

Imported extensively drug-resistant *Mycobacterium tuberculosis* Beijing genotype, Marseilles, France, 2011

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Multidrug- (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) are reported to gradually spread across European countries with low TB prevalence including France. Some isolates may even accumulate traits of resistance in addition to the XDR profile, as a result of therapeutic mismanagement. We report here the first case of XDR TB in Marseilles and discuss the potential effectiveness of sulfamide treatment in such cases.

Extensively drug-resistant (XDR) *Mycobacterium tuberculosis* strains are defined as resistant to isoniazid and rifampicin (multidrug-resistant, MDR) and at least to one fluoroquinolone and one of the following antibiotics: amikacin, capreomycin or kanamycin [1]. Hence, treatment options become very restricted, further complicating the management of cases and constituting a potential public health threat calling for particular vigilance [2]. By the end of 2010, 68 countries including 19 of the 27 countries in the European Union had reported at least one XDR-TB case [1]. XDR-TB is an emerging issue in Europe, illustrated by the present report of the first case infected with an XDR *M. tuberculosis* strain detected in Marseilles.

Case report

A 63-year-old Russian woman originating from the Republic of Dagestan was admitted on 11 January 2011 to our Departement for Infectious Diseases and Tropical Medicine in Marseille, France. The patient had a medical history of pulmonary tuberculosis (TB) first diagnosed in 2008 in Russia. She reported having undergone three sequential lines of anti-tuberculosis treatments including a combination of isoniazid, rifampicin, pyrazinamide, ofloxacin and kanamycin, but could not provide any further details. At the time of her arrival in France on 21 December 2010, she was treated by levofloxacin alone and spent three weeks in her daughter's home before being admitted to our department for persistent febrile cough and a major

weight loss. At the time of her admission, the physical examination found a body-mass index of 17 and diffuse rhonchi. Chest radiography and computed tomography scan highlighted multiple excavated lesions and infiltrates of both upper lobes of the lungs. The first sputum and stool specimens (a non-invasive specimen replacing gastric fluid) processed in our laboratory [3] on 13 January 2011 exhibited >100 acid-fast bacilli per power field after Ziehl-Neelsen staining.

Molecular analyses

Subsequent molecular identification by 16S-23S intergenic spacer-based real-time PCR [4] and the GeneXpert system (Cepheid, Toulouse, France) [5] detected the presence of *M. tuberculosis* complex (MTC) DNA and drug resistance to rifampicin. Multispacer sequence typing (MST) specified an MST4 profile [6] and the pyrosequencing analysis of the Rv0927c gene and the Rv0927c-pstS3 intergenic region showed a Beijing genotype [7]. A bacterial growth was detected after 14 days of automated liquid culture (BD Bactec MGIT 960, Sparks, Maryland); further culture in the presence of drugs confirmed resistance to first-line anti-tuberculosis drugs including isoniazid, rifampicin, streptomycin, ethambutol, pyrazinamide, and further to kanamycin. The minimum inhibitory concentration (MIC) of trimethoprim-sulfamethoxazole (TMP-SMX) was determined by the dilution method using the Bactec MGIT supplemented medium tubes, in the presence of a growth control. After a five-day incubation period, no bacterial growth was detected at concentrations of TMP-SMX $\geq 1.6/8.3$ µg/ml. Sequencing showed the -15C/T and S315T mutation in the *inhA* regulatory region and in the *katG* gene, which confirmed high level resistance to isoniazid and associated resistance to ethionamide, the D516Y mutation in the rifampicin resistance-determining region of the *rpoB* gene, the K43R mutation in the *rpsL* gene (resistance to streptomycin) and the G to A substitution at position 119 resulting in a nonsense mutation in the *pncA* gene (locus associated with

resistance to pyrazinamide). While no mutation was found in the *gyrB* gene, we detected two mutations D94G and S95Y in the *gyrA* gene known to cause fluoroquinolone resistance. No additional mutation was found in the 526 bp partial sequence of the *embB* gene (including the 306 codons associated with ethambutol resistance) or in the *rrs* gene (locus associated with resistance to aminoglycosides).

Treatment

Based on these laboratory results, the treatment regimen was switched to the combination of ethambutol, linezolid, para-aminosalicylic acid, cycloserin, amikacin and TMP-SMX. At the time of publication of the present report, the patient is still under treatment with this antibiotic combination and a clinical improvement has been observed so far including apyrexia. Additionally, two sputum specimens collected eight weeks after initiation of this antibiotic combination treatment showed, for the first time, no acid-fast bacilli after Ziehl staining and direct microscopic examination.

Discussion

The 1 % prevalence of resistant TB observed in Marseilles has been unexpectedly low so far, even compared to the prevalence reported at national level in France [8]. Our mycobacteriology reference laboratory routinely collects respiratory tract specimens from all four tertiary care hospitals of Marseilles covering a population of approximately 1 million. Between 1 January 2001 and 1 January 2011, 18,778 respiratory tract samples yielded a low prevalence of 384 *M. tuberculosis* isolates (2%), including a very low prevalence of five MDR isolates and no XDR isolate. In France, between 38 and 60 MDR TB cases were reported annually from 2001 through 2009 [8]. The first XDR TB case was detected in 2002, one to two cases were reported annually from 2003 through 2008 and four cases in 2009 (amounting to 8% of the MDR cases) [8].

The isolate reported here had accumulated several phenotypic and molecular traits of resistance in addition to the XDR profile. Such a highly resistance pattern may have resulted from a multistep process combining both primary and secondary resistance. The patient had been treated in Russia, a country ranking third for the prevalence of resistant tuberculosis [9]. The acquisition of additional resistances may have been secondarily facilitated by the sequential lines of anti-tuberculosis treatments. The resulting resistance pattern inherently limited the choice of anti-tuberculosis drugs available for treating the patient, leading to the initiation of long-term potentially harmful intravenous therapy.

The lack of active anti-tuberculosis drugs has recently led to a renewed interest in neglected molecules such as TMP-SMX [10], a combination known to be particularly effective for the treatment of pulmonary infections such as pneumocystosis. Currently, no standardised method based on clinical correlation studies has been validated to determine the susceptibility of

M. tuberculosis strains to sulfamides. Based on a daily oral dose of 960 mg/4,800 mg TMP/SMX prescribed to eight non-tuberculosis patients in our hospital, we observed a mean serum concentration of 4.4/81.5 µg/ml, nearly 10 times higher than the MIC of the *M. tuberculosis* isolate described here. TMP-SMX might be considered as an alternative, cheap and overall well tolerated second-line antibiotic to treat highly resistant pulmonary tuberculosis, warranting further investigations.

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Patient consent

The patient had given written informed consent to the publication of this report.

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