Cross-resistance of *Mycobacterium tuberculosis* isolates among streptomycin, kanamycin and amikacin

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Received 8 December 2008

Seventy-four streptomycin (SM)-resistant *M. tuberculosis* clinical isolates were subjected to cross-resistance drug testing against two major aminoglycosides, kanamycin (KM) and amikacin (AMK). Among them, 15 clinical isolates (20.3%) were resistant to both KM and AMK. Fifteen (80%) of 19 KM-resistant isolates were AMK-resistant. Fifteen SM, KM, and AMK resistant isolates harbored *rrs* mutation, but only two had *rrs* and *rpsL* double mutations. Low-level SM resistance was associated with *rpsL* mutation, whereas high-level SM resistance was linked to *rrs* mutation.

**Keywords:** Amikacin, Cross-resistance, Kanamycin, MDR-TB, Streptomycin

Streptomycin (SM) is one of the aminoglycosides and is used as a first-line anti-tuberculosis (anti-TB) drug. Its structure is o-2-deoxy-2-(methylamino)-α-L-glucopyranosyl-(1→2)-o-5-deoxy-3-C-formyl-α-L-lyxofuranosyl-(1→4)-N,N’-bis(aminoiminomethyl)-D-streptamine. Earlier in a study, we used 115 streptomycin (SM)-resistant clinical isolates from Beijing, China, of which 85.2% harbored *rpsL* or *rrs* mutation, while *rpsL* mutation (76.5%) dominated among the isolates1. Among them, 45 clinical isolates were resistant to SM at more than 100 μg/mL and regarded as high-level SM-resistant. These 115 SM-resistant clinical isolates were collected from local farmers (treated previously with several anti-TB drugs) from all over China and sent to Beijing Tuberculosis and Lung Tumor Research Institute for further survey. There are reports that kanamycin (KM) and/or amikacin (AMK) are used as second-line anti-TB drugs for patients with MDR-TB and KM and AMK kill SM-resistant isolates2.

KM and AMK are aminoglycosides with the structures o-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-o-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→4)]-2-deoxy-D-streptamine and o-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-o-[6-amino-6-deoxy-α-D-glucopyranosyl-[1→4]]-N1-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-D-streptamine, respectively. It indicates that SM, KM and AMK have similar structures. Moreover, KM and AMK are used as second-line anti-TB drugs for patients with MDR-TB, but there is no report on cross-resistance among SM, KM and AMK based on large clinical samples3. In view of this, the present study was undertaken to elucidate the cross-resistance among SM, KM, and AMK.

Seventy-four streptomycin (SM)-resistant and 11 SM-sensitive clinical isolates of *M. tuberculosis* from China were considered for the study. The 74 patients with SM-resistant TB had been treated with several anti-TB drugs, but had no previous history of KM and AMK treatment. There were no mono-resistant SM clinical isolates. Several isolates did not grow well and hence, were omitted from this study. Minimum inhibitory concentrations (MICs) of SM, KM, and AMK were detected by absolute concentration method in L-J medium at 1, 2, 5, 10, 20, 40, 80, 100, 200, 400, 800 and 1,000 μg/mL (Ref. 1). Isolates with a MIC exceeding 10 μg/mL are defined as SM-resistant4,5. However, for KM and AMK resistance, MIC was 20 μg/mL (Refs 4, 6).

Forty-five isolates (61%) were resistant to SM at more than 100 μg/mL and referred to as high-level SM resistance (Fig. 1). Twelve isolates had a MIC of less than 40 μg/mL. On the other hand, there were 19 KM-resistant isolates, and 15 of them were resistant to concentrations exceeding 100 μg/mL. There were 15 AMK-resistant isolates, of which 9 were resistant at more than 100 μg/mL of concentration. There were 15 isolates resistant to both KM and AMK, and 9 (60%) of them were resistant to concentrations exceeding 100 μg/mL (Table 1).
It has been considered worth to examine these 15 SM, KM and AMK resistant clinical isolates genetically in terms of rpsL and rrs mutations. We utilized a denaturing HPLC (DHPLC) system to detect point mutation of target genes as reported previously. All the isolates displayed rrs mutation and three of them had rpsL mutation. The most common rrs mutation was found in 7 isolates. Interestingly, high-level SM resistance was closely associated with both KM and AMK resistance. The target genes of SM were rpsL and rrs, while a target gene of KM and AMK was rrs. As all SM-resistant clinical isolates possessed rrs mutation, they were also resistant to KM and AMK.

Among 11 SM-sensitive isolates, only two were resistant to KM and AMK. Thus, there were cases that KM or AMK could not be used as a second-line drug for MDR-TB patients with high-level SM resistance.

In the present study, it was found that MDR-TB cases were not only confined to China, but also found three SM-, KM- and AMK-resistant cases in Osaka, Japan. There were not many such cases in Japan (Personal communication with Dr. K. Tsuyuguchi, Japan).

In conclusion, it seems that there are few reports on no cross-resistance between SM and KM or SM and AMK, as long as we searched in the literature. It is concluded that high-level SM resistance is closely linked to mutation of rrs, which is a target gene of KM and AMK, whereas low-level SM is linked to rpsL mutation.

**References**


