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1 Rapid emergence of *Mycobacterium tuberculosis* bedaquiline resistance: lessons not
2 to repeat past errors

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43 Bedaquiline (BDQ), has demonstrated potent clinical activity against multi (MDR) and
44 extensively (XDR) drug resistant *M. tuberculosis* complex strains [1–3]. It has now
45 been used in more than 50 countries, and it is estimated that approximately 2500
46 patients have been treated by BDQ by the end of 2015. In spite of a recent clinical use,
47 there are few reports of BDQ resistant strains [4, 5]. Mutations in the *rv0678* gene
48 encoding the MmpL5 efflux pump repressor generate low-level BDQ resistance and
49 clofazimine (CFZ) cross-resistance [6]. This mechanism is, to our knowledge, the sole
50 mechanism of BDQ resistance described in clinical strains [4, 5]. Despite a recent
51 introduction in France in 2011 for XDR and MDR-TB treatment, we report herein four
52 BDQ-resistant cases, and discuss strategies to avoid a surge of BDQ resistance.

53 In France, all MDR *M. tuberculosis* complex strains are sent to the National Reference
54 Center for Mycobacteria (NRC) for complete genotypic and phenotypic drug
55 susceptibility testing (DST), including BDQ since 2014. BDQ minimum inhibitory
56 concentration (MIC) was measured in 7H11 medium in polystyrene petri dishes for all
57 strains that were resistant to a screening concentration of 64 mg/L in Lowenstein-
58 Jensen medium. For each MIC measure, H37Rv was included as susceptible control.
59 For all strains screened as BDQ resistant, the *atpE* and *rv0678* genes were sequenced
60 [4]. BDQ dry powder was kindly supplied by Janssen. The clinical history of patients
61 harbouring BDQ-resistant strains was retrospectively abstracted from medical records.
62 Ethics approval of the study protocol was granted by Bligny Hospital Institutional
63 Review Board.

64 Between January 1st 2014 and December 31st 2015, 209 MDR, including 40 XDR, *M.*
65 *tuberculosis* complex strains were routinely tested at the NRC for BDQ susceptibility.
66 Among these, 4 (2%) had elevated BDQ MICs, between 0.25 and 0.5 mg/L as

67 compared to 0.03 mg/L for the H37Rv reference strain. The genotypic and phenotypic
68 characteristics of these 4 unrelated strains are summarized in the table.

69 Patient 1, born in Pakistan, had no past history of tuberculosis or leprosy treatment
70 and never received BDQ nor CFZ. He was diagnosed with bone and pulmonary MDR-
71 TB on arrival in France in January 2015. His strain isolated before any tuberculosis
72 treatment displayed BDQ resistance.

73 Patient 2 was treated for MDR-TB in Romania from September 2013 to November
74 2014 but has never received BDQ nor CFZ. He was diagnosed in France with cavitary
75 lung MDR-TB in October 2015. The strain isolated at that time displayed BDQ
76 resistance.

77 Patient 3 has been treated in Georgia for 24 months from 2011 to 2013 for MDR-TB
78 with ethionamide (ETH), para-aminosalicylic acid (PAS), capreomycin (CAP),
79 cycloserine (CYC), amoxicillin-clavulanate (AMC), a regimen subsequently
80 supplemented with pyrazinamide (PZA) and BDQ for the last 4 months. In January
81 2014, he was diagnosed in France with cavitary lung XDR-TB, with a BDQ-resistant
82 strain.

83 Patient 4 arrived in France from Georgia in 2013 after a 10-year history of tuberculosis
84 treatment, with extensive bilateral cavitary lung lesions. In July 2013, he started
85 treatment with PZA, amikacin (AMK), ETH, PAS, linezolid (LNZ), ethambutol (EMB)
86 and BDQ. The strain isolated on arrival in France was only susceptible to AMK, PAS,
87 LNZ, and had BDQ MIC in the normal range. In August 2013, EMB and LNZ were
88 withdrawn, due respectively to in vitro resistance and to peripheral neuropathy, and
89 imipenem (IMP)/AMC was introduced. He became sputum culture negative in October
90 2013. In December 2013, IMP/AMC was stopped and CFZ was introduced. In

91 September 2014, AMK was stopped. In October 2014, the patient reverted to sputum
92 culture positivity. DST of the 2014 strain showed the same susceptibility profile as the
93 initial 2013 strain, except for appearance of BDQ resistance.

94 The rapid appearance of BDQ resistance is alarming. These 4 cases can be grouped
95 into 2 profiles, which raise different questions.

96 The first profile is the one of patients 1 and 2 who had never been treated with BDQ.
97 Considering that tuberculosis is transmitted from a patient to another, one can
98 hypothesize that BDQ resistance was selected by BDQ or CFZ treatment in the
99 transmission chain. This is unlikely for BDQ, because of its recent use. CFZ however,
100 may have been prescribed for a non-tuberculous disease such as leprosy. Finally,
101 other compounds could select for BDQ resistance through *rv0678* mutations, because
102 the recognized mechanism of resistance affects an efflux pump. Altogether, these data
103 underline the importance of including BDQ DST in national programs implementing
104 BDQ for the treatment of MDR or XDR TB.

105 In patients 3 and 4, the background regimen failed to prevent the selection of drug
106 resistance. Reviewing DST results and treatment histories, it appears that, except for
107 BDQ, the only likely active drugs were PAS (for both patients) and AMK (for patient 4).
108 We previously described a similar case in which fluoroquinolone resistance was
109 selected despite PAS and AMK co-administration [7]. Recently, a case of BDQ
110 resistance was reported despite EMB, PAS, CAP, and CYC combined therapy [8]. Of
111 note, when BDQ was switched for delamanid (DLM), DLM resistance was
112 subsequently selected [8]. The 2013 WHO recommendations on BDQ use do not
113 address the effectiveness of BDQ companion drugs. Regimens in which BDQ is only
114 combined with drugs with poor bactericidal activity or poor tissue diffusion such as
115 PZA, PAS, CYC, EMB, CFZ or aminoglycosides, may not prevent the selection of BDQ

116 resistance. We suggest that BDQ should be always associated with at least one drug
117 with both bactericidal and sterilizing activity for the full treatment duration in order to
118 avoid selection of BDQ resistance. Fluoroquinolones, LNZ, DLM, and maybe ETH,
119 may be considered in this category since they have proved in vivo activity [3, 9].
120 Furthermore, recent data suggest that the combination of DLM and BDQ is safe [10,
121 11]. If the number of remaining bactericidal drugs is low and there are important
122 destructive lung lesions, surgery should be considered [12].

123 The definition of BDQ resistance is currently not completely established. EUCAST
124 adopted 0.25 mg/L as susceptibility breakpoint in 7H11 [13] which is above the MIC 90
125 (0.12 mg/L) [14]. However, with such breakpoint, the strain responsible for clinical
126 failure in patient-4 (MIC 0.25 mg/L, 16-fold higher than before BDQ treatment) would
127 have been categorized as susceptible. The clinical impact of *rv0678* mutations
128 conferring elevated MICs in the 0.25-0.5 mg/L range, i.e. below the usual BDQ serum
129 level (3-4 mg/L) could be a matter of discussion. A murine experiment has shown that
130 *rv0678* mutations increasing BDQ MICs by 4- to 8-fold reduce the 1-month BDQ
131 bactericidal activity by 1 log₁₀ CFU [4]. Conversely, a clinical study suggested that
132 there was no impact on treatment efficacy of BDQ containing combined regimen [3].
133 Nevertheless, the case of patient 4, who became initially sputum culture negative but
134 relapsed with positive cultures after 16 months of a BDQ-containing regimen, suggests
135 that the clinical impact of such mutations could be meaningful. Finally, it seems that
136 there is an overlap between MICs of wild-type and mutant strains which may require a
137 2 breakpoint classification with an intermediate category as for other antituberculous
138 drugs.

139

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145

146

147 Table: characteristics of the 4 bedaquiline resistant *M. tuberculosis* strains

Patient	Previous treatments	<i>M. tuberculosis</i> strain				
		Lineage	BDQ MIC (mg/L)	<i>atpE</i>	<i>rv0678</i>	MIRU-VNTR
H37Rv			0.03	WT	WT	
1	No	Delhi/CAS	0.5	WT	del gg 18-19	242 235 442 244 425 173 344 742
2	INH, RFB, EMB, PZA, CAP, MXF, LNZ, TER	S	0.5	WT	WT*	233 353 212 434 215 133 336 A22
3	BDQ combined with PZA, ETH, CAP, CYC, PAS and AMC but only PAS susceptible on DST	Beijing	0.25	WT	ins g140	244 233 352 644 425 153 353 823
4	INH, RIF, EMB, PZA, SM then KAN, MXF, ETH, CYC, PAS BDQ combined with EMB, PZA, AMK, ETH, LNZ, PAS, CFZ, PAS, IMP/AMC but only PAS and AMK susceptible on DST	Beijing	0.015	WT	WT	244 233 352 644 425 173 353 623
			0.25	WT	M139T ^a	244 233 352 644 425 173 353 623

148 a : amino acid substitution in the Rv0678 protein

149 **pepQ* was sequenced and was also wild-type[16]

150 Abbreviations: bedaquiline (BDQ), minimal inhibitory concentration (MIC), drug susceptibility
151 testing (DST), Mycobacterial Interspersed Repetitive Units -Variable Number of Tandem
152 Repeats (MIRU-VNTR)

153

154 INH : isoniazid, RIF : rifampin, EMB : ethambutol, PZA : pyrazinamide, SM : streptomycin,
155 KAN : kanamycin, AMK : amikacin, CAP : capreomycin, MXF : moxifloxacin, CYC :
156 cycloserine, PAS : para-amino-salicylic acid, ETH : ethionamide, LNZ : linezolid, CFZ :
157 clofazimine, RFB : rifabutin ; AMC amoxicillin-clavulanate, IMP imipenem, TER: terizidone,
158 WT : wild-type
159

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