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Public health impact of isoniazid-resistant *Mycobacterium tuberculosis* strains with a mutation at amino-acid position 315 of *katG*: a decade of experience in The Netherlands

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ABSTRACT

A previous limited study demonstrated that *Mycobacterium tuberculosis* isolates with a mutation at amino-acid position 315 of *katG* (Δ315) exhibited high-level resistance to isoniazid and were more frequently resistant to streptomycin. In the present study, isoniazid-resistant *M. tuberculosis* isolates from 8332 patients in The Netherlands (1993–2002) were screened for the Δ315 mutation. Isoniazid resistance was found in 592 (7%) isolates, of which 323 (55%) carried Δ315. IS*6110* restriction fragment length polymorphism analysis showed that Δ315 isolates occurred in clusters, suggesting recent transmission, at the same frequency as isoniazid-susceptible isolates. In contrast, other isoniazid-resistant isolates clustered significantly less frequently. Δ315 isolates were high-level isoniazid-resistant, streptomycin-resistant and multidrug-resistant significantly more often, and may have a greater impact on public health, than other isoniazid-resistant isolates.

Keywords IS*6110*, isoniazid resistance, *katG* (Δ315 mutation), *Mycobacterium tuberculosis*, public health impact, restriction fragment length polymorphism analysis, The Netherlands

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INTRODUCTION

Despite the availability of various drugs with activity against *Mycobacterium tuberculosis* and worldwide bacille Calmette–Guérrin vaccination, tuberculosis (TB) is the second most frequent infectious cause of death worldwide [1]. The emergence of drug resistance, and especially multidrug resistance (i.e., resistance to at least isoniazid and rifampicin), among strains of *M. tuberculosis* has become a major health threat in various parts of the world [2]. One of the mainstay drugs for the treatment of TB is isoniazid. Its effectiveness against *M. tuberculosis* was discovered simultaneously by three groups in 1952 [3–5], but resistant strains were reported shortly thereafter [6]. In The Netherlands, an isoniazid resistance level of 7% was reached in 1993–1997 [7], but resistance levels of up to 30–40% have been reached in several high-incidence countries [8].

Resistance against isoniazid is associated mostly with mutations or deletions in *katG*. This gene encodes the enzyme catalase peroxidase, which converts isoniazid into an active compound (isoniazid itself has no mycobactericidal activity) [9–11]. Other resistance mutations occur in the *inhA* gene (or its promoter), which encodes an enoyl acyl carrier protein reductase involved in fatty acid synthesis, which is the

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target of the active derivative of isoniazid [12]. Mutations in several other genes have been reported to be associated with isoniazid resistance, but occur less frequently, and their association with isoniazid resistance is less clear [13]. The most frequent mutation occurs at amino-acid position 315 of *katG* (Δ315), and this mutation accounts for 53–96% of resistance mutations among isoniazid-resistant isolates [7,14–18].

Part of the success of the Δ315 isolates is probably caused by the fact that catalase peroxidase is still active in these mutants; indeed, 30–40% of the initial catalase activity remains when this mutation is introduced into *katG* with site-directed mutagenesis [19]. A previous study in The Netherlands revealed that Δ315 isolates were found in clusters as frequently as isoniazid-susceptible isolates, whereas isoniazid-resistant isolates with another mechanism of resistance appeared to be transmitted less frequently (reflected by a lower percentage of clustered isolates). However, these differences were not significant. Furthermore, Δ315 isolates were reported to be associated significantly with high-level resistance to isoniazid and with additional streptomycin resistance [7].

In the present study, the prevalence of the Δ315 mutation was determined among 8332 *M. tuberculosis* isolates sent to the National Institute of Public Health and the Environment (RIVM, Bilthoven, The Netherlands) between 1993 and 2002. The associations between the presence of this mutation and other laboratory and clinical data were examined to determine the significance of the basis of isoniazid resistance in relation to the potential impact on public health.

MATERIALS AND METHODS

Between 1993 and 2002, *c.*10 000 isolates of *M. tuberculosis* from TB patients in The Netherlands were submitted to the RIVM for species identification, drug susceptibility testing and IS*6110* restriction fragment length polymorphism (RFLP) typing [20,21]. Patient information was obtained from The Netherlands Tuberculosis Register, maintained by the KNCV Tuberculosis Foundation, which has been in place since 1993. The Netherlands Tuberculosis Register lists patient information anonymously; therefore, patient information was matched with laboratory information, using gender, date of birth, postal area code and year of diagnosis to identify matches.

The susceptibility of all isolates to isoniazid, streptomycin and rifampicin was determined with the MIC method [22], testing concentrations of 0.1, 0.5, 1, 2, 5, 10, 20 and 50 mg/L in 7H10 medium (Difco, Detroit, MI, USA). The isolates were considered to be resistant if >1% of the original inoculum grew on concentrations of at least 0.5, 10 and 2 mg/L for isoniazid, streptomycin and rifampicin, respectively. Multidrug resistance was defined, according to the definition of the WHO, as resistance to at least isoniazid and rifampicin.

Isoniazid-resistant isolates were investigated for the presence of the Δ315 mutation by PCR restriction endonuclease analysis with either *Aci*I [17] or *Msp*A1I [23] for the isolates obtained in 1993–1997, or by DNA sequencing of a 127-bp fragment of *katG*, using primers 315MGB-s and 315MGB-as [24], for the isolates obtained in 1998–2002. On the basis of these assays, the isoniazid-resistant isolates were divided into two groups: those with the Δ315 mutation, and other isoniazid-resistant isolates.

Standard RFLP typing was performed with IS*6110* as a probe [21]. If fewer than five bands were present in the RFLP pattern, polymorphic GC-rich sequence RFLP typing was also performed [20]. The term 'cluster' was used for two or more *M. tuberculosis* isolates with completely identical RFLP patterns, or for the respective patients. Based on IS*6110* RFLP analysis or spoligotyping, isolates were assigned to the Beijing genotype according to international guidelines [25].

ORs were calculated with the Epi6 program (CDC, Atlanta, GA, USA). Binary logistic regression to adjust for possible confounders (all variables were taken into account) was performed with SPSS v.11.5.2 software (SPSS Inc., Chicago, IL, USA).

RESULTS

During the period 1993–2002, *c.*15 000 cases of TB were recorded in The Netherlands. Bacteria belonging to the *M. tuberculosis* complex were cultured from *c.*10 000 cases and were submitted to the RIVM [26]. Laboratory data from the RIVM were matched successfully with clinical data from the Netherlands Tuberculosis Register for 8332 patients. For these patients, all variables were known, except the results of microscopy (3495; 42%) and data concerning previous episodes of TB (7305; 88%). Of the 8332 patients, 592 (7.1%) yielded isoniazid-resistant *M. tuberculosis*, and 74 (0.89%) yielded multidrug-resistant (MDR) *M. tuberculosis*. PCR analysis showed that 323 isoniazid-resistant isolates (55%) had the Δ315 mutation, and of these, 95% had an isoniazid MIC >2 mg/L. In contrast, only 14% of isoniazid-resistant isolates without this mutation had an MIC >2 mg/L. Among all isoniazid-resistant isolates with an MIC >2 mg/L, 89% had the Δ315 mutation (Fig. 1).

Because of the different characteristics of isolates from Dutch patients and immigrants, the dataset was split into Dutch (n = 3437; 41%) and non-Dutch (n = 4895; 59%) patients. The countries