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Abstract (poster session)

Tuberculosis: molecular susceptibility testing reveals mixed infections with three strains

F. Kontos, M. Bobola, V. Mollaki, T. Karonis, M. Souli, G. Dimopoulos, N. Siafakas*, A. Armaganidis, G. Petrikkos, L. Zerva (Athens, GR)

Objectives: Mixed pulmonary infections, usually involving two strains of M. tuberculosis (MTB), have been reported to occur, mostly in settings of high disease incidence. Methods: A 55 year-old male patient from Kazakhstan was admitted for possible pulmonary tuberculosis. During 45 days of hospitalization five samples of bronchial secretions, all Ziehl-Neelsen (+) were obtained for culture and susceptibility testing (MGIT960, Becton-Dickinson). The following assays were applied on both clinical samples and (+) MGIT vials: MTBDRplus, Genotype Mycobacterium CM and AS, MTBDRsl (Hain-Lifesciences), a Real Time PCR identifying nontuberculous mycobacteria (NTM) and a PCR detecting the characteristic deletion in the Region of Difference 105 (RD105) of Beijing MTB strains. The MIRU-VNTR method in 24 polymorphic loci was used for genotyping. Results: MTBDRplus showed that both clinical samples and (+) MGIT vials were MuTauBetapositive, however, results of simultaneous resistance and susceptibility were detected for rifampin (rpoB: hybridization with both S531L mutation and wild type probes) and isoniazid (katG: hybridization with both S315T mutation and wild type probes). Coinfections with a NTM strain and laboratory contamination were excluded. The MIRU-VNTR method showed the presence of three distinct MTB strains in MGIT culture (double genotype in 12 loci, triple genotype in 4). Only one of them (strain RIF-R) was isolated by sub culturing in MGIT vials with rifampin (1µg/ml) and was found to be molecularly and phenotypically resistant to rifampin/isoniazid, and phenotypically resistant to streptomycin, ethambutol and pyrazinamide. The same phenotype was obtained by testing the mixed culture, while MTBDRsl showed susceptibility to aminoglycosides, quinolones and ethambutol. The genetic fingerprint of strain RIF-R and the detection of the RD105 deletion demonstrated that it belongs to the Beijing family. After 40 days of treatment (second line drugs), the time to positivity of MGIT cultures increased from 5 to 20 days. The strain that prevailed was one of the two more susceptible strains. The patient died, while it was found that 4 years ago, he was diagnosed with multidrug resistant tuberculosis in two other hospitals in Athens. Conclusion: Molecular direct susceptibility testing enabled the detection of mixed infection, which apparently may be encountered even in a low incidence setting.