Article/Artigo

Improving tuberculosis control through the partnership between university and the health system

Qualificar o controle da tuberculose mediante a parceria entre a Universidade e o Sistema de Saúde

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ABSTRACT

Introduction: Tuberculosis (TB) control is linked to the availability of qualified methods for microbiological diagnostics; however, microscopy with limited sensitivity is the only method available in many locations. The objective of this study was to evaluate the introduction of culture, drug susceptibility testing (DST), and genotyping in the routine of a Municipal Program of Tuberculosis Control. **Methods:** Direct microscopy of sputum and culture in Ogawa-Kudoh were performed on 1,636 samples from 787 patients. DST of positive cultures was performed by resazurin microtiter assay and genotyping by mycobacterial interspersed repetitive units–variable number tandem repeat. **Results:** A total 91 patients with TB were identified. The culture increased case detection by 32% compared with the microscopy; acquired resistance was 3.3% and the genotyping showed high genetic diversity. **Conclusions:** Ogawa-Kudoh contributed significantly to the increase in case detection and is suitable for implementation in poor-resource locations. The acquired resistance rate was lower than that reported in a recent Brazilian survey. The high genetic diversity is possibly related to the high TB prevalence in the population, as well as to early detection and suitable treatment of patients. The interaction between research and health care is important for reorienting the practice, transferring technology, and improving TB control.

Keywords: *Mycobacterium tuberculosis*. Diagnostic. Molecular epidemiology.

RESUMO

Introdução: O controle da tuberculose (TB) está relacionado com a disponibilidade de métodos de diagnóstico microbiológico qualificados, no entanto a microscopia com a sua limitada sensibilidade é o único método disponível em muitos locais.O objetivo deste estudo foi avaliar a introdução da cultura, teste de sensibilidade aos antimicrobianos (TSA) e genotipagem na rotina de um Programa Municipal de Controle da Tuberculose. **Métodos:**A baciloscopia direta do escarro e cultura em Ogawa-Kudoh foram realizadas em 1.636 amostras de 787 pacientes. O TSA das culturas positivas foi realizado pelo método de microdiluição e a genotipagem por *Mycobacterial Interspersed Repetitive Units – Variable Number Tandem Repeat* (MIRU-VNTR). **Resultados:** Foram identificados 91 pacientes com TB, com a cultura aumentando em 32% a detecção de casos em comparação com a microscopia; a resistência adquirida foi de 3,3% e a genotipagem mostrou alta diversidade genética. **Conclusões:** O cultivo em Ogawa-Kudoh contribuiu significativamente para o aumento na detecção de casos e é adequado para ser implementado em locais com poucos recursos. A taxa de resistência adquirida foi menor do que a relatada em recente inquérito nacional. A alta diversidade genética está possivelmente relacionada à alevada prevalência de TB na população, detecção precoce e tratamento adequado dos pacientes.A interação entre a pesquisa e serviço de saúde pública é importante para reorientar a prática, transferir tecnologia e melhorar o controle da TB.

Palavras-chaves: *Mycobacterium tuberculosis*. Diagnóstico. Epidemiologia molecular.

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INTRODUCTION

Tuberculosis (TB) control is a challenge for health professionals and society as a whole. According to estimates by the World Health Organization (WHO), the overall TB incidence is 137 per 100,000 inhabitants, while Brazil, among the 22 countries with the highest number of disease cases[1], has an incidence of 37 per 100,000 inhabitants[2]. The State of Rio Grande do Sul, located in Southern Brazil, has an incidence of 46 per 100,000 inhabitants[2], while the City of Pelotas, located in the south of this state, reported 64 cases per 100,000 habitants[3] in 2009.

TB control is necessarily linked to the availability of accurate, robust, and rapid methods for disease detection. Although several methods have been developed in recent years, the traditional sputum microscopy, with its limited sensitivity, remains virtually the only method available for TB laboratory diagnosis. Fast, effective, and inexpensive new laboratory methods have been validated, which makes their implementation in routine attractive. However, the transfer of these tools has been slow; this has been due, among other factors, to the difficulty of transferring knowledge between the academic and the health services. Thus, the aim of this study was to evaluate the introduction of culture, drug susceptibility testing (DST), and genotyping in the routine of a Municipal Program for TB Control (MPTC).

METHODS

Samples

The sample studied was composed of 787 patients with suspected TB, which represents the total number of patients seen in the MPTC laboratory of bacteriology from May 2009 to July 2010.

Culture, identification of clinical isolates, and susceptibility test

A total of 1,636 spontaneous sputum samples were processed by the method of culture in Ogawa-Kudoh[4] and were subsequently incubated at 37°C. Cultures were monitored weekly until 60 days of incubation. For all cultures identified as acid-fast bacilli, DNA extraction proceeded by thermal shock[5] and isolate identification through IS*6110* DNA amplification by polymerase chain reaction[6]. In addition, susceptibility testing to isoniazid (INH) and rifampin (RIF) was conducted using the resazurin microtiter assay method[7].

The contribution of culture to TB diagnosis was calculated as recommended by the Ministry of Health/Brazil, 2008[8].

$$\text{Culture contribution to the diagnosis} = \frac{(c)}{(a) + (b) + (c) + (d) + (e)} \times 100$$

where *a* is the number of cases with positive microscopy and culture, *b* is the number of cases with positive microscopy and unperformed culture, *c* is the number of cases with negative microscopy and positive culture, *d* is the number of cases with positive microscopy and negative culture, and *e* is the number of cases with positive microscopy and contaminated culture.

Genotyping

Genotyping of isolates was performed by the technique 12 loci – mycobacterial interspersed repetitive units–variable number tandem repeat (MIRU-VNTR) as described by Supply and colleagues[9]. For the analysis of genetic relationships dendrograms were built, performed via http://www.miru-vntrplus.org[10]. The discriminatory power of MIRU-VNTR was calculated using the Hunter Gaston index[11].

Ethical considerations

This research was approved by the Ethics Committee in Research of the Federal University of Rio Grande, under report number 29/2010 and was licensed by the Health Department of the Municipality of Pelotas/RS.

RESULTS

Culture in Ogawa-Kudoh and sputum direct microscopy

From the 787 patients included in the study 91 (11.6%) clinical isolates were obtained, identified as belonging to the *M. tuberculosis* complex; 35 (38.5%) of these patients had false negative results by sputum direct microscopy, of which eight (23%) were HIV positive. Eight cases with positive microscopy were characterized as contamination in the culture, while ten cases had positive microscopy and negative culture. Culture contribution to the TB diagnosis was 32%.

Epidemiological profile of TB cases identified by the culture

Of the 91 positive patients by culture, 72 were included in the analysis of epidemiological profile (**Table 1**), since 19 patients who were positive by culture were not found in the MPTC records. Fifty (69%) patients with TB were male, the average age was 39 years, and 51 (70.8%) were in the age group of 20 to 49 years. Seventeen (23.6%) cases studied corresponded to patients with previous TB history, and of these, 11 (64.7%) were cases of readmission after abandonment of treatment and six (35.3%) were relapse cases after cure. The use of alcohol and other drugs was recorded for 14 (19.4%) patients, among whom six (42.8%) were cases of readmission after abandonment of treatment.

Three resistant isolates were identified: one case of mono-resistance to INH and two cases of resistance to INH and RIF, characterizing as multidrug-resistant strains (MDR). All patients with TB caused by resistant strains had been previously treated for TB, representing a 3.3% rate of acquired resistance. None of the patients infected by resistant strains came from prison or had positive HIV serology. Regarding the geographic distribution of TB cases, 18% were concentrated in the most populous neighborhood in the city, followed by downtown (15%) and prison (11%). The remaining cases were scattered in neighborhoods of greater social vulnerability. Among the TB cases from prison, six (60%) were false negative by microscopic approach. A check of MPTC records showed that five were not on the list of patients initiated on treatment and one started the treatment without bacteriological confirmation. Two patients with false negative results were no longer prisoners of the penitentiary when the positive result by culture was received, so it was not possible to find them.

TABLE 1 - Epidemiological profile of tuberculosis cases identified by culture and registered in the Municipal Control Program of Tuberculosis, in Pelotas, State of Rio Grande do Sul, Brazil.

	Positive Ogawa-Kudoh culture	
Characteristics	**n**	**%**
Gender		
male	50	69.0
female	22	31.0
Age (in years)		
≥10	1	1.4
11 to 19	5	6.9
20 to 29	18	25.0
30 to 39	15	20.8
40 to 49	18	25.0
50 to 59	8	11.1
≥ 60	7	9.7
TB previous history		
yes	17	23.6
no	55	76.4
TB/HIV co-infection		
yes	10	3.9
no	51	70.8
no information on HIV test	11	15.3
Use of alcohol and other drugs		
alcoholic patients	7	9.7
patients taking other drugs	6	8.3
alcohol and other drugs	1	.4
total patients using alcohol or other drugs	14	9.4
non-users of alcohol or other drugs	58	80.5
Total patients	72	100.0

TB: tuberculosis; **HIV:** human immunodeficiency virus.

TABLE 2 -Allelic distribution of MIRU-VNTR 12 loci among 91 clinical isolates in Pelotas, State of Rio Grande do Sul, Brazil.

	Locus											
Copy number MIRU-VNTR	**2**	**4**	**10**	**16**	**20**	**23**	**24**	**26**	**27**	**31**	**39**	**40**
0	1	0	1	0	0	0	4	0	0	0	0	0
1	3	1	2	13	11	0	87	0	14	5	27	33
2	87	78	8	30	62	0	0	0	23	14	49	12
3	0	0	22	43	15	6	0	7	49	67	14	5
4	0	0	45	4	3	5	0	19	5	2	1	10
5	0	0	13	1	0	32	0	46	0	3	0	5
6	0	12	0	0	0	35	0	17	0	0	0	0
7	0	0	0	0	0	10	0	2	0	0	0	0
8	0	0	0	0	0	3	0	0	0	0	0	0
Allelic diversity												
H*	0.07	0.24	0.66	0.64	0.49	0.70	0.07	0.65	0.61	0.42	0.59	0.75
ID**	Low	Low	High	High	Mod	High	High	High	High	Mod	Mod	High

*$\mathbf{H:}\ 1 - \Sigma x_i^2\ [n/(n-1)]$; **ID: discriminatory index defined as high if greater than or equal to 0.6, moderate if less than 0.6 and greater than or equal to 0.3; and low if less than 0.3. **Mod:** moderate; **MIRU-VNTR:** mycobacterial interspersed repetitive units– variable number tandem repeat.

Genotyping of clinical isolates

Of the 91 isolates, 84 presented orphan pattern while seven were distributed into three clusters. No epidemiological link was identified among patients with isolates that contained the same molecular profile. The MIRU exhibited a high discriminatory power with a 0.999 Hunter-Gaston Discrimination Index (HGDI). The alleles with the highest discriminatory capacity were 10, 16, 23, 26, 27, and 40, while 2, 4, and 24 presented low differentiation power (**Table 2**). The 10 isolates from the prison, though not sharing an identical profile, were located near the dendrogram, differing in one or two loci (**Figure 1**).

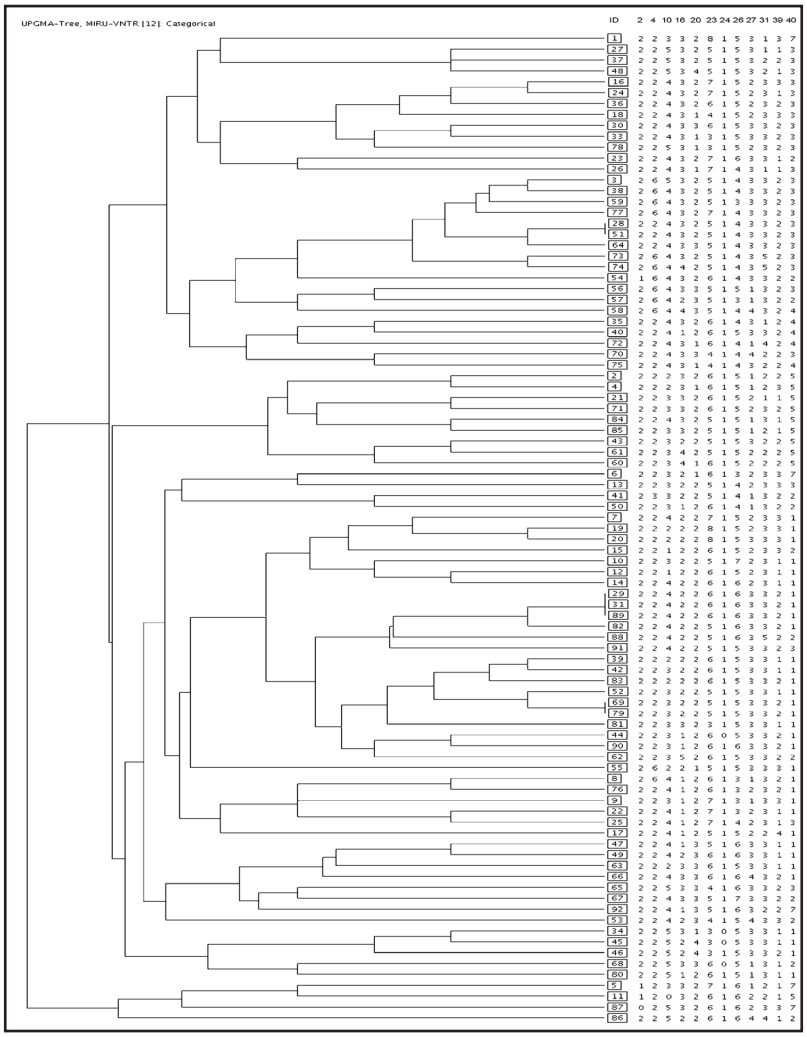

FIGURE 1 - Genotyping dendrogram by mycobacterial interspersed repetitive units-variable number of tandem repeat of clinical isolates in Pelotas, State of Rio Grande do Sul, Brazil*.

*Strains 3, 28, 38, 54, 56, 59, 64, 73, 74, and 77 were isolated from regional prison inmates.

DISCUSSION

The introduction of culture in routine TB diagnosis contributed to a 32% increase in TB case detection. This result is within the expected rate of 30% to 40% in sites whose samples are grown consistently for all respiratory symptomatics[8]. A similar study showed that the introduction of culture increased case detection by 21%[12]. The 10 cases of positive microscopy with negative cultures may indicate the presence of unviable bacilli in the sample, explained in five patients who were being treated. This fact confirms the validity of culture, not only for the diagnosis of new cases but also for the control of treatment effectiveness. The use of 2% NaOH for sample decontamination, although effective, is drastic for microorganisms and may also influence the viability of bacteria[7,12].

The 3.3% acquired resistance rate observed in this study is below the 15.3% and 7.5% acquired resistance to INH and MDR, respectively, found in the recent epidemiological survey conducted in seven states of Brazil[13].

Among the patients with resistant strains, none was HIV positive; the same was reported by a study conducted in Rio de Janeiro, Brazil[14]. Another study, also conducted in Rio de Janeiro, found no significant association between TB/HIV co-infection and resistance[15]. However, this is not the trend described in the international literature[16,17], and according to the WHO, a large proportion of missing data on MDR-TB makes it impossible to conclude whether there is an overall association between MDR-TB and the HIV epidemic[18]. This possibility of an association must

still be subject to further analysis in the municipality of Pelotas, since 50% of patients with HIV had a history of prior exposure to anti-TB drugs, and the TB/HIV co-infection rate found in this study (14%) corresponds to a WHO estimate for Brazil of 5% to 19% co-infection in 2009[19].

The WHO estimates that the overall incidence of MDR-TB is 3.6%[18]; however, less than 30,000 MDR-TB cases were reported in 2009, which corresponds to 12% of the total estimate (250,000)[19]. Thus, it is likely that a significant number of cases have not been diagnosed. The culture and DST are not performed routinely in most developing countries, collaborating with the underreporting of MDR-TB cases, hampering the resistance profile identification, increasing morbidity and mortality, and promoting the spread of resistant strains.

Regarding TB frequency by age and sex, a previous study in a similar model reported higher TB prevalence among men, with greater occurrence in the economically productive age group[12], which is also described in this work and other researches[20,21]. This epidemiological pattern, however, differs from that found in countries where the disease is better controlled and as a result of past exposure, the elderly population is the most affected[22].

Cases of patients with previous TB history included in this study accounted for 23.6%, and 64.7% of these had poor adherence to treatment. Factors related to non-compliance with anti-TB treatment may be of a different nature and have been linked, among others, to the use of alcohol and other drugs[23-25]. A study conducted between 1995 and 1996 in the same city showed that 20% of patients diagnosed in a year abandoned the anti-TB treatment; 24.2% of these patients were alcohol users[26]. Similar findings are in line with the situation revealed in this study; among patients who reported using alcohol and other drugs (19.4%), 50% were readmission cases after treatment abandonment.

Late diagnosis, such as that which occurred in the penitentiary, has serious implications when it deprives ill individuals of treatment, in addition to creating transmission risk beyond the prison walls. This fact reflects the need to use laboratory methods that may qualify the diagnosis in special populations, such as those deprived of liberty.

The fact that this study detected 91 TB cases by culture and only 72 were enrolled in the MPTC imposes questions about the reasons that led to the loss of 19 patients for epidemiological analysis. It is possible that these patients were not found in the MPTC records because the service does not have a computerized system for the registration of patients seen, and the search for data in medical record books may be susceptible to losses. On the other hand, this may be associated with MPTC difficulties in making an active search for TB cases. It should also be considered that methodological limitations imposed by retrospective data collection resulted in weaknesses in the analysis of the patients' epidemiological profile and suggest that data collection is prospective for studies of this nature.

Genotyping showed a high genetic diversity of isolates; a similar profile was described by previous studies in southern Brazil[5,27,28]. The high genetic diversity may be related to the high TB prevalence in the studied site, as well as to the early detection and appropriate treatment of patients. The alleles with higher and lower differentiation capacity agree with a previous study[5]. Some studies have shown that the use of MIRU isolates on their format of loci 12 has a limited discriminatory power, suggesting the use of 15 or 24 MIRU loci as tools for epidemiological studies. Our results, however, show a high discriminatory power of MIRU 12, which is consistent with previous studies[28,29]. This discrepancy may be related to differences in the TB prevalence in the population studied.

No epidemiological link was established among isolates with identical genetic patterns. This fact might suggest the occurrence of cross-contamination among these samples. However, this hypothesis was discarded since none of these samples was processed in the same period, there being months of difference on the date of entry into the laboratory; moreover, each patient had more than one positive culture. In this case, it can also be inferred that the investigation of contacts, as well as the treatment of index cases are properly carried out by MPTC[30]. Interestingly, isolates from populations in prisons, all in the same penitentiary, although not holding an identical genotypic profile, were phylogenetically close, differing in one or two loci. It has been shown that MIRU-VNTR has temporal stability that qualifies it as a robust method for studies on molecular epidemiology[31]. Nevertheless, a study proposing MIRU standardization as an epidemiological biomarker showed a possible variation among strains isolated from cases that are epidemiologically related[32].

Although we did not find clusters among strains coming from prison, the fact that these were institutional TB cases and were clinical isolates that were phylogenetically close shows the need to establish control programs suitable to the local reality. In this sense, interventions such as screening at the time of entry into prison using clinical evaluation, chest X-ray, sputum microbiological examination, and intradermal sensitivity test are recommended and are shown to be effective[33].

Culture in Ogawa-Kudoh contributed to a 32% increase in case detection and is an appropriate method to implement in poor-resource sites. The 3.3% acquired resistance rate is below those rates identified in a survey in Brazil. The MIRU-VNTR genotyping differentiated the 91 isolated strains in 84 orphan patterns and seven were distributed into three clusters. Therefore, the method obtained high discriminatory power with a 0.999 HGDI. The diversity of genotypes found may be associated with high TB prevalence in the city as well as the early detection and treatment of patients. The profile of patients included in this study shows no peculiarities, similar to that described by other studies performed in areas with high TB levels.

The interaction between research and health care mobilized professionals to search for measures that would enable the municipality to implement the culture method in Ogawa-Kudoh in the TB diagnostic routine. As a result, the method was no longer performed as a research project, as it was subsidized and carried out by the municipality. In this context, the knowledge and experience produced by the research contributed to the improvement of the TB control program, not only by transferring technology and information but also by generating data that provided subsidies to re-orient the practice.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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