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COMPARISON OF N-ACETYL-L-CYSTEINE AND SULFURIC ACID DECONTAMINATION METHODS FOR RECOVERY OF MYCOBACTERIA

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Comparison of N-Acetyl-L-Cysteine and Sulfuric Acid Decontamination Methods for Recovery of Mycobacteria

Pawan Kumar

Research Scholar

Abstract – The most frequently isolated NTM were Mycobacterium lentiflavum and Mycobacterium intracellulare. Comparing the two decontamination methods the recovery of NTM in the sulfuric acid group was significant higher than in the NaOH-NALC group (p=0.001). In contrast, no significant difference was found for the recovery. These results show that the decontamination method used affects the recovery of nontuberculous mycobacteria in particular. These considerations have prompted us to compare the effect of these two decontamination procedures on the sensitivity of the detection of M. tuberculosis complex and NTM in sputum samples. The study was carried out in two rural hospitals HIV prevalence was approximately 20%. In the present study we found a high rate of positive cultures for mycobacteria. Of the 261 isolates positive for mycobacteria 62 (24%) were identified as M. tuberculosis and 139 (53%) were identified as NTM. Significantly more NTM were recovered by the sulfuric acid decontamination method than by the NaOH-NALC method (84 versus 55 respectively). There was no significant difference between the sulfuric acid decontamination method and the NaOH-NALC method.

Keywords: NALC, Sulfuric Acid, Decontamination, Method, NTM, Cultures, Hospitals

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INTRODUCTION

The collection and processing of specimens was done in a way to avoid cross contamination. All disposables needed were imported from The Netherlands and for sterilization the autoclave of the hospital was used. The restriction fragment length polymorphism (RFLP) patterns of the isolated M. tuberculosis strains were almost all different (data not shown) virtually excluding the possibility of a cross contamination in the laboratory in Zambia. Furthermore, to assure that the isolated NTM were not contaminants introduced in the laboratory, sputum specimens (stored at −20°C) of 20 patients were checked and confirmed positive for mycobacteria by amplified rDNA restriction analysis (4). In addition AFLP DNA typing was performed from 10 M. lentiflavum strains that showed that different strains were isolated and therefore a laboratory contamination could be considered unlikely (1). Although not all M. lentiflavum isolates have clinical significance the first case of human disease was a patient with spondylodiscitis who markedly improved on multiple ant tuberculous agents (2). Other clinical cases were reported later: four cases of cervical lymphadenitis, a case of cavitary pulmonary disease, one disseminated infection in a woman undergoing steroid therapy, a disseminated infection and hepatic abscess in an AIDS patient (3,6,7,5,8). Both decontamination methods eliminate most of the contaminating bacteria in the sputum specimen but also reduce the viability of the mycobacteria in the specimen. The alkaline or acid environment is toxic not only to contaminating organisms but to mycobacteria as well. In the present study a complete shift is observed in the ratio of M. intracellulare and M. lentiflavum. In the NaOH-NALC method the ratio is 17/7 and in the sulfuric acid method the ratio is 13/26. The cell viability of different species of mycobacteria is probably not the same for various decontamination methods. The effect of genetic variation of mycobacteria on the sensitivity to the decontamination process is probably another reason (9).

REVIEW OF LITERATURE:

In the present study NTM were apparently more susceptible to killing by Na OH. The final concentrations for Na OH (1.7%) and sulfuric acid (3.0%) employed in this study were higher than the recommended concentrations (1.0% and 2.0% respectively). These concentrations were according to protocols used in The Netherlands because of a higher contamination rate of the sputum. This might be another reason for the different percentage of recovery of NTM between Na OH-NALC and sulfuric acid decontamination. The type of decontamination method applied definitely influences the recovery of

mycobacteria, NTM in particular. This has clear consequences for the quality of both microbiological diagnosis and treatment of patients. More research is needed for the evaluation of the clinical relevance of the various NTM isolated.

- **Materials and methods:**

The medical officer in charge documented the medical history and a general physical examination was performed. A daily follow-up of signs and symptoms was done. A sputum specimen was cultured for mycobacteria. The sputum was divided into two equal parts. One half was decontaminated using 6% sulphuric acid and cultured on Löwenstein- Jensen (LJ) medium and the other half was stored at −20°C in Zambia. In The Netherlands the latter half was decontaminated with N-acetyl-l-cysteine (NALC)-NaOH and cultured in Mycobacteria Growth Indicator tubes (Becton Dickinson Microbiology Systems, Cockeysville, Md.) (6). Serological testing for HIV was performed using a particle agglutination test (Serodia HIV1/2, Fujirebio Inc., Tokyo, Japan) and the AxSYM HIV Ag-Ab Combination Assay (Abbott GmbH Diagnostika, Wiebaden-Delkenheim, Germany). Mycobacterial isolates were identified by the Accuprobe culture confirmation test for the M. tuberculosis complex (Accuprobe, bioMérieux, Marcy l'Etoile, France) or by 16S rRNA gene sequencing (7). and subjected to culture in Mycobacteria Growth Indicator tubes (Becton Dickinson Microbiology Systems, Cockeysville, Md.). Decontamination of the sputum was done using N-acetyl-l-cysteine (NALC)-NaOH and 6% sulfuric acid after dividing the sputum specimen into two equal parts to compare decontamination procedures for the detection of mycobacteria (4). In St. Francis Hospital in Katete and Yeta District Hospital in Sesheke, sputum from patients was collected and cultured on two consecutive days. In Our Lady's Hospital in Chilonga only a single sputum was collected and cultured because of logistic reasons. Serological testing for HIV was performed using a qualitative immunoassay (Abbott Determine HIV-1/2) and the Vidas HIV DUO assay (bioMérieux, Marcy l'Etoile, France). Chest X-rays were taken and evaluated blind in The Netherlands. The Accuprobe culture confirmation test for M. tuberculosis complex (Accuprobe, bioMérieux, Marcy l'Etoile, France) and/or 16S rRNA gene sequencing were used to identify mycobacterial isolates (10).

- **Laboratory methods:**

Sputum or gargle specimens were divided into two equal parts: one half was decontaminated with N-acetyl-l-cysteine (NALC)-NaOH and the other half was decontaminated using 6% sulfuric acid in order to compare decontamination procedures for the culture of mycobacteria (12). The specimens were cultured in Mycobacteria Growth Indicator tubes (Becton Dickinson Microbiology Systems, Cockeysville, Md., US) according to the manufacturer's instructions and the guidelines described previously by Master (11). Mycobacterial isolates were identified by the Accuprobe culture confirmation test for the M. tuberculosis complex (Accuprobe, bioMérieux, Marcy l'Etoile, France) or by 16S rRNA gene sequencing (10). Serological testing for HIV was performed using a qualitative immunoassay (Abbott Determine HIV-1/2) and the Vidas HIV DUO assay (bioMérieux, Marcy l'Etoile, France). Materials needed for collection and culture were imported from The Netherlands, and an experienced Dutch technician performed the work in the Zambian laboratory. All possible efforts were made to prevent laboratory cross-contamination, including the collection of a sputum specimen in a brand-new container imported from The Netherlands. The stock of decontamination fluid was sterilized twice a week, and each day a fresh, sterile tube or bottle was opened. The samples in the laboratory were processed one by one, away from other samples, and negative control cultures were included in each batch of samples. Microscopic slides were also prepared one at a time. All work associated with this project was conducted by the same experienced laboratory technician. All culture procedures were performed in a new class I biosafety cabinet.

CONCLUSION:

In none of the eight patients diagnosed with NTM infection of normally sterile body sites was the diagnosis made before hospital discharge or death. No specific therapy was given for the mycobacteria isolated, except for patient 2, who received empirical treatment with rifampin, isoniazid, and pyrazinamide before the results of the culture became available. In five of the six specimens of sterile body sites, which were decontaminated with sulfuric acid and NALC-NaOH, NTM were cultured only after decontamination with sulfuric acid. However, the commonly used decontamination method is the use of NALC-NaOH. It has been shown that the decontamination method used affects the recovery of NTM. Culture is considered the gold standard for the diagnosis of tuberculosis, but most clinical sputum samples contain a variety of micro-organisms that may overgrow M. tuberculosis. Decontamination of these samples is therefore crucial in preventing contamination of the mycobacterial culture. However, decontamination inhibits the recovery of mycobacteria. In the study described , we compared the effect of two decontamination procedures, NaOH-N-actetyl cysteine (NaOH-NALC) and sulphuric acid, on the sensitivity to detect M. tuberculosis complex and NTM in sputum samples. The recovery of NTM in the sulphuric acid group was significant higher than in the NaOH-NALC group (p = 0.001). In contrast, no significant difference was found for the recovery of M. tuberculosis.

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