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# The demise of multidrug-resistant HIV-1: the national time trend in Portugal

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**Objectives:** Despite a decreasing mortality and morbidity in treated HIV-1 patients, highly active antiretroviral treatment (HAART) can still fail due to the development of drug resistance. Especially, multidrug-resistant viruses pose a threat to efficient therapy. We studied the changing prevalence of multidrug resistance (MDR) over time in a cohort of HIV-1-infected patients in Portugal.

**Patients and methods:** We used data of 8065 HIV-1-infected patients followed from July 2001 up to April 2012 in 22 hospitals located in Portugal. MDR at a specific date of sampling was defined as no more than one fully active drug (excluding integrase and entry inhibitors) at that time authorized by the Portuguese National Authority of Medicines and Health Products (INFARMED), as interpreted with the Rega algorithm version 8.0.2. A generalized linear mixed model was used to study the time trend of the prevalence of MDR.

**Results:** We observed a statistically significant decrease in the prevalence of MDR over the last decade, from 6.9% (95% CI: 5.7–8.4) in 2001–03, 6.0% (95% CI: 4.9–7.2) in 2003–05, 3.7% (95% CI: 2.8–4.8) in 2005–07 and 1.6% (95% CI: 1.1–2.2) in 2007–09 down to 0.6% (95% CI: 0.3–0.9) in 2009–12 [OR=0.80 (95% CI: 0.75–0.86); $P<0.001$]. In July 2011 the last new case of MDR was seen.

**Conclusions:** The prevalence of multidrug-resistant HIV-1 is decreasing over time in Portugal, reflecting the increasing efficiency of HAART and the availability of new drugs. Therefore, in designing a new drug, safety and practical aspects, e.g. less toxicity and ease of use, may need more attention than focusing mainly on efficacy against resistant strains.

**Keywords:** resistance, drug susceptibility, therapy failure, antiretroviral therapy, AIDS

## Introduction

In 2012, clinicians in higher-income countries can choose out of 25 different FDA-approved anti-HIV-1 drugs to set up a potent highly active antiretroviral therapy (HAART).[1] Nevertheless, in many patients not all options can be used, due to intolerance or side effects for certain drugs and because of the presence of antiviral drug (cross-)resistance.[2] This cross-resistance can be so severe that no efficient HAART can be composed, because nearly all drugs are affected.[3–5] Fortunately, multidrug

resistance (MDR) to anti-HIV-1 drugs has been decreasing in recent years in patients failing therapy and drug developers have been encouraged to focus their research on new potent drugs with a better tolerability, ease of use and less toxicity.[6,7] This paper analysed the trend of MDR in Portugal from the moment that resistance testing became routine clinical practice in cases of treatment failure—i.e. July 2001—until the current date—i.e. April 2012. We aimed to quantify to what extent the issue of MDR still exists today.

## Patients and methods

A Portuguese database, covering 22 hospitals located over the entire country and containing HIV-drug resistance data and clinical data of HIV-infected patients, was consulted in April 2012. Resistance data and, if available, start date of initial therapy were retrieved for 8065 HIV-1-infected patients who had undergone at least one drug resistance test from July 2001 to March 2012. No Ethics Committee approval was required. The vast majority of samples were tested at Hospital Egas Moniz in Lisbon, the major reference laboratory in Portugal. The Rega algorithm (version 8.0.2) was used to interpret the susceptibility to drugs for 10286 viral *pol* sequences. The studied drugs belong to the three major drug classes (nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors) and all are authorized by the National Authority of Medicines and Health Products (INFARMED) in Portugal.[8] A virus was defined to be multidrug resistant at a specific date of sampling, when no more than one drug authorized by the Portuguese INFARMED (but excluding entry and integrase inhibitors) was still fully active at that timepoint (see Figure 1), reflecting the difficulty of selecting an efficient HAART regimen. The prevalence of MDR in a certain time interval was calculated as the proportion of patients with MDR among those who had at least one sample for resistance testing in that time interval. Prevalence per 2 years was modelled over time and graphically visualized using a (univariate) Poisson regression model. The majority of the patients (81.1%) had only one resistance test in the dataset. Nevertheless, a generalized linear mixed model (GLMM) was used in order to test the significance of the time trend of drug resistance, taking into account the correlation among multiple samples per patient. Finally, the model was adjusted for those confounding factors for which there were data available: duration on therapy and incomplete initial start date of therapy. The data were analysed using the free statistical software R (version 2.10.1).

## Results

We observed a decrease in the prevalence of MDR over the last decade, from 6.9% (95% CI: 5.7–8.4) in 2001–03, 6.0% (95% CI: 4.9–7.2) in 2003–05, 3.7% (95% CI: 2.8–4.8) in 2005–07 and 1.6% (95% CI: 1.1–2.2) in 2007–09 down to 0.6% (95% CI: 0.3–0.9) in 2009–12. Prevalence and Poisson trend lines are shown in Figure 2. This decreasing trend was confirmed to be statistically significant by GLMM: for every consecutive year, the odds of having multidrug-resistant cases decreased by 17% (OR=0.83; 95% CI: 0.82–0.84; $P<0.001$). Furthermore, GLMM showed significant effects for the confounding factors: for every extra year on therapy, the odds of evolving to MDR increased by 23% (OR=1.23; 95% CI: 1.21–1.24; $P<0.001$). Patients with an incomplete initial start date of therapy ($n=1148$, 14.2%) were more than three times more likely to develop MDR (OR=2.67; 95% CI: 2.41–2.96; $P<0.001$), which may reflect the fact that these patients started therapy before the HAART era, when therapy initiation records were not recorded electronically.

## Discussion

Over the last three decades, antiretroviral therapy has become more effective and drug resistance testing strategies have improved. Despite a clear pattern of increasing antiretroviral resistance in lower-income settings,[9,10] there have been recent reports on declining trends of resistance in high-income countries.[6,7,11,12] Therefore, we questioned whether current regimens are still selecting for MDR. Our previous analysis showed a significant decreasing trend of MDR from 2001 to 2006, but used a rather conservative approach by taking into account only those drugs available over the total study period.[7] Moreover, if a patient had more than one sample for resistance testing, consecutive samples were only taken into consideration if an earlier sample did not show resistance, leading to the calculation of incidence, instead of the more intuitive prevalence, and causing data loss. In this study, all anti-HIV-1 drugs were considered as potential candidates for setting up a new treatment, from the moment they were authorized by the Portuguese INFARMED. This is clinically more relevant as these drugs

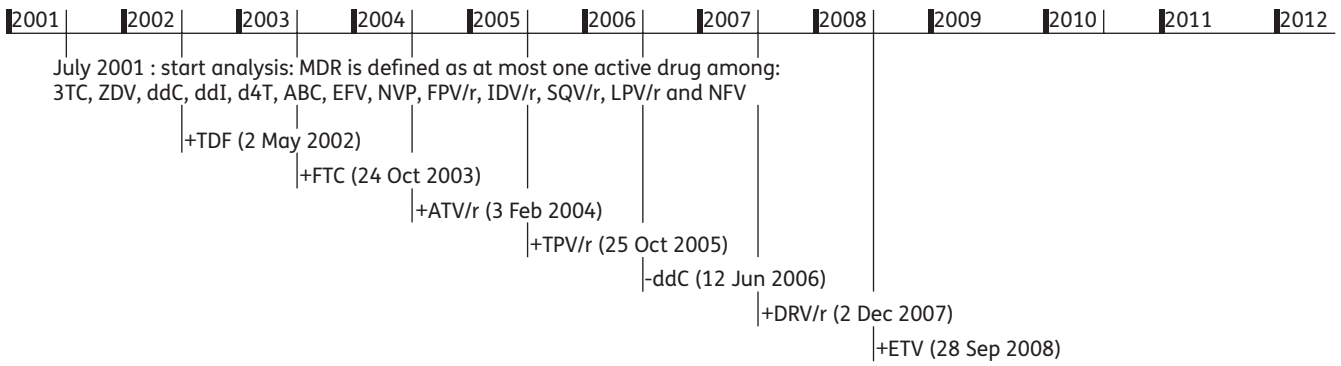


**Figure 1.** Overview of anti-HIV drugs used in the definition of MDR based on dates of authorization by the Portuguese INFARMED. A virus was defined to be multidrug resistant at a specific date of sampling, when no more than one drug on this list was still fully active at that timepoint. 3TC, lamivudine; ZDV, zidovudine; ddC, zalcitabine; ddI, didanosine; d4T, stavudine; ABC, abacavir; EFV, efavirenz; NVP, nevirapine; FPV/r, boosted fosamprenavir; IDV/r, boosted indinavir; SQV/r, boosted saquinavir; LPV/r, boosted lopinavir; NFV, nelfinavir; TDF, tenofovir; FTC, emtricitabine; ATV/r, boosted atazanavir; TPV/r, boosted tipranavir; DRV/r, boosted darunavir; ETV, etravirine.

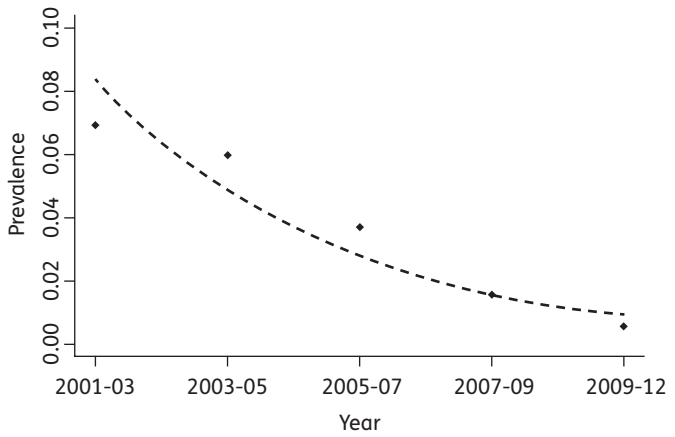


**Figure 2.** Time trend of prevalence per 2 years of multidrug-resistant HIV-1 in Portugal. The prevalence in a certain 2 year interval was calculated as the proportion of patients with MDR among those who had at least one sample for resistance testing in that time interval. The time trend was graphically visualized using a (univariate) Poisson regression model (broken line).

represent the choices that the treating clinician had at the time of therapy failure. The following drugs were not taken into account and can still exhibit antiviral activity: maraviroc (information on viral tropism was not available for all patients), enfuvirtide (information on *gp41*-associated resistance was not available), raltegravir (integrase genotype information was not available for all patients) and rilpivirine (no rules for this newest drug are available as yet in the Rega algorithm); thus, the latest MDR prevalence may even be lower. Furthermore, by using a GLMM, the correlation between resistance information of consecutive samples of the same patient is taken into account. Nevertheless, since routine resistance testing in cases of treatment failure in Portugal was consistently conducted only from July 2001 onwards, only samples from that date onwards were taken into consideration, reducing the bias of left censoring. Following this approach, we saw a statistically significant downwards trend of MDR (2001–12). This result was corrected for two confounding factors: (i) the duration on therapy at which patients are genotypically tested; and (ii) the fact that for a number of patients the initial start date of therapy was not exactly known, which may reflect the fact that these patients started therapy before 1998, when therapy initiation records were finally kept electronically. It can be argued that at the beginning of our study period, resistance testing started to be implemented in routine clinical practice, such that relatively more advanced and drug resistance patients were tested for the first time (left-censoring bias). To cope with this problem, patients with samples that showed MDR before July 2001 were excluded from the analysis of MDR. While this may not have eliminated the bias entirely, the continuing decreasing trends of MDR even in the most recent years suggests that these are genuine.

In Portugal, multidrug-resistant HIV-1 seems to vanish over time: in July 2011 the last new case of MDR was seen (incidence data not shown). No more new cases of MDR does not mean that resistance will disappear completely, as can be seen by several drugs that are still showing a high prevalence of resistance in 2012.[13] Resistance to the newest agents can emerge, resulting in the appearance of novel drug resistance mutations in the HIV-1 polymerase, integrase and envelope genes.[5] Moreover, as a consequence of ongoing transmission of drug resistance, newly infected patients may already have drug-resistant virus. However, as the list of candidate drugs for use in follow-up regimens after therapy failure is still expanding, the occurrence of multidrug-resistant HIV-1 for which an efficient treatment with at least three potent drugs is not possible is increasingly rare. Moreover, the demise of MDR follows the improving therapy and drug resistance testing strategies in high-income countries.

These results are based on nationwide data from the past decade in Portugal and, in our opinion, are likely to reflect resistance trends in other high-income countries with similar access to antiretroviral therapy regimens. This information is of great importance for drug developers, who are encouraged to focus more on better tolerability and ease of use of the drug rather than developing potent antiretroviral agents targeted at overcoming resistance.

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## Transparency declarations

None to declare.

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