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DOI: 10.1183/09031936.00162311

Proficiency testing of first- and second-line anti-tuberculosis drugs in Italy

To the Editors:

The emergence of drug-resistant tuberculosis (TB) is an increasing threat to public health in industrialised countries; thus, it is important to supervise mycobacteriology laboratories by performing periodic proficiency of anti-TB drug susceptibility testing (DST). In 1994, the World Health Organization (WHO) and the International Union against Tuberculosis and Lung Diseases developed a global project of anti-TB drug resistance surveillance to assist countries *via* a network of supranational reference laboratories (SRLs). Proficiency test (PT) results of first-line anti-TB drugs have been reported for the SRL network [1] and for some individual countries [2, 3].

The SRL in Rome, Italy, coordinated two PTs of first-line drugs in endemic countries in 2002–2006 [4] and two PTs of first-line drugs in Italy in 1998–2000 [5, 6]. The present study aims to verify whether the quality of DST in Italy changed after that time; to this end, a comprehensive survey of five PTs during a 13-yr period (1998–2010) is reported here, together with a pilot round of second-line drug PTs in 2010.

Laboratories covering 18 out of 20 Italian regions participated in the PT exercise: 22 laboratories in 1998, 20 in 2000, 28 in 2003, 29 in 2007 and 30 in 2010. To maintain knowledge and skills, the laboratories were selected by the SRL on the basis of the number of patient samples analysed for DST. For instance, a mean of 88 first-line DSTs per laboratory (range 21–357) were performed in 2009. In 2010, 13 laboratories with a mean of 113 first-line and six second-line DSTs per laboratory in 2009 also performed the second-line drug PT. A mean of nine second-line DSTs per laboratory was performed in 2010. The *Mycobacterium tuberculosis* panels for first- and second-line drug PTs distributed by the Rome SRL to the mycobacteriology laboratories (all from public hospitals) contained only WHO-characterised strains received annually from the WHO coordinating centres in Ottawa, Canada (4th and 5th rounds [7]) and Antwerp, Belgium (8th, 10th and 14th rounds [1]). Panel strains were selected with the aim of achieving a 50% representation of resistance to streptomycin (SM), isoniazid (INH), rifampicin (RMP), ethambutol (EMB), kanamycin (KM), amikacin (AK), capreomycin (CP) and ofloxacin (OFL) in various combinations [1]. In 1998–2007, 20 strains (10 pairs) were sent to the laboratories for first-line PTs. The pattern of resistance/susceptibility to SM, INH, RMP and EMB, respectively, was: 10/10, 14/6, 10/10 and 6/14 in 1998; 8/12, 16/4, 10/10 and 12/8 in 2000; 8/12, 12/8, 6/14 and 10/10 in 2003; and 8/12, 10/10, 6/14 and 8/12 in 2007. In 2010, 10 strains (five pairs) of the 14th WHO round were sent to the laboratories for both first- and second-line PTs. The pattern of resistance/susceptibility to SM, INH, RMP, EMB, KM, AK, CP and OFL was 6/4, 4/6, 6/4, 2/8, 6/4, 2/8, 4/6 and 2/8, respectively. The use of 10 strains for second-line PT was adequate to assess the quality of the laboratories, which analysed a mean of nine patient samples for second-line DST in 2010 (see earlier). Each laboratory used their routine DST method to test the samples they received. Procedures for first-line drugs included testing on solid media (proportion method in Löwenstein–Jensen (LJ) medium) and liquid media (BACTEC 460TB system and Manual MGIT or MGIT 960 system (Becton Dickinson, Sparks, MD, USA), or MB/BacT (MB) (Organon Teknika, Boxtel, the Netherlands)). For the first-line drug PTs, the use of LJ and BACTEC decreased from 43.5% and 39.1% in 1998 to 0% and 0% in 2010, respectively. Conversely, the use of MGIT increased from 17.4% in 1998 to 100% in 2010. Only one laboratory used MB in 2000. Second-line drug PTs were performed with the MGIT system or in LJ medium using 5, 1, 2.5 or 2 $\mu g \cdot mL^{-1}$, or 30, 40, 40 or 2 $\mu g \cdot mL^{-1}$ of KM, AK, CP or OFL, respectively [8, 9]. Sensitivity testing for one first-line drug (pyrazinamide) and for some second-line drugs (*e.g.* cycloserine) are not reliable and, therefore, were not compared in a PT programme. Results were reported as ''resistant'', ''susceptible'' or ''no result due to lack of strain growth''. Data were evaluated for sensitivity (ability to detect true resistance), specificity (ability to detect true susceptibility) and efficiency (ratio between the number of correct results and the total number of results), according to the WHO format [1, 7]. In each round, the laboratories that obtained <90% efficiency retested the same strain panel for that/those drug(s); laboratories were informed of the total numbers of discrepancies for one or more drugs, but not about individual strain discrepancies [4].

Average sensitivity, specificity and efficiency, with 95% confidence intervals, for first- and second-line drugs, are shown in figure 1. For the first-line drugs (fig. 1a–l), in 1998, there was low sensitivity (<90%) in detecting resistance to SM and EMB (87.7% and 85.5%, respectively), compared with INH and RMP (93.2% and 94.9%, respectively). In the same year, specificity and efficiency were >90% for all the four drugs, but INH and RMP showed the highest values (98.5% and 98.6% for specificity, and

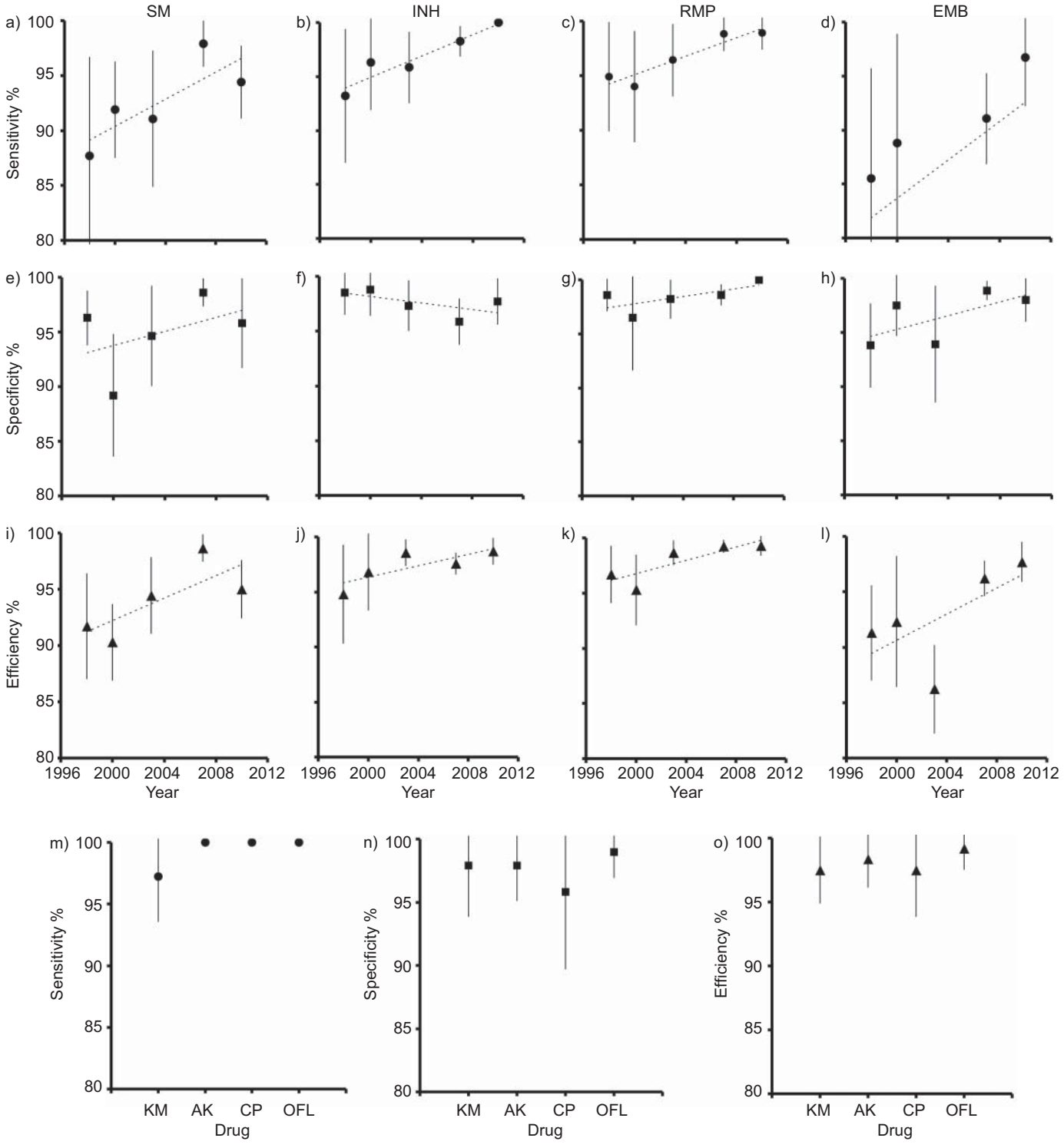

FIGURE 1. Average values and 95% confidence intervals of a–d) sensitivity, e–h) specificity and i–l) efficiency for the first-line drugs streptomycin (SM), isoniazid (INH), rifampicin (RMP), ethambutol (EMB) in the proficiency tests (PTs) of 1998, 2000, 2003, 2007 and 2010, and m–o) for the second-line drugs kanamycin (KM), amikacin (AK), capreomycin (CP) and ofloxacin (OFL) in the PT of 2010. -----: linear trend.

94.8% and 96.7% for efficiency, respectively). A progressive increase was generally observed in the next four rounds, as shown by a linear trend-line of all the three indicators consistently increasing over the 13-yr period examined. Indeed, in 2010, sensitivity in detecting resistance to SM and EMB was 94.4% and 96.7%, respectively, while the corresponding values for INH and RMP were 100% and 98.9%, respectively. Specificity was also high (95.8%, 97.7%, 100% and 98% for SM, INH, RMP and EMB, respectively). Efficiency was $\geqslant$95% for SM and EMB and >98% for INH and RMP. Overall, performance for SM and EMB was poorer than for INH and RMP, similarly to other PTs [1–7], but a clear improvement of quality assurance for each drug was observed in the period examined. Differences in sensitivity, specificity and efficiency between the 2010 first-line PT and the 1999–2007 WHO SRL first-line PT [1] were low ($\pm$1.4% for INH-RMP and $\pm$3.5% for SM-EMB).

For the second-line drugs (fig. 1m–o), one laboratory scored $\leqslant$80% efficiency and was excluded from analysis; in the remaining 12 laboratories, sensitivity was 97.2% for KM, and 100% for AK, CP and OFL. Specificity and efficiency ranged between 95.8% and 98.9%, and 97.5% and 99.2%, respectively, with the lowest and the highest values being those of CP and OFL, respectively.

Reproducibility (intra-laboratory agreement between duplicate cultures), and predictive values for resistance (ratio of true resistance to total resistance) and susceptibility (ratio of true susceptibility to total susceptibility) for first-line drug PTs also increased from 1998 to 2010 and were >94% for second-line drug PT (data not shown).

Overall, the upward trend in the indicators of first-line drug PT from 1998 to 2010 showed that repeated exercises were beneficial to improve the DST in Italy. Difficulties of poor performance were observed mainly in the first three rounds, particularly for EMB. Proper corrective actions were suggested by the SRL, including advice to use WHO-recommended drug concentrations and promotion of adoption of new methods. Indeed, the proportion method in LJ medium and the radiometric BACTEC system were gradually replaced by the MGIT 960 system, which was used by 30 (100%) out of 30 laboratories for first-line drug PTs and 12 (92%) out of 13 laboratories for second-line drug PTs in 2010. The major advantage of the MGIT 960 instrument is that it is a fully automated, nonradiometric apparatus that does not have the problem of disposal of radioactive materials; this is the main reason why the BACTEC 460 system has been replaced. Several studies showed that the MGIT 960 is as accurate as the BACTEC for testing the susceptibility of *M. tuberculosis* to first- and second-line drugs. Progressive introduction of the MGIT system correlated with decrease of the confidence intervals for all drugs from 1998 to 2010. No apparent correlation was seen between confidence intervals and the extent of samples tested per year by the laboratories.

The pilot study of second-line drug PTs demonstrated the feasibility of this exercise in Italy; to the best of our knowledge, this is the first report on the issue at a national level. The good quality of second-line PTs in 12 out of 30 laboratories in nine regions entitles the Rome SRL to consider them as qualified laboratories for participation in the network of second-line DST. This pilot study will be extended to include more laboratories in the future, with the final purpose of identifying one laboratory per region performing this activity. This will allow the SRL to collect data on second-line drug resistance in Italy only from these centralised laboratories in the future.

Reliable DST for first- and second-line drugs is essential for diagnosis of TB caused by multidrug-resistant (MDR) *M. tuberculosis* strains (resistant to at least INH and RMP), and extensively drug-resistant (XDR) strains (MDR strains resistant to any quinolone and at least one injectable drug (KM, CP or AK)). Isolation of MDR and XDR strains is increasing in Italy, particularly from foreign-born TB patients [10], hence the importance of improving accuracy in detecting resistance to first- and second-line drugs. This activity needs to be continuously supported by health authorities to perform the PTs once a year, as recommended by the WHO, for a better management of this difficult-to-treat disease.

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Support Statement: This work was supported by the CCM Project of the Italian Ministry of Health.

Statement of Interest: None declared.

Acknowledgements: The members of the SMIRA Group are: C. Piersimoni (Ospedali Riuniti, Ancona); D. Costa (Policlinico di Bari, UOC Igiene, Bari); A. Grimaldi and A. De Santis (Ospedale Fallacara, Triggiano, Bari); M. Arosio and A. Goglio (Ospedali Riuniti, Bergamo); C. Mazza, L. Squintani, B. Di Pede and G. Gaspari (Ospedale Maggiore, Bologna); D. Marchetti (Ospedale Bellaria, Bologna); A. Nanetti (Ospedale S. Orsola-Malpighi, Bologna); C. Larcher, E. Frizzera and F. Rizza (Azienda Sanitaria dell'Alto Adige, Bolzano); G. Pinsi and A. Turano (Spedali Civili, Brescia); R. Caddeu and A.G. Farris (Ospedale Binaghi, Cagliari); C. Di Naso (Policlinico Ospedaliero Garibaldi Centro, Catania); P. Cavalcanti (Policlinico Ospedaliero Annunziata, Cosenza); G. Tomei and G. Mantini (Ospedale di Chieti, Chieti); T. Ceruti and L. Ferrari (Ospedale di Cremona, Cremona); M.R. Rossi (Ospedale S. Anna, Ferrara); E. Tortoli (Ospedale Careggi, Florence); G. Montini (Ospedale di Forlì, Forli); E. Senno (Ospedale S. Martino, Genoa); S. Nisticò (Ospedale di Lamezia Terme, Catanzaro); C. Colonna and L. Buono (Ospedale Contrada Ambulante, Matera); E. Mazzola, G. Gesu, V. Penati, P. Vaccarino and F. Piana (Azienda Ospedaliera Niguarda Ca Granda, Milan); P. Cichero (Laboraf,

Ospedale S. Raffaele, Milan); A. Lombardi (Ospedale L. Sacco, Milan); G. Mantovani (Ospedale di Mirandola, Modena); A. Fabio, G. Bertoli and F. Rumpianesi (Policlinico di Modena, Modena); G. Santoro (Azienda Ospedaliera dei Colli, Ospedale V. Monaldi, Naples); G.L. Molinari and A. Camaggi (Azienda Ospedaliero-Universitaria Maggiore della Carità, Novara); M.G. Chirillo (Azienda Sanitaria Ospedaliera S. Luigi Gonzaga, Orbassano, Turin); M. Peracchi, L. Fallico (Azienda Ospedaliera di Padova, Padua); M. Menozzi (Ospedale di Palermo, Palerno); G. Dettori (Ospedale di Parma, Parma); P. Marone and L. Bono (IRCCS S. Matteo, Pavia); R. Mazzolla, T. Sposini and C. Tiecco (Ospedale S. Maria della Misericordia, Perugia); A. Barbaro (Ospedale di Reggio Calabria, Reggio Calabria); A. Fabio and L. Vecchia (Ospedale di Reggio Emilia, Reggio Emilia); A. Piscina (Ospedale di Rimini, Rimini); P. Chiaradonna and M. Tronci (Ospedale S. Camillo-Forlanini, Rome); E. Bordi and P. De Mori (INMI, Ospedale L. Spallanzani, Rome); F. Diamare (Ospedale di Salerno, Salerno); E. Libanori and T. Panaiota (Azienda Ospedaliera Valtellina e Valchiavenna, Sondalo, Sondrio); R. Milano and A. Mondo (Ospedale A. Di Savoia, Turin); A. Barbui (Azienda Ospedaliero-Universitaria S. Giovanni Battista, Turin); L. Fabbro (Azienda Ospedaliera S. Giovanni Bosco, Turin); R. Centis, L. D'Ambrosio and A. Spanevello (Fondazione S. Maugeri, Tradate); I. Caola (Ospedale di Trento, Trento); A. Mottola (Ospedale di Treviso, Treviso); C. Fabris (Azienda Ospedaliero-Universitaria Ospedali Riuniti, Trieste); M. Scagnelli (Ospedale di Vicenza, Vicenza); and M.C. Screm and C. Scarparo (Azienda Ospedaliero-Universitaria S. Maria della Misericordia, Udine; all Italy).

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DOI: 10.1183/09031936.00129011

Risk factors for drug-resistant tuberculosis patients in Lithuania, 2002–2008

To the Editors:

Lithuania, a high-priority country for tuberculosis (TB) control in the World Health Organization European Region, has one of the world's highest rates of multidrug-resistant (MDR)-TB. It has recently seen an increase in the rates of both primary and acquired MDR-TB (9% of new and 50% of re-treatment cases were MDR in 2010), and the appearance of extensively drug-resistant (XDR)-TB cases constituting 4.3% of all MDR-TB cases [1, 2]. Drug resistance is accompanied by low treatment success rates (40% in newly diagnosed and 19% in re-treatment cases in 2009) among MDR-TB patients despite a well-established TB control programme with relatively good indicators of treatment success and low default rates (7%) among patients with sensitive TB [2].

Although there are data describing the molecular epidemiology of drug resistance in Lithuania [3], relatively little is known about risk factors for drug resistance. We analysed 7 yrs of Lithuanian national surveillance data: all treated culture-confirmed TB cases, including new and re-treatment cases, registered from 2002 to 2008 in the national TB register (established in 2002). Our aim was to describe the epidemiological, clinical and socioeconomic features of MDR-/XDR-TB cases, and to establish risk factors for drug resistance acquisition and development during re-treatment.

Standard case reporting included demographic and clinical information with initial and follow-up drug susceptibility testing (DST) results. Individual patients suspected of having a high risk of HIV/AIDS were offered testing for HIV according to the national policy. A randomly selected proportion of strains (~18%) was genotyped (by IS6110 restriction fragment length polymorphism typing and spoligotyping) within the routine service by the Lithuanian Institute of Biotechnology (Vilnius, Lithuania).

Non-MDR-TB patients who received a second treatment cycle, and were reported as susceptible to isoniazid and rifampicin in

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