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Surveillance of Drug-Resistant Tuberculosis in Spain (2001–2015)

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Multidrug-resistant (MDR) tuberculosis (TB) is a cause of increasing concern. This study investigated first-line anti-TB drug resistance in *Mycobacterium tuberculosis* strains submitted to the Tuberculosis Reference Center in Córdoba (Spain) between 2001 and 2015. A total of 1,207 cultures were tested against first-line drugs using the BACTEC MGIT 960 system. Resistance to first-line drugs was detected in 207 strains (17.2%), the greatest resistance being found in INH (5.3%) followed by streptomycin (3%), pyrazinamide (2.2%), rifampicin (1%), and ethambutol (0.2%). A total of 1.9% of strains were MDR. k strains displayed resistance to four drugs, and three strains to five drugs. In view of resistance observed, careful surveillance of drug resistance is recommended.

Keywords: tuberculosis, resistance, mycobacterium

Introduction

TUBERCULOSIS (TB) REMAINS A major global health problem, and multidrug-resistant (MDR) TB is a cause of increasing concern. In 2016, according to the World Health Organization (WHO), an estimated 10.4 million new cases of TB were notified, and an estimated 1.3 million people died of the disease, which remained one of the top 10 causes of death worldwide, ranking above HIV infection and malaria.¹

MDR-TB continues to constitute a major public health crisis, affecting an estimated 53 million people. The WHO reported 490.00 new cases of MDR-TB in 2016.¹

MDR in TB is by no means a new phenomenon; its incidence has varied over the years, depending on the drugs used to treat the disease.

As early as the 1950s, when the first specific anti-TB drugs were introduced, resistance soon developed to streptomycin when used alone.² To tackle this problem, combined treatments were developed, associating streptomycin with para-aminosalicylic acid and isoniazid (INH); this combined therapy, used over a period of 18–24 months, cured most treatment-naïve patients whose compliance was ensured by admission to hospitals or sanatoria.

In the course of the 1960s, the success of this therapy prompted a degree of overconfidence; rules were relaxed, and treatments started to be administered on an outpatient basis with insufficient supervision. This led to the emergence of resistance to INH, which proved problematic in some regions, where patients displayed resistance to both

streptomycin and INH.³ These early cases of MDR-TB could then infect others, who developed initial drug-resistant TB. This contagion prompted an increase in the number of patients with chronic disease, who—though not dying—increased the incidence of environmental drug resistance.⁴

In the 1970s, the situation was alleviated by the introduction of rifampicin and pyrazinamide; closely supervised short-term combined therapy (6–9 months) succeeded in curing cases of initial INH-resistant TB.^{5,6}

By the 1980s, excessive confidence in the theoretical efficacy of existing therapies—coupled with a lack of resources, insufficient healthcare, and other factors including emigration, AIDS, and drug addiction—prompted the emergence of resistance not only to rifampicin but also, more worryingly in health terms, to both INH and rifampicin, the two drugs most widely used to treat TB.⁷ It was at this point that the term MDR was coined.⁸ In 2014, an estimated 480,000 people developed MDR-TB worldwide. Reports suggest that MDR-TB currently accounts for over 1.2% of every 10,000 cases of TB, and over 4% of all new cases, causing around 150,000 deaths per year, of which the WHO estimates that only around 7%, are diagnosed.¹

The present study reports on resistance to first-line anti-TB drugs in *Mycobacterium tuberculosis* strains studied between 2001 and 2015 at the Cordoba Tuberculosis Reference Centre (Spain). The Reference Center receives samples from Andalusia, with a population of 8,379,880; and the proportion of samples with respect to the total number of samples in Spain represents a percentage of 18%.

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Materials and Methods

Study design

This cross-sectional study sought to investigate drug resistance in *M. tuberculosis* isolates recovered from culture-positive cases diagnosed at the Cordoba Tuberculosis Reference Centre (Spain) between 2001 and 2015. The study was conducted in accordance with international recommendations for drug-resistance surveillance.

Over the 15-year period under study, a total of 56,708 clinical specimens from patients with suspected TB were processed. *M. tuberculosis* was detected in 1,207 cultures from 1,207 patients, all of whom were tested for resistance to first-line anti-TB drugs: streptomycin, isoniazid, ethambutol, rifampicin, and pyrazinamide.

Cultures were viewed under a fluorescent microscope, decontaminated using a NaCl-NaOH solution, and inoculated into Bactec MGIT 960 TB tubes (Becton-Dickinson, Sparks) containing Middlebrook 7H9 liquid broth medium supplemented with PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) and OADC (oleic acid, albumin, dextrose, and catalase). Cultures were also grown on Lowenstein-Jensen and Coletsos media. Positive cultures were confirmed by Ziehl-Neelsen staining, and *M. tuberculosis* was identified first using the AccuProbe rRNA hybridization assay (San Diego, California).⁹ All *M. tuberculosis* cultures were tested for drug resistance, using ESPII system (Trek Diagnostic Systems, Westlake, Ohio).¹⁰

When the polymerase chain reaction was introduced, cultures were identified using the GenoType mycobacterium assays: CM (for 20 common mycobacterial species) and AS (for 17 additional species).¹¹ Both assays are based on DNA strip technology involving multiplex amplification with biotinylated primers and reverse hybridization.

All cultures were tested for sensitivity to streptomycin (SM), rifampicin (RIF), ethambutol (EB), isoniazid (INH), and pyrazinamide (PZA), following the MGIT 960 protocol, all in the form of lyophilized powder in vials.

Once reconstituted, 0.1 ml of each drug was added to vials containing Middlebrook 7H12 medium; the strain grown on Lowenstein-Jensen medium was then inoculated. Vials were incubated at 37°C and read daily until growth was sufficient to enable interpretation.

Statistical analyses

Epi Info v.7.2 software (Centers for Disease Control, Atlanta, GA) was used for data input and for statistical analyses, which took into account study design. The standard error (SE) was adjusted.

Where there were four or fewer drug-resistant cases, the exact binomial confidence interval was used, without reference to sample weights and without adjusting SE. For statistical analysis, Pearson χ^2 test, $p=0.05$ was used as the cutoff for statistical significance.

Results

All *M. tuberculosis* strains (1,207) were tested for first-line drug resistance. A total of 1,000 strains (82.8%) were sensitive to all the drugs, while 207 (17.2%) were resistant to one or more drugs (95%CI 14.9–19.3). Overall resistance

T1 results are shown in Table 1.

TABLE 1. RESISTANCE TO FIRST-LINE DRUGS (2001–2015)

Specimens	No.	% (95% CI)
Sensitives	1,000	82.8 (80.7–85.0)
Resistants	207	17.2 (15.0–19.3) <AUS
I. 1 ATM		17,2 (14,9-19,3)
ST	36	3.0 (2.0–4.0)
INH	64	5.3 (4.0–6.6)
EB	3	0.2 (0.1–0.7)
RF	12	1.0 (0.4–1.6)
PZ	27	2.2 (1.4–3.1)
II. 2 ATM		
ST+INH	19	1.6 (0.8–2.3)
ST+EB	2	0.2 (0.0–0.6)
ST+RF	2	0.2 (0.0–0.6)
ST+PZ	1	0.1 (0.0–0.5)
INH+EB	5	0.4 (0.1–1.0)
INH+RF	14	1.2 (0.5–1.8)
INH+PZ	4	0.3 (0.1–0.8)
III. 3 ATM		
ST+INH+EB	2	0.2 (0.0–0.6)
ST+INH+RF	2	0.2 (0.0–0.6)
INH+EB+RF	2	0.2 (0.0–0.6)
INH+EB+PZ	2	0.2 (0.0–0.6)
INH+RF+PZ	1	0.1 (0.0–0.5)
IV. 4 ATM		
ST+INH+EB+PZ	5	0.4 (0.1–1.0)
ST+INH+RF+PZ	1	0.1 (0.0–0.5)
V. 5 ATM		
ST+INH+EB+RF+PZ	3	0.2 (0.1–0.7)
Total	194	16.1 (14.0–18.2)
MDR	23	1.9 (1.1–2.7)

Cordoba Mycobacterial Reference Center.

ST, streptomycin; INH, isoniazide; EB, ethambutol; RF, rifampicin; PZ, pyrazinamide; MDR, multidrug-resistant.

The most widespread resistance was recorded for isoniazid (5.3% of all resistant strains; 95%CI 4.0–6.6), followed by streptomycin (3%; 95%CI 2.0–4.0), pyrazinamide (2.2%; 95%CI 1.4–3.1), rifampicin (1%; 95%CI 0.4–1.6), and finally ethambutol (0.2%; 95%CI 0.1–0.7).

Among strains resistant to more than one drug, the greatest resistance was recorded for streptomycin+isoniazid (1.6%; 95%CI 0.8–2.3), followed by streptomycin+rifampicin (1.2%; 95%CI 0.5–1.8); MDR strains resistant to other combinations accounted for far less than 1% (95%CI 0.1–1.0). Only six strains (0.5%; 95%CI 0.1–0.9) were resistant to streptomycin, isoniazid, ethambutol, and rifampicin, while three strains were resistant to all five drugs tested (0.4%; 95%CI 0.1–1.0).


A total of 1.9% (95%CI 1.0–2.8) of resistant strains displayed multi-resistance to isoniazid+rifampicin. However, resistance rates varied considerably over the 15-year study period, ranging from 7.6% (95%CI 5.9–9.3) in 2006, to 5.1% (95%CI 3.7–6.5) in 2005 and 3% (95%CI 1.9–4.1) in 2002; in other years, resistance rates approached the average, or no MDR strain was detected (2001, 2010, 2013, 2014).

Discussion

The overall resistance rates obtained here (11.8%) are higher than those reported for centers elsewhere in the Iberian Peninsula (Table 2), with the exception of those <T2

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TABLE 2. RESISTANCE TO FIRST-LINE DRUGS (2001–2015)

 Autor/specimens	Blanquer No. 519	Jiménez No. 956	Torres No. 428	Caminal No. 268	Alberte No. 918	Remache No. 258	Martin No. 1749
I. 1 ATM (%)							
ST	1.5	2.2	1.6	1.1	1.2	0.7	1.3
INH	2.7	3.3	2.6	1.1	3.3	5.0	3.1
EB	1.0	0.4	0.2		0.1	0.7	
RF	1.9	0.4	0.9	0.7	0.3	3.8	0.1
PZ	2.1	0.4	0.9				0.6
II. 2 ATM (%)							
ST+INH	1.9	0.1	2.1				0.3
ST+EB				0.4			
ST+RF							
ST+PZ							0.6
INH+EB	0.6	0.3	0.9	1.5			0.9
INH+RF	0.2						0.6
INH+PZ							
III. 3 ATM (%)							
ST+INH+EB		0.1					
ST+INH+RF	0.2		0.9				0.2
INH+EB+RF							0.6
INH+EB+PZ	0.2						
INH+RF+PZ			0.2				0.2
IV. 4 ATM (%)							
ST+INH+EB+PZ			0.5				
ST+INH+RF+PZ							
ST+INH+RF+PZ	0.2	0.1					0.1
INH+EB+RF+PZ	0.2						
V. 5 ATM (%)							
ST+INH+EB+RF+PZ	0.6	0.1					0.4
Total (%)	9.2	2.1	6.3	3.0	4.9	10.5	5.1
MDR (%)	1.9	2.2	0.9	1.5	0.1	2.7	1.9
Autor/specimens	Alvarez No. 100	Aznar No. 244	Arago No. 77	Idígoras No. 1011	Frutado No. 2358	Gomes No. 827	Perdigao No.3025
I. 1 ATM (%)							
ST	3.0	16.9	6.5	1.6	7.6		26.9
INH	4.0	7.4	3.9	4.9	2.6	9.4	9.1
EB	1.0	3.3	3.9	0.3	0		0.3
RF	1.0	2.5	2.6	1.1	0.6	3.9	0.1
PZ	1.0	4.0	2.6		1.3		1.2
II. 2 ATM (%)							
ST+INH	2.0	0.4	2.6				9.4
ST+EB							0.1
ST+RF							0.2
ST+PZ							0.3
INH+EB		0.8			8.0		2.9
INH+RF							1.2
INH+PZ							0.1
III. 3 ATM (%)							
ST+INH+EB	1.0		1.3				0.1
ST+INH+RF					18.9		4.1
INH+EB+RF							0.5
INH+EB+PZ			0.6				
INH+RF+PZ	1.0	0.4	6.2				
IV. 4 ATM (%)							
ST+INH+EB+PZ			9.1				0.1
ST+INH+RF+PZ			27.0				0.3
ST+INH+RF+PZ			4.0				15.0
INH+EB+RF+PZ							2.9
V. 5 ATM (%)							
ST+INH+EB+RF+PZ		1.2			25.7		18.6
Total (%)	10.0	34.0	19.5	7.9	12.1	12.1	24.2
MDR (%)	2.0	3.4	0.6	1.0	5.9	13.3	46.6

Centers consulted.

reported by Aznar for Madrid (34%) and by Aragón for Sautunto (19.5%).^{13,14}

The multi-resistance rate recorded here (1.9%) is lower than that reported by the authors themselves in an earlier study for the same area (5.8%), and considerably lower than the 34.0%, 10.5%, and 10% reported by Aznar (Madrid), Remacha (León), and Alvarez (Galicia), respectively.^{13,15,16}

With regard to findings for individual anti-TB drugs, resistance to streptomycin (3.0%) was higher than that reported by all except Aznar (16.9%) and Aragón (6.5%); other authors noted rates similar to those obtained here.^{17–25} Resistance to isoniazid (5.3%) exceeded that recorded by any other author except Aznar (7.4%), while the ethambutol resistance rate (0.2%) was lower than those obtained by all except Aragón (3.9%) and Aznar (3.3%). Resistance to rifampicin (1.0%) was also lower than that reported in the literature for Spain; Remacha obtained rates of 3.8%, Aragón 2.6%, and Aznar 2.5%. By contrast, resistance to pyrazinamide (2.2%) was higher than that reported by all authors except Aznar (4.0%) and Aragón (2.6%). Comparing our results to those published by WHO (Global Tuberculosis Report 2017),¹ the % of resistance is low in most countries, whereas it is maintained in Spain.

In terms of resistance to two or more drugs, the most striking finding was for the ST+R (1.6% resistance); higher figures are reported by Aragón,¹⁴ Torres (Zaragoza),¹⁶ Alvarez,¹⁷ and Remacha (2.6%, 2.1%, 2.0%, and 1.9%, respectively); all other authors report lower resistance rates. Resistance to the INH+RF combination (1.2%) was comparable only to that noted by Caminal (27%), lower rates being reported by all other centers. Resistance to other drug combinations tested (1% or lower) was relatively negligible.

Overall resistance findings were considerably lower than in Portugal (Table 2): Pertigao, for example, reported overall resistance of 24.16%, while Furtado recorded rates of 12.13%.^{29,30}

Multi-resistance rates also appear to be much higher in Portugal; Perdigao, Furtado, and Gomes report rates of 46.6%, 5.9%, and 13.3%, respectively.^{29–31}

Recorded rates of resistance to individual anti-TB drugs in Portugal are in some cases higher than those obtained here: Perdigao, for example, reported 26.9% resistance to streptomycin and 9.1% resistance to isoniazid; findings for other drugs, however, were similar to those recorded here.^{32–34}

It should be noted that resistance rates in our study were generally lower than those recorded in previous studies by the same authors. This may be due to earlier and swifter disease diagnosis, and improved clinical attention; constant patient surveillance ensures better compliance with combined drug therapies.

Even so, the findings reported here suggest that MDR, a phenomenon that emerged in the 1950s in response to early anti-TB drugs, continues to represent a challenge in the 21st century.

Swifter and more precise microbiological diagnosis—based on improved systems able to cut diagnosis times from days or weeks to a matter of hours—is essential to combat MDR-TB; the development of new, more efficacious, drugs would help to limit cross-resistance and could reduce treatment time from months to days. All this should be underpinned by the fastest-possible sensitivity testing.³⁵

Although resistance rates and MDR-TB prevalence remain relatively low, constant surveillance of MDR is essential to adequate health control.

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Disclosure Statement

No competing financial interests exist.

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