Detection of Multidrug Resistance Genes of Mycobacterium tuberculosis among Patients referred to the NTBRL

Reham A. S. Hamedto¹, Elamin I. Elnima¹ & Elsheik A. Elobied²

¹Department of Pharmaceutics, Faculty of Pharmacy, University of Khartoum.
²Ahfad University for Women, College of Pharmacy, Khartoum, Sudan.

Introduction

The spread of multidrug resistant (MDR) strains of Mycobacterium tuberculosis has become a major public health concern as these bacteria often cause incurable disease, even when expensive second and third line drugs are available.

Objective

This study aimed to identify M. tuberculosis among suspected tuberculosis patients in Khartoum State by using conventional methods also to identify rifampicin and isoniazid resistant M. tuberculosis by amplifying (rpo B and kat G, kasA) genes respectively, using polymerase chain reaction (PCR).

Method

Verbal consent of patients participated in the current study was taken from every participant and then epidemiological data were collected. Two hundred and fifty sputum samples were collected from suspected tuberculosis patients. Direct smears were performed using Ziehl-Neelsen stain, then all positive sputum samples were inoculated on Lowenstein Jensen medium and inoculated aerobically at 37°C. selected biochemical tests were performed to all Mycobacterium spp. Drug sensitivity testing were performed to all isolates, IS6110 primers were used to confirm the Mycobacterium tuberculosis type and PCR was used for all species to identify resistant gene to rifampicin, (rpoB gene) and isoniazid (katG and kasA genes).

Results

The results of ZN showed that 91 isolates (36.4%) were positive to AFB, and 83(91.2%) of them showed growth of Mycobacterium spp. On the other hand 159 (63.6%) were negative, and 40 (25.1%) of them showed growth of mycobacteria.

According to the results of biochemical tests all strains were identified as Mycobacterium tuberculosis complex and concordant results were obtained by molecular technique using IS6110.

One hundred twenty three clinical isolates were subjected to drug susceptibility testing using proportion method. 45/123 (18%) were MDR, 45(18%) were sensitive to all 1st line anti tuberculosis drugs, 18(7.2%) were resistant to streptomycin, and 15(6%) showed triple resistance to isoniazid, ethambutol and streptomycin.

Forty five resistant isolates were subjected to PCR searching for rifampicin resistance genes. The
results showed the presence of expected mutant genes in 43(95.5%) of rifampicin resistant, while isoniazid resistance genes were found in 42(93.3%) of katG genes and 1(2.2%) due to kas gene only while other 4(8.8%) isolates harbour kasA and katG genes in the same strains that have two points of mutation.

**Conclusion and recommendations**

The study revealed that the molecular technique was less sensitive than conventional technique in detection of multidrug resistance. However molecular technique is not time consuming, rapid, reproducible and it may replace conventional methods in the future due to low sensitivity of Ziehl-Neelsen stain, and the long time consumed in culture and drug sensitivity test. It also represents rapid tools for diagnosis and detection of mutation to drugs. From this study it is clear that MDR TB is a serious problem in Sudan that needs special attention from the health authorities.

**Development of Derivative Spectrophotometric and HPLC Methods for Determination of Cefquinome sulphate and Niclosamide**

Shaza Wagiealla Shantier & Elrasheed Ahmed Gadkariem  
*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum*

Simple, sensitive and accurate spectrophotometric and HPLC methods were developed for the determination of cefquinome sulphate (CS) and niclosamide (NA) in bulk and dosage forms. The spectrophotometric method was based on the measurement of the first and second derivative spectra for the aqueous solution of cefquinome sulphate and the first derivative of the niclosamide methanolic solution at λmax 286nm, 300nm and 351nm, respectively. The HPLC-separation was conducted on Shimpac C-18 (25x4.6mm) column using suitable mobile phases consisted of 80:20 v/v ammonium acetate: acetonitrile and 70: 30 v/v methanol: water for the elution of cefquinome sulphate and niclosamide respectively. System suitability was assessed by measurement of factors affecting column efficiency. Beer’s law was applied over the concentration range 2-12µg/ml with a correlation coefficient not less than 0.999. The added recovery results were 100.1± 0.575% (n=3) for CS and 100.80 ± 0.59 (n=3) for NA, which indicates the absence of interference by the suspension and tablets excipients. The results obtained by the developed methods were statistically compared with those of reported methods and evaluated at 95% confidence limits.