

9. Neilands JB. Siderophores: structure and function of microbial iron transport compounds. *J Biol Chem* 1995; **270**: 26723–26726.
10. Rabsch W, Reissbrodt R. Investigations of *Salmonella* strains from different clinical-epidemiological origin with phenolate and hydroxamate (aerobactin) siderophore bioassays. *J Hyg Epidemiol Microbiol Immunol* 1988; **32**: 353–360.
11. Rabsch W, Reissbrodt R. Biotest zum Nachweis von hydroxamat-Fe-chelatoren (Aerobactin). *J Basic Microbiol* 1985; **25**: 663–667.
12. Ang-Kucuker M, Kucukbasmaci O, Tekin M, Akbulut D, Buyukbaba-Boral O, Ang O. Serotypes, siderophore synthesis, and serum resistance of uropathogenic *Klebsiella* isolates. *Adv Exp Med Biol* 2000; **485**: 237–241.
13. Marrs CF, Zhang L, Tallman P *et al.* Variations in 10 putative uropathogen virulence genes among urinary, faecal and peri-urethral *Escherichia coli*. *J Med Microbiol* 2002; **51**: 138–142.
14. Carbonetti NH, Boonchal S, Parry SH, Vaisanen-Rhen V, Korhonen TK, Williams PH. Aerobactin-mediated iron uptake by *Escherichia coli* isolates from human extraintestinal infections. *Infect Immun* 1986; **51**: 966–968.
15. Vidotto MC, Furlaneto MC, Perugini MRE. Virulence factors of *Escherichia coli* in urinary isolates. *Brazil J Med Biol Res* 1991; **24**: 365–373.
16. Vila J, Simon K, Ruiz J *et al.* Are quinolone-resistant uropathogenic *Escherichia coli* less virulent? *J Infect Dis* 2002; **186**: 1039–1042.
17. Brook I. Enhancement of growth of aerobic and facultative bacteria in mixed infections with *Bacteroides* species. *Infect Immun* 1985; **50**: 929–931.
18. Morin MD, Hopkins WJ. Identification of virulence genes in uropathogenic *Escherichia coli* by multiplex polymerase chain reaction and their association with infectivity in mice. *Urology* 2002; **60**: 537–541.
19. Torres AG, Redford P, Welch RA, Payne SM. Ton-B dependent systems of uropathogenic *Escherichia coli*: aerobactin and heme transport and Ton B are required for virulence in the mouse. *Infect Immun* 2001; **69**: 6179–6185.
20. Ou Said AM, Contrefois MG, Der Vartanian M, Girardeau JP. Virulence factors and markers in *Escherichia coli* from calves with bacteremia. *Am J Vet Res* 1988; **49**: 657–660.
21. Sharma S, Harjai K, Mittal R. Enhanced siderophore production and mouse kidney pathogenicity in *Escherichia coli* grown in urine. *J Med Microbiol* 1991; **35**: 325–329.
22. Roy D, Expert D, Razafindratsita A *et al.* Activity and specificity of a mouse monoclonal antibody to ferric aerobactin. *Infect Immun* 1992; **60**: 768–772.

RESEARCH NOTE

Mycobacterial testing in hospital laboratories: results from a questionnaire survey in Italy

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ABSTRACT

Between 1999 and 2001, 355 hospital laboratories in Italy were asked to complete a questionnaire addressing mycobacterial test methods, 1-year workloads and laboratory safety features. Analysis of the data showed that rapid methods for mycobacterial testing were being used by most larger laboratories; however, sub-optimal methods were still in use in small and medium-size laboratories. In a country such as Italy, which has a low prevalence of tuberculosis cases, implementation of rapid technologies, combined with regionalisation of mycobacterial diagnostic services, seems to be the most reasonable and cost-effective strategy.

Keywords Diagnostic tests, *Mycobacterium tuberculosis*, rapid methods, safety, survey

Original Submission: 14 August 2003; **Revised Submission:** 6 February 2004; **Accepted:** 16 March 2004

Clin Microbiol Infect 2004; **10**: 1014–1017
10.1111/j.1469-0691.2004.00950.x

Tuberculosis (TB) may be considered a global emergency, with >2 million people dying and 8 million new cases each year [1]. Although the prevalence of TB in the industrialised world is relatively low, outbreaks caused by multiresistant strains of the *Mycobacterium tuberculosis* complex (MTB) have occurred in hospitals, prisons and shelters for homeless people, often involving

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HIV-infected individuals and immigrants [2]. To control the spread of disease, individuals with TB must be detected as quickly as possible and placed on effective chemotherapy [3]. Consequently, laboratories must adopt methods that provide rapid and accurate detection, isolation, identification and drug susceptibility testing (DST) of MTB in clinical specimens [4,5]. This issue has already been addressed in the USA [6–9]. The present report describes the results of a questionnaire developed by the Mycobacteria Committee (CoSMic) of the Italian Association of Clinical Microbiology (AMCLI) to assess current practice in hospital clinical laboratories in Italy that perform mycobacterial testing.

The Italian national health service is governed at two political levels: nationally by the Parliament, and regionally by elected councils. Italy comprises 20 regions whose councils implement the health system locally through regional laws, and who are also responsible for planning, financing, monitoring and control [10]. In this context, regional health institutions (Assessorati alla Sanità) were asked to co-manage the survey and provide institutional coverage. Some regions supported the survey actively, while others unfortunately did not. Consequently, the survey was completed over a 3-year period (1999–2001) by the AMCLI regional offices. The laboratory component covered the 12-month period (from 1 January to 31 December) closest to the data collection.

The questionnaire requested information on methods used for acid-fast microscopy, decontamination, routine culture, species identification, DST and reporting, as well as details of safety equipment. Each laboratory was also asked to indicate the number of samples processed, number of referred mycobacterial isolates, number of MTB strains identified, number of isolates tested for drug susceptibility, and whether novel molecular amplification-based systems for direct detection were being used. Information was returned from AMCLI regional offices to the CoSMic-appointed survey coordinator. Data were coded, entered and analysed with the use of either Epi Info 5.0 or Microsoft Excel software packages.

Information was available for 355 (59.6%) of 596 public hospital laboratories contacted (including two regional reference laboratories), but the response varied by region. Of the 20 regions, all

hospital-based laboratories were surveyed in ten regions, 60–90% in four regions, and 30–50% in four regions; in two regions, the survey could not be performed at all. However, even in those regions where coverage was not 100%, all the largest laboratories were included.

Microscopy for acid-fast bacilli (AFB) was performed by all laboratories, primary culture by 298 (83.9%), identification of MTB by 133 (37.5%), DST by 83 (23.4%), and direct amplification by 73 (20.6%). Larger hospital laboratories were more likely to perform multiple mycobacteriology procedures. The total numbers of specimens processed for microscopy for AFB and culture during the surveyed 12-month interval were 242 381 and 218 991, respectively; thus, even though the sensitivity of smear microscopy is low, >23 000 specimens were not cultured for AFB.

In total, 179 (50.4%) laboratories processed ≤500 clinical specimens/year, 105 (29.6%) laboratories processed 500–3000 clinical specimens/year, and only 14 (3.9%) laboratories processed >3000 specimens/year. Of the 57 (16.1%) laboratories that did not perform culture for AFB, 35 (9.9%) sent specimens to larger laboratories. The laboratories processing <500 specimens/year seemed to be located mostly in central and southern Italy. MTB identification and DST were performed on 10 045 and 5649 isolates, respectively. In total, 22 740 MTB direct amplification tests were performed during a 12-month period, with 2723 (11.9%) positive results. Further information concerning the methods used by different laboratories is listed in Tables 1 and 2. On average, results of smear microscopy for AFB were available 3 days after specimen receipt

Table 1. Procedures used for smear detection and cultivation of mycobacteria in Italian laboratories

| Test method | No. (%) of laboratories |
|----------------------------|-------------------------|
| Smear microscopy stains | |
| Ziehl–Neelsen | 289 (81.4) |
| Fluorochrome | 51 (14.4) |
| Kinyoun | 15 (4.2) |
| Decontamination | |
| Standard NALC–NaOH [18] | 166 (55.7) |
| NALC–NaOH 3% v/v | 5 (1.7) |
| Dithiothreitol–NaOH 2% v/v | 8 (2.7) |
| NaOH 4% v/v | 24 (8.1) |
| Trisodium phosphate | 15 (5.0) |
| Others | 80 (26.8) |
| Primary culture medium | |
| Solid | 141 (47.3) |
| Solid and radiometric | 18 (6.0) |
| Solid and non-radiometric | 139 (46.7) |

NALC, N-acetyl-L-cysteine.

Table 2. Methods used in Italian laboratories for identification, drug susceptibility testing and direct detection by amplification tests of members of the *Mycobacterium tuberculosis* complex from clinical samples

| Test method(s) | No. (%) of laboratories |
|--|-------------------------|
| <i>Mycobacterium tuberculosis</i> identification tests | |
| Nucleic acid probes/DATs | 85 (28.6) |
| BACTEC NAP | 20 (6.7) |
| Biochemical tests | 28 (9.4) |
| HPLC | 0 |
| None | 165 (55.3) |
| Drug susceptibility tests | |
| Egg-medium proportional method | 27 (9.1) |
| Agar proportional method | 1 (0.3) |
| Radiometric | 34 (11.4) |
| Liquid non-radiometric | 20 (6.7) |
| DATs | |
| Local laboratory-formulated test | 5 (1.4) |
| GenProbe AMTD II | 29 (8.2) |
| Abbott LCx | 22 (6.2) |
| Roche Amplicor | 11 (3.1) |
| BD ProbeTec ET | 6 (1.7) |

DAT, direct amplification test; HPLC, high-performance liquid chromatography.

(mean times of 2.6, 2.9 and 3.5 days for northern, central and southern Italy, respectively).

Of the 317 hospital laboratories that provided information on biosafety measures [11,12], 107 (33.7%) reported a dedicated room or an isolation room, and four (1.3%) had negative air ventilation equipment. HEPA filter-equipped biological safety cabinets were used in 269 (84.8%) laboratories, while a centrifuge dedicated to procedures for AFB was used in 117 (36.9%) laboratories. However, only 146 (46.1%) centrifuges used for mycobacterial testing were equipped with sealed O-ring carriers. For decontamination, 55.7% of laboratories used the standard n-acetyl-l-cysteine (NALC)-NaOH method, with variations on the most widely used protocol being used in many other laboratories. Unfortunately, most of these variations are considered to be harsh decontamination systems that may significantly reduce the number of viable mycobacteria.

The American Thoracic Society [13] has suggested that, in order to maintain proficiency, laboratories should process 10–15 specimens/week and identify 20 cultures/week. Fewer than 20% of surveyed laboratories in Italy can fulfil these criteria. These findings suggest a need for increased referral of samples and/or isolates to larger laboratories, or the grouping together of low-volume laboratories. Such a strategy would reduce the risk of false-positive cultures, which is associated significantly with laboratories dealing with a low sample load [14], and would fit well with the regional government network in Italy.

The major weakness of the survey was the 3-year data collection period and the uneven response rate of hospital laboratories, but the surveyed laboratories were representative (in number and size) of the current Italian situation. A previous Italian survey [15] of 99 clinical laboratories located in larger hospitals (all including infectious diseases units) found that 57.6% did not identify MTB and 42.6% did not perform any DST. The biosafety picture was similar, in that 15.1% of the laboratories did not use a Class II safety cabinet, and 28% used unsealed-rotor centrifuges.

In order to make the laboratory diagnosis of TB in Italy more rapid, sensitive and safer for laboratory personnel, it seems advisable to concentrate the diagnostic service in a limited number of specialised laboratories, chosen on the basis of workload and professional expertise. Factors affecting the efficiency of reference laboratories should also be addressed [6–9,16]. The American Thoracic Society has recommended the classification of laboratories into Levels I, II and III, according to workload and expertise. Level II should serve 1 million people (a regional reference laboratory) and Level III should serve 5–10 million people (a national reference laboratory). In Italy, this structure of laboratory services was introduced in 1998 by a national law (Guidelines for TB control) [17], but only a minority of regions have so far provided implementation plans, regional laws and proper financial support. Regrettably, it seems that much remains to be done by Italian public health institutions to ensure that hospital diagnostic laboratories are properly equipped and prepared for mycobacteriology testing.

ACKNOWLEDGEMENTS

The authors thank the following participants for their assistance in providing the data: M. G. Mazzarello (Ovada), P. Troupioti (Sondalo), G. Mucignat (Pordenone), G. De Fina (Bolzano), I. Caola (Trento), M. L. Moro (Bologna), G. Sbaraglia (Perugia), M. G. Proietti (Terni), P. Chiaradonna (Rome), P. Fazii (Pescara), M. Bucci (Larino), M. Liguori (Cagliari), G. Miragliotta (Bari), M. Conte (Naples) and P. Cavalcanti (Cosenza).

REFERENCES

1. World Health Organization. *Global tuberculosis control*. WHO Report 2001. Geneva: World Health Organization, 2001; 18–19.

2. Kochi A, Vareldzis B, Styblo K. Multidrug-resistant tuberculosis and its control. *Res Microbiol* 1993; **144**: 104–110.
3. World Health Organization. *Anti-tuberculosis drug resistance in the world. The WHO/IUATLD global project on anti-tuberculosis drug resistance surveillance*. WHO/TB/97.229. Geneva: WHO, 1997; 1–227.
4. Hinman AR, Hughes JM, Snider DE, Cohen ML. Meeting the challenge of multidrug-resistant tuberculosis: summary of a conference. *MMWR* 1992; **41**(RR-11): 51–57.
5. Tenover FC, Crawford JT, Huebner RE, Geiter LJ, Horsburgh CR, Good RC. The resurgence of tuberculosis: is your laboratory ready? *J Clin Microbiol* 1993; **31**: 767–770.
6. Huebner RE, Goods RC, Tokars JL. Current practices in mycobacteriology: results of a survey of state public health laboratories. *J Clin Microbiol* 1993; **31**: 771–775.
7. Bird BR, Denniston MD, Huebner RE, Good RC. Changing practice in mycobacteriology: a follow-up survey of state and territorial public health laboratories. *J Clin Microbiol* 1996; **34**: 554–559.
8. Woods GL, Witebsky FG. Mycobacterial testing in clinical laboratories that participate in the college of American pathologists' mycobacteriology E survey: results of a 1993 questionnaire. *J Clin Microbiol* 1995; **33**: 407–412.
9. Tokars JL, Rudnick JR, Kroc K *et al*. US hospitals mycobacteriology laboratories: status and comparison with state public health department laboratories. *J Clin Microbiol* 1996; **34**: 680–685.
10. Remuzzi G. Country profile: Italy. *Lancet* 1996; **348**: 167–175.
11. Barenfanger J. Making your lab safe against multi-drug-resistant *Mycobacterium tuberculosis*. *Clin Microbiol News* 1993; **15**: 76–80.
12. Centers for Disease Control/National Institutes of Health. *Proposed guidelines for goals for working safely with Mycobacterium tuberculosis in clinical, public health and research laboratories*. Atlanta: US Department of Health and Human Services, Centers for Disease Control and Prevention, 1997.
13. American Thoracic Society. Level of laboratory services for mycobacterial diseases. *Am Rev Respir Dis* 1983; **128**: 213.
14. de Boer AS, Blommerde B, de Haas PEW *et al*. False-positive *Mycobacterium tuberculosis* cultures in 44 laboratories in the Netherlands (1993–2000): incidence, risk factors, and consequences. *J Clin Microbiol* 2002; **40**: 4004–4009.
15. Chiaradonna P, Girardi E, Spanò A, Tronci M. Risultati dell'indagine conoscitiva nazionale sulla diagnostica delle infezioni da micobatteri negli ospedali sede di reparti di Malattie Infettive. *Microbiol Med* 1996; **11**: 59–62.
16. Drobniowski FA, Watt B, Smith EG *et al*. A national audit of the laboratory diagnosis of tuberculosis and other mycobacterial diseases in the United Kingdom. *J Clin Pathol* 1999; **52**: 334–337.
17. Anonymous. Linee-guida per il controllo della malattia tubercolare, su proposta del Ministero della Sanità, ai sensi dell'art. 115, comma 1, lettera b), del decreto legislativo 31 marzo 1998, n. 112.
18. Metchock B, Nolte FS, Wallace RJ. *Mycobacterium*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH, eds. *Manual of clinical microbiology*, 7th edn. Washington DC: ASM Press, 1999; 399–437.

RESEARCH NOTE

Isolation of a novel sequevar of *Mycobacterium flavescens* from the synovial fluid of an AIDS patient

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ABSTRACT

This report describes the characterisation of a mycobacterium involved in a case of septic arthritis in an AIDS patient that was treated successfully with specific anti-mycobacterial drugs. The biochemical and cultural features, and the mycolic acid pattern as assessed by high-performance liquid chromatography, were fully compatible with the isolate being *Mycobacterium flavescens*. However, the isolate's 16S rDNA sequence differed by five nucleotides from the two known sequevars of *M. flavescens*, thus indicating that this isolate belonged to a new 16S rDNA sequevar.

Keywords AIDS, identification, *Mycobacterium flavescens*, 16S rDNA

Original Submission: 6 November 2003; **Revised Submission:** 25 February 2004; **Accepted:** 20 March 2004

Clin Microbiol Infect 2004; **10**: 1017–1019
10.1111/j.1469-0691.2004.00947.x

Mycobacterium flavescens is a scotochromogenic mycobacterium, characterised by an intermediate

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