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BRIEF REPORT

Resistance status of *Mycobacterium tuberculosis* on the island of Crete, Greece

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The present study was conducted in order to assess the level of resistance of *Mycobacterium tuberculosis* to isoniazid (0.2 μg/ml, INH), rifampicin (40 μg/ml, RMP), streptomycin (10 μg/ml, SM), ethambutol (3 μg/m, EMB), and para-aminosalicyclic acid (1 μg/ml, PAS) on the island of Crete, Greece.

A total of 155 consecutive *M. tuberculosis* isolates from different patients, isolated over a 7-year period (2000–2006), were analyzed. Isolation of the mycobacteria and differentiation to the species level was performed using AccuProbe (GenProbe, San Diego, Calif.), biochemical analysis and/or the GenoType MTBC assay (Hain Lifescience, Nehren, Germany), as previously described [1]. Information on previous drug treatment was limited. Susceptibilities were determined by culture on Lowenstein-Jensen slants incorporating the relevant antibiotics (BioMerieux, Marcy l' Etoile, France) using the proportions method. In order to determine the underlying resistance mechanism, all RMP- and INH-resistant strains were further analyzed with GenoType MTBDR (Hain Lifescience, Nehren, Germany), a recently developed commercial DNA strip assay [2], according to manufacturer's instructions. Statistical analysis was performed using the Pearson chi-square test. Probability values (p values) less than 0.05 were considered to be statistically significant. SPSS ver. 11.5 software (SPSS, Chicago, Ill.) was used for all two-sided statistical analyses.

Multi-drug resistant tuberculosis (MDR-TB) is TB showing resistance to both INH and RMP. According to the World Health Organization, extensively drug-resistant TB (XDR-TB) is TB showing resistance to at least INH and RMP, in addition to any fluoroquinolone, and to at least one of the following three injectable drugs used in anti-TB treatment: capreomycin, kanamycin and amikacin [3].

Classified along ethnic background, the 155 patients of the study cohort consisted of 122 Greeks (78.7%), ten Bulgarians (6.4%), eight Romanians (5.2%), seven Albanians (4.5%), three Georgians (1.9%), two Ukrainians (1.3%), two Indians (1.3%) and one Finn (0.6%). The overall resistance rates along with the resistance rates for the Greek and non-Greek patients separately are shown in Table 1. A statistically significant difference between the rate of resistance to SM for Greeks and that for immigrants was found. A total of 137 patients (88.39%) were susceptible to all

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Table 1 Overall resistance rates (%), separate resistance rates for Greek and non-Greek patients, respectively, and statistical analysis of the differences

Antibiotic[a]	Overall	Greeks	Foreign-born	*p*
INH	5.81 (9/155)	4.09 (5/122)	12.12 (4/33)	0.08
RMP	0.64 (1/155)	0 (0/122)	3.03 (1/33)	0.054
SM	9.03 (14/155)	5.74 (7/122)	21.21 (7/33)	0.006
EMB	0.64 (1/155)	0.82 (1/122)	0 (0/33)	0.602
PAS	0.64 (1/155)	0 (0/122)	3.03 (1/33)	0.054

[a] INH, Isoniazid; RMP, rifampicin; SM, streptomycin; EMB, ethambutol; PAS, para-aminosalicyclic acid

of the anti-TB drugs (ATD) tested. There was only one MDR strain (0.64%); this was isolated from the sputum of a hepatitis B virus-positive 44-year-old Romanian male with numerous cases of non-compliance to treatment in the past. The strain, which was also resistant to SM and susceptible to EMB and PAS, was further tested with an extended panel of ATDs (Liofilchem, Roseto, Italy). It was found to be susceptible to rifabutine (10 μg/ml), ofloxacine (5 μg/ml), cycloserine (30 μg/ml), kanamycin (10 μg/ml) and amikacin (5 μg/ml), and resistant to ethionamide (10 μg/m), nicotinamide (10 μg/ml), capreomycin (10 μg/ml), pyruvate 0.2%, pefloxacin (2 μg/ml) and rifapentin (9 μg/ml). Although it is generally accepted that there is a cross-resistance/susceptibility amongst fluoroquinolones, ofloxacin has been shown to be a more active molecule than pefloxacin against *M. tuberculosis* [4, 5]. Based on the above resistance profile, the strain was considered to be an XDR-TB strain. To our knowledge, this constitutes the first case of XDR-TB reported in Greece.

In order to obtain an estimation of the resistance trends, the isolates were divided into two groups: group A included the initial 78 isolates (isolated up to 29 November 2002), and group B included the remaining 77 isolates. Resistance rates for INH and SM were 3.85 (3/78) and 6.41% (5/78), respectively, for group A compared with 7.79 (6/77), and 11.68% (9/77), respectively, for group B. The increases for INH and SM were statistically significant at $p=0.046$ and $p=0.036$, respectively.

Hybridization patterns of the nine INH-resistant strains revealed three different genotypes: (1) a mutation at *katG* codon 315 (Ser→Thr) (seven patients); (2) a mutation within the *katG* not registered by the mutation probes (one patient); (3) a wild-type pattern, indicative of INH-resistance related to mutations in other DNA locations, such as *inhA, ahpC, kasA* (one patient). In terms of the RMP resistance, a mutation at the *rpoB* codon 516 (Asp→Val) was found.

To the best of our knowledge, there are only two studies published to date on the resistance status of *Mycobacterium tuberculosis* in Greece. In the first one, Trakada et al. assessed the status of *M. tuberculosis* in the area of Patras, which is located in the mid-western part of the Greek mainland [6]. In the second, Kanavaki et al. assessed the resistance of *M. tuberculosis* to INH, RMP and MDR among native Greeks, immigrants and repatriated Greeks using samples originating mostly from the metropolitan area of Athens [7]. When comparing our results with those of the Kanavaki et al. study, there was no statistically significant differences between the resistance rates of both groups of immigrants (INH: 12.1 vs. 11.4%; RMP: 3 vs. 6.0%; MDR: 3 vs. 5.4%) [7]. Such similarities were expected as the dispersion of the foreign-born population is homogeneous throughout Greece. Conversely, the resistance ratios for native Greeks in our study were significantly lower than those found in Athens (INH: 4.1 vs. 6.6%, $p=0.001$; RMP: 0 vs. 2.9%, $p=0.001$; MDR: 0 vs. 2.5%, $p=0.001$). These differences are possibly due to differences in TB control programs in the two areas. TB control programs in big cities usually struggle to obtain good cure rates due to a multitude of factors, including homelessness and a high drug-addicted population. In the study of Trakada et al., the overall mono-resistance to INH (combined primary and secondary resistance) was found to be 14.5% [(12 + 18)/207] [6]. Such a high level of resistance (even higher than that found in immigrants in both the present study and that of Kanavaki et al.) is likely to be attributed to local epidemiological reasons.

The resistance rates to INH, RMP, EMB and SM that we found in the present study were similar to Europe's median values (6.0, 1.6, 1.1 and 5.0%, respectively) and the rates found in major Western European countries [8].

In conclusion, the resistance rates of *M. tuberculosis* were found to be comparable to those reported by other European countries. The level of resistance to SM among immigrants was significantly higher than that found among members of the indigenous population. In addition, an XDR-TB case was detected.

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