damsker B. *Mycobacterium avium–Mycobacterium intracellulare* from intestinal tracts of patients with Aquired Immunodeficiency Syndrome. *J Infect Dis* 1985;151:179-81.
19. Nightingale SD, Byrd LT, Southern PM, Jockusch FJ, Cal SX, Wynne BA. *Mycobacterium avium – intracellulare* complex bacteremia in human immunodeficiency virus positive patients. *J Infect Dis* 1992;165:1082-5.
20. Pierce PF, De Young DR, Roberts GD. Mycobacteremia and the new blood culture systems. *Ann Int Med* 1983;99:786-9.
21. Abe C, Hosojima SM, Fukasawa Y, Kazumi Y, Takahashi M, Hirano K, Mori T. Comparison of MB-Check, BACTEC and Egg-Based Media for recovery of mycobacteria. *J Clin Microbiol* 1992;30(4):878-81.
22. McClatchy JK, Waggoner RF, Kanes W Cernich, MS, Bolton TL. Isolation of mycobacteria from clinical specimens by use of selective 7H medium. *Am J Clin Pathol* 1976;65:412-5.
23. Mitchison DA, Aber VR. Culture of specimens other than sputum for *Mycobacteria*. *J Clin Pathol* 1974;27:883-7.