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Poster Session III
Mycobacterial susceptibility and molecular epidemiology

POLYCLONAL SPREAD OF ANTIMICROBIAL RESISTANT MYCOBACTERIUM TUBERCULOSIS AND CHARACTERIZATION OF RESISTANCE MUTATIONS IN A UNIVERSITY HOSPITAL, CENTRAL GREECE

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Objectives: Despite the control measures, tuberculosis (TB) still remains prevalent worldwide, and important issues regarding drug resistance have emerged. In Greece, the incidence of isolates resistant to at least one drug was 13% during the period 1995-2009, according to previously published data of the Greek National Reference Laboratory for Mycobacteria (Papaventsis D et al., Euro Surveill. 2010; available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19614). The aim of the present study was: a) the determination of the rates of antimicrobial resistance in *M. tuberculosis* recovered the past five years (2009-2013) in the University Hospital of Larissa (UHL), Central Greece, b) characterization of the mutations associated with antimicrobial resistance, and c) the investigation of the molecular epidemiology of antimicrobial resistant strains.

Methods: Identification of isolates was performed by a commercial molecular assay (Genotype MTBC, Hain Lifescience). Antimicrobial susceptibility testing for rifampicin, streptomycin, isioniazid and pyrazinamide was performed by the Bactec MGIT 960 system. PCR amplification and sequencing of the *rpoB*, *katG*, *inhA*, *rpsL* and *pncA* genes was performed using primers and conditions as described previously (http://www.tbdreamdb.com). BOX-PCR and Multispacers-Sequence Typing (MST) were used for genotyping of drug-resistant (TB-DR) isolates, as described previously.

Results: From 2009-2013, a total of 171 *M. tuberculosis* were recovered at the Department of Microbiology of UHL. Out of 171, 24 (14%) isolates displayed resistance to several antimicrobial agents, including streptomycin (SM-R; 14 isolates), isoniazid (INH-R; 12 isolates), pyrazinamide (PZA-R; 3 isolates), rifampicin (RIF-R; one isolate). Six of the 24 TB-DR isolates, displayed co-resistance to streptomycin and isoniazid. PCR and sequencing of genes associated with antimicrobial drug-resistance revealed the presence of several substitutions, which were described previously among TB-DR isolates. Among the INH-R isolates, the S315T and S463L were identified in KatG, and the S94A in the structural region of the InhA, whereas no substitutions were detected in the promoter region of *inhA*. The K43R in RpsL of SM-R isolates, the H57D in the PncA of PZA-R isolates, and the I491F in RpoB of a RIF-R isolate were also identified. The 24 drug-resistant TB were distributed into eight different BOX-PCR profiles and 20 different STs, as shown by MST. Four TB-DR isolates belonged to MST-groups 40, 42 and 62, whereas the remaining (n=20) isolates were assigned to 17 novel MST groups. No associations were observed between the MST groups and the antimicrobial resistance patterns or the substitution patterns in the antimicrobial resistance genes of the TB-DR isolates.

Conclusion: During 2009-2013, the rate of isolation of TB-DR was 14% in UHL. PCR amplification and sequencing of the genes associated with rifampicin (*rpoB*), isioniazid (*katG*, *inhA*), streptomycin (*rpsL*) and pyrazinamide (*pncA*) resistance has revealed the presence of previously described substitutions. Genotyping of isolates by BOX-PCR and MST showed polyclonal spread of TB-DR strains.