test for smoking behaviour [2]. Cotinine is specific to nicotine intake and has a half-life of 18 h, meaning results are indicative of smoking over a number of days. However, cotinine measurements are largely confined to sophisticated laboratory tests, which are expensive and time consuming. We would like to take this opportunity to describe a new point of care saliva test, which measures cotinine and the other nicotine metabolites.

A previous colorimetric urine test [3] called SmokeScreen was modified. The same testing device was used, but the reagents were changed to improve sensitivity to detect the lower levels of cotinine found in saliva. The new, more sensitive assay based on the König reaction has been developed and evaluated in the laboratory and then using a group of healthy volunteers (n=124, age range 21–67 yrs), including 61 smokers with a cigarette consumption of four or more cigarettes per day, (mean 16.6 cigarettes per day), 25% of whom smoked hand rolled cigarettes.

Each provided a saliva sample using a manufactured collecting sponge and collecting bottle. 1 mL of saliva was eluted using the test's fixed-volume syringe. The sample was introduced onto freeze-dried reagents and quickly shaken. A sample positive for nicotine metabolites would be expected to turn pink within 1 min, but up to 10 min was allowed for full colour development. The resultant colour was compared with a four-point colour chart (*i.e.* 1–4 point to represent weak to very intense colour) and the level of smoking recorded. Samples from nonsmokers remained unchanged. A positive colour change was obtained from 55 of the 61 smokers and a negative result from 62 of the 63 nonsmokers, giving a sensitivity of 90%, specificity of 98% and accuracy of 94% ($p<0.05$). There is a significant difference in saliva test results between the smoking and nonsmoking groups (Chi-squared test, $p<0.01$), indicating that the test is specific for screening smokers with a cigarette consumption of four or more cigarettes a day.

The new test was found to have a sensitivity and specificity comparable with the other commercial point of care saliva cotinine test available [4], but was quicker and is less expensive. A dedicated colorimeter to quantify the result is under development. This test could be an important adjunct for identifying smokers and treating smoking-related diseases by providing instantaneous results at a fraction of the cost of laboratory cotinine analysis.

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Drug-resistant tuberculosis among foreign-born persons in Italy

To the Editors:

Over the last few years, drug-resistant tuberculosis (TB) has emerged as an important threat to public health in industrialised countries. In Italy, the most recent data on resistance to the first-line drugs (FLDs) streptomycin (S), isoniazid (H), rifampicin (R) and ethambutol (E) were reported for the period 1998–2001 [1]. These studies determined the prevalence of resistance among new cases and previously treated cases, but no information was available on the contribution of immigration, which plays an important role on TB epidemiology in low-incidence countries [2].

In the last decade, while the notified incidence of TB in Italy was stable at approximately seven cases per 100,000 people annually, the proportion of foreign-born persons (FBPs) with TB increased from 22% in 1999 to 46% in 2008 [3]. In the same period, the proportion of African-born persons with TB decreased from 51% to 30%, whereas the proportion of European cases increased from 16% to 33%, most of them being born in Eastern Europe, including Former Soviet Union (FSU) countries.

Eastern European countries are among those with the highest TB rates caused by multidrug-resistant (MDR) *Mycobacterium*

tuberculosis strains (*i.e.* resistant to at least H and R) and extensively drug-resistant (XDR) strains (*i.e.* MDR strains resistant to any fluoroquinolone and to at least one injectable second-line drug (SLD): kanamycin (KM), capreomycin (CM), amikacin (AK)) [4].

Reliable drug susceptibility testing (DST) is essential to diagnose TB caused by drug-resistant strains. In Italy, a network of laboratories coordinated by the World Health Organization (WHO) Supranational Reference Laboratory (SRL) in Rome performs drug susceptibility proficiency testing for S, H, R, E (five rounds from 1997 to 2010) and SLD (KM, AK, CM and ofloxacin (OFL)) (one round in 2010) [5].

In order to understand the impact of immigration on drug-resistant TB in Italy, we conducted a retrospective study over the period 2008–2010 to investigate drug-resistance proportions and drug-resistance profiles of *M. tuberculosis* strains circulating among FBPs and Italian-born persons (IBPs).

Our laboratory network (Italian Multicentre Study on Resistance to Antituberculosis drugs (SMIRA)) is composed of 30 hospital mycobacteriology laboratories located in 19 out of 20 Italian regions, selected on the basis of: 1) technical skills for DST, periodically evaluated by proficiency testing [5]; 2) number of first-line DSTs performed annually (a mean of 72, 88 and 78 DSTs per laboratory in 2008, 2009 and 2010, respectively); and 3) convenient geographic location reflecting different TB-specific settings. In 2010, SMIRA covered 59% of nationwide notified cases, allowing preparation of the national annual report on drug resistance [3].

TB cases with positive *M. tuberculosis* cultures were routinely examined by SMIRA laboratories for susceptibility to S, H, R and E. DST procedures included testing on solid media (proportion method in Löwenstein–Jensen (LJ) medium) and liquid media (BACTEC 460 TB (BACTEC) and MGIT 960 (MGIT) systems; Becton Dickinson, Sparks, MD, USA). In 2008, the LJ, BACTEC and MGIT procedures were used by 14%, 10% and 76% of laboratories, respectively. In 2009, the use of MGIT increased, and reached 100% in 2010. MDR-TB isolates were sent to the SRL to retest susceptibility to FLDs and test susceptibility to SLDs. Susceptibility to S, H, R, E, KM, AK, CM, OFL, moxifloxacin (MX), ethionamide (ETH), linezolid (LZ) was determined by the MGIT system, using the following concentrations: 1.0, 0.1, 1.0, 5.0, 5.0, 1.0, 2.5, 2.0, 0.25, 5.0 and 1.0 $\mu g \cdot mL^{-1}$, respectively [5, 6].

Data on resistance to FLDs and SLDs of strains isolated in 2008–2010 from IBPs and FBPs are given in table 1. The five countries mainly contributing to the FBP group were Romania (28.7%), Morocco (9.9%), Peru (5.8%), Pakistan (5.8%) and India (5.6%). A lower FBP proportion emigrated from high MDR-TB burden FSU countries [4], including Ukraine (2.5%), Moldova (2.2%) and others (Russia, Georgia, Latvia, Armenia and Belarus (<1%)). We stratified FBP data in three groups: Romania (the largest TB group), FSU (the highest MDR-TB prevalence group) and all others.

Out of 5,267 TB cases with known country of birth (table 1), FBPs were significantly younger that IBPs (mean$\pm$SD 35$\pm$14 *versus* 58$\pm$22 yrs, respectively; p<0.0001) and arrived in Italy from 84 countries. 40% came from Europe (29% from Romania, 6% from FSU countries and 5% from other European countries), 27% from Africa, 21% from Asia and 12% from the Americas. 61% of IBPs and 63% of FBPs were male (p=0.14). 81% of IBPs and 78% of FBPs were new cases (p=0.007). Noticeably, cases from the FSU were more likely to harbour strains resistant to one or more FLDs (47.6%) than those isolated from IBPs, Romanian-born persons and other FBPs (27.0% (p<0.0001), 29.8% (p<0.0001) and 35.3% (p=0.003), respectively). Overall, the highest prevalence of monoresistance was seen for S and H, while monoresistance to R and E was low (<1%). Monoresistance to H in patients from the FSU (6.9%) was significantly higher than in IBPs (3.2%; p=0.02). The prevalence of any form of resistance to S and H was higher than those to R and E.

The overall MDR-TB prevalence was 3.8%, with large differences between groups. The MDR rate was low in IBPs (1.4%), but high in immigrants from the FSU, Romania and all other foreign countries (30.3% (p<0.0001), 5.9% (p<0.0001) and 4.1% (p<0.0001), respectively). Out of 44 MDR-TB patients from the FSU, most came from Ukraine (47.8%) and Moldova (34%), and a minority from Russia, Armenia and Belarus. All these countries are included in the WHO list of high MDR-TB burden countries responsible for 85% of the global MDR-TB burden [4]. FBPs with MDR-TB were younger than IBPs (mean$\pm$SD 33$\pm$12 *versus* 53$\pm$21 yrs, respectively; p<0.0001) and most of them were new cases (55%), while most IBPs were previously treated cases (62%). As for drug combinations, MDR strains showed low resistance to HR and HRE, but were frequently resistant to SHRE and SHR. Almost 90% of MDR-TB cases (175 out of 198) were resistant to at least three FLDs. The highest frequency of the SHRE resistance pattern was seen in strains collected from patients coming from FSU (20.7%) and the lowest in strains collected from IBPs (0.5%) (p<0.0001). Other H and R plus S or E resistances were rare.

Among 80 MDR strains tested for susceptibility to SLDs (68 from FBPs and 12 from IBPs), the highest total percentage of resistance was seen to ETH (47.5%), followed by KM, AK and CM ($\geqslant$20%). Overall, the drugs potentially active for clinical use were OFL, MX and LZ (18.8%, 16.2% and 12.5% resistance, respectively). Six MDR-TB isolates were XDR strains (two from Ukraine, and one each from Moldova, Romania, Peru and Bangladesh), with LZ being the only drug active against all of them.

Few nationwide data have been reported on FLD and SLD resistance in FBPs [7]. In Italy, the MDR-TB prevalence in FBPs was consistent with that of their native countries (*e.g.* in 2009: Romania, 11.2%; Ukraine, 19%; Moldova, 44.3%) [4, 8]. The study results demonstrated, for the first time under a national perspective, that: 1) IBP contribution to MDR-TB is low; and 2) MDR-TB strains isolated from FBPs (particularly from the FSU) are highly resistant to FLDs. Thus, efficient strategies for rapid identification and treatment of MDR-TB cases in FBPs are imperative. SLD data showed that resistance to LZ seemed to be still low, suggesting its use for difficult-to-treat cases. Indeed, LZ-containing combinations are administered for off-label therapy of MDR/XDR-TB in Italy and other countries [9, 10]. However, new drugs are necessary to treat these life-threatening cases.

TABLE 1 First- and second-line anti-tuberculosis drug resistance in *Mycobacterium tuberculosis* strains isolated from Italian-born persons (IBPs) and foreign-born persons (FBPs) in the period 2008–2010

	IBPs	FBPs			Total
		Romania	FSU[#]	All others	
Total tested	2596 (100.0)	766 (100.0)	145 (100.0)	1760 (100.0)	5267 (100.0)
Resistance to any FLD	701 (27.0)	228 (29.8)	69 (47.6)	621 (35.3)	1619 (30.7)
Monoresistance					
S	139 (5.4)	23 (3.0)	5 (3.4)	71 (4.0)	238 (4.5)
H	82 (3.2)	42 (5.5)	10 (6.9)	89 (5.1)	223 (4.2)
R	18 (0.7)	4 (0.5)	0 (0.0)	3 (0.2)	25 (0.5)
E	15 (0.6)	3 (0.4)	1 (0.7)	8 (0.5)	27 (0.5)
Any resistance					
S	239 (9.2)	80 (10.4)	56 (38.6)	184 (10.4)	559 (10.6)
H	199 (7.7)	108 (14.1)	63 (43.4)	231 (13.1)	601 (11.4)
R	58 (2.2)	45 (5.9)	36 (24.8)	123 (7.0)	262 (5.0)
E	34 (1.3)	26 (3.4)	36 (24.8)	57 (3.2)	153 (2.9)
H and R resistance					
MDR[¶]	36 (1.4)	45 (5.9)	44 (30.3)	73 (4.1)	198 (3.8)
SHRE	12 (0.5)	20 (2.6)	30 (20.7)	29 (1.6)	91 (1.7)
SHR	14 (0.5)	18 (2.3)	13 (9.0)	32 (1.8)	77 (1.5)
HRE	2 (0.1)	2 (0.3)	0 (0.0)	3 (0.2)	7 (0.1)
HR	8 (0.3)	5 (0.7)	1 (0.7)	9 (0.5)	23 (0.4)
H plus other resistances					
HS	62 (2.4)	16 (2.1)	4 (2.8)	45 (2.6)	127 (2.4)
HSE	14 (0.5)	18 (2.3)	13 (9.0)	30 (1.7)	75 (1.4)
HE	7 (0.3)	3 (0.4)	0 (0.0)	4 (0.2)	14 (0.3)
R plus other resistances					
RE	3 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	4 (0.1)
RS	3 (0.1)	4 (0.5)	0 (0.0)	2 (0.1)	9 (0.2)
RES	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
MDR strains tested for resistance to SLDs[+]	12 (100.0)	24 (100.0)	15 (100.0)	29 (100.0)	80 (100.0)
ETH	5 (41.7)	11 (45.8)	8 (53.3)	14 (48.3)	38 (47.5)
KM	3 (25.0)	7 (29.1)	4 (26.7)	7 (24.1)	21 (26.2)
AK	3 (25.0)	5 (20.8)	4 (26.7)	6 (20.7)	18 (22.5)
CM	2 (16.7)	7 (29.2)	2 (13.3)	5 (17.2)	16 (20.0)
OFL	3 (25.0)	2 (8.3)	5 (33.3)	5 (17.2)	15 (18.8)
MX	2 (16.7)	1 (4.2)	4 (26.7)	6 (20.7)	13 (16.2)
LZ	1 (8.3)	5 (20.8)	1 (6.7)	3 (10.3)	10 (12.5)

Data are presented as n (%). FSU: Former Soviet Union; FLD: first-line drug; S: streptomycin; H: isoniazid; R; rifampicin; E: ethambutol; MDR: multidrug-resistant; SLD: second-line drug; ETH: ethionamide; KM: kanamycin; AK: amikacin; CM: capreomycin; OFL: ofloxacin; MX: moxifloxacin; LZ; linezolid. [#]: Armenia, Belarus, Georgia, Latvia, Moldova, Russia and Ukraine; [¶]: resistant to at least H and R; [+]: values in parenthesis are the percentages of MDR strains.

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Availability of anti-tuberculosis drugs in Europe

To the Editors:

The emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) represents a major threat to TB control globally and, specifically, in Europe [1–3]. MDR-/XDR-TB is at large due to clinical mismanagement of drug-susceptible and drug-resistant TB cases as well as to transmission of resistant strains [1–3]. Continuous availability of quality-controlled drugs is a prerequisite to ensure correct clinical management of TB patients [2, 4].

Comprehensive and updated information on the availability and registration procedures of first-line (FLD) and second-line (SLD) anti-TB drugs is not available, neither in Europe nor elsewhere. Anecdotal evidence suggested that in most European Union (EU) countries, where TB has a low incidence, procurement procedures are decentralised (not through Global Drug Facility, GDF), and with no specific responsibility for TB drug procurement available at the ministerial level. Despite high costs of SLD, registration procedures are strong enough to potentially prevent marketing and prescription of poor quality drugs. FLD and SLD are usually available (with mechanisms to prevent stock-outs), although the low number of drug doses sold can create challenges in assuring their continuous availability [5].

In EU countries with TB incidence >20 per 100,000 people, high MDR-TB prevalence and intermediate income, drug-procurement procedures are centralised (through GDF), drug