

Mixed *Mycobacterium tuberculosis* infection: Changing drug-resistance patterns through antibiotic selection.

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Multiple infections with different strains of *Mycobacterium tuberculosis* may frequently occur in settings where the infection pressure is high. The epidemiological relevance of mixed infections for the patient, clinician and TB control program remains unclear. This study aimed to investigate the role of mixed infections by examining the mechanisms leading to drug-susceptibility variation in *M. tuberculosis* cultures collected from 48 patients diagnosed with multi-drug resistant (MDR) tuberculosis. Serial *M. tuberculosis* isolates were evaluated by phenotypic drug-susceptibility testing and mutation detection methods. Genotypic analysis was done by IS6110 DNA fingerprinting and a novel strain-specific PCR amplification method. DNA fingerprinting showed the presence genetically distinct strains in serial isolates from the 11 of 48 (23%) patients diagnosed with MDR tuberculosis. Strain-specific PCR amplification demonstrated the presence of both strains in the initial isolates from 5 patients (mixed infection), while in 3 patients the second strain appeared during therapy (reinfection). In 1 patient the appearance of the second strain was possibly due to laboratory error. In the absence of strains specific markers, isolates from the remaining two patients could not be analysed. Antibiotic treatment resulted in the selection of the resistant sub-population, while poor adherence or second-line therapy resulted in the re-emergence of the drug-susceptible sub-populations. We demonstrate that drug-susceptibility patterns change through the presence or absence of antibiotic pressure in patients infected with both drug-susceptible and drug-resistant strains. The inability to accurately determine resistance patterns in cases of mixed infection exacerbates diagnostic delays which could have implications for the patient and the spread of drug-resistant strains.