Figure S1 – BDQ MIC for all isolates

BDQ MIC using the CRyPTIC (UKMYC6) plates for all isolates for which phenotypic data was available.

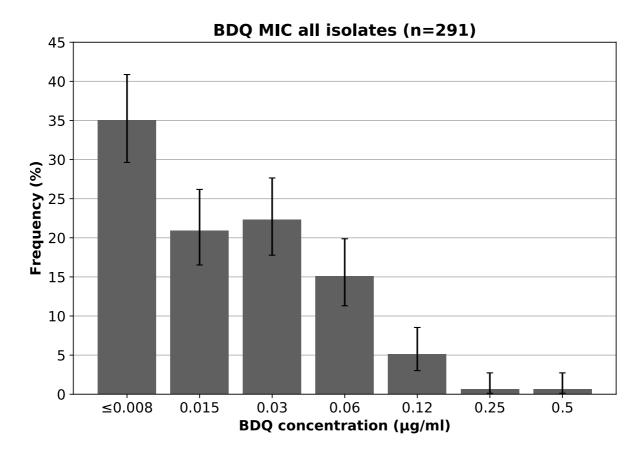


Figure S2 – BDQ MIC for individual mutations

Observed BDQ MIC values for each mutation in the dataset for which phenotypic data was available. MIC reading was done after two weeks of incubation. # Synonymous SNPs

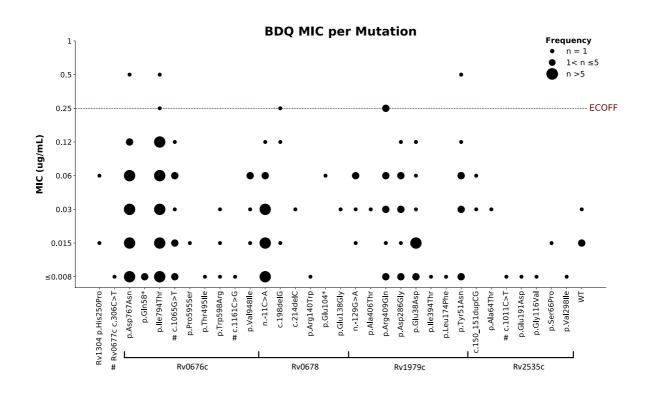


Figure S3: Sensitivity analysis – BDQ per combination of mutations Observed BDQ MIC values for each combination of mutations in the dataset for which phenotypic data was available. MIC reading was done after two weeks of incubation.

		MIC (ug/mL)						
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5
	Rv0676c p.lle794Thr							•
+ Rv0676c p.lle794Thr	Rv0676c c.1065G>T			•				
	Rv0676c c.1161C>G							
	Rv0676c p.Trp598Arg	•	•	•				
	Rv0676c c.2611G>T	- ·	•					
	Rv0677c c.306C>T	•						
	Rv0678 p.Arg140Trp	•						
	Rv1304 p.His250Pro	- ·	•					
	Rv1979c p.Glu38Asp		•	•	•	•		
	Rv1979c p.Ala406Thr	- ·		•				
	Rv2535c p.Ala64Thr	- ·		•				
	Rv2535c p.Ser66Pro		•					
	Rv2535c p.Glu191Asp	•	÷					
	Rv2535c p.Val298lle	•						
Rv2535c c.1011C>T		•						
Rv0676c p.Val948lle, Rv1979c n129G>A		- ·	٠	•	•			
	Rv0676c p.Asp767Asn	•	•	•		•		÷
+ Rv0676c	Rv0678 n11C>A				•			
p.Asp767Asn	Rv1979c p.Tyr51Asn	-		ĕ	•	•		•
p.Asp707ASII	Rv1979c p.Leu174Phe	•						
Rv0678	c.198delG, Rv0678 n11C>A		•					
Rv0678 p.Glu138Gly, Rv1979c p.Tyr51Asn		- ·		٠				
	Rv1979c p.Asp286Gly			•	•	•		
+ Rv1979c	Rv0676c p.Gln58*	•						
p.Asp286Gly	Rv0676c p.Pro595Ser	- ·	•					
p	Rv0678 p.Glu104*	- ·			•			
	Rv1979c p.lle394Thr	•						
	Rv1979c p.Arg409Gln	•	•	•	•		•	Frequen
+ Rv1979c	Rv0678 c.214delC	- ·		•				• n=1
p.Arg409Gln	Rv2535c c.150_151dupCG	- ·	÷	•	•			● 1< n
-	WT	' - ·	•	•				, e n, >5,
							ECOFF	

BDQ MIC per Combination of Mutations

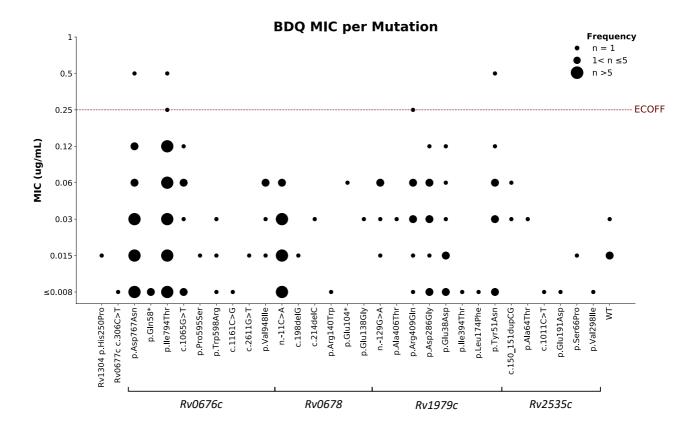


Figure S4: Sensitivity analysis – BDQ MIC for individual mutations

Observed BDQ MIC values for each mutation in the dataset for which phenotypic data was available. MIC reading was done after two weeks of incubation.

Figure S5: Sensitivity analysis – BDQ MIC per lineage

(A) BDQ MIC using the CRyPTIC (UKMYC6) plates for lineage 1 isolates. (B) BDQ MIC using the CRyPTIC (UKMYC6) plates for lineage 2 isolates. (C) BDQ MIC using the CRyPTIC (UKMYC6) plates for lineage 3 isolates. (D) BDQ MIC using the CRyPTIC (UKMYC6) plates for lineage 4 isolates. (E) BDQ MIC using the CRyPTIC (UKMYC6) plates for all isolates. (F) Kernel density estimation (KDE) of BDQ MIC of lineage 1, 2 and 4 isolates. KDE and corresponding confidence intervals were calculated using the prop.test function in the R *stats* package (version 4.0.0). MIC reading was done after two weeks of incubation.

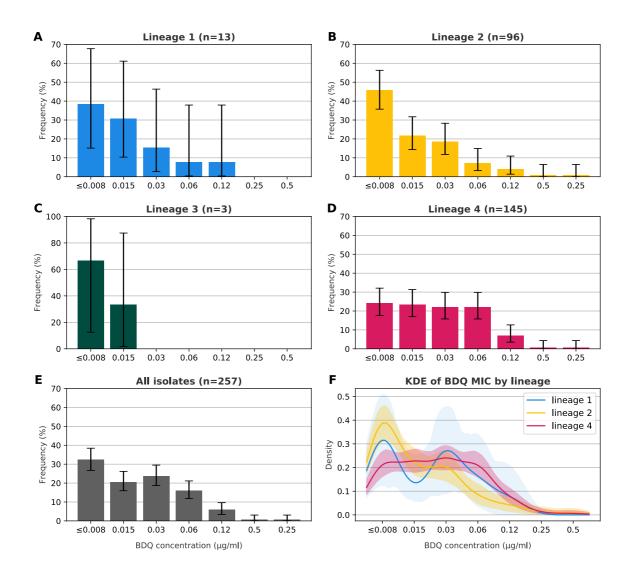


Table S1 – Genomic regions investigated

Gene	Region	H37Rv Coordinates		
Bu120E (ataE)	coding	1461045 - 1461290		
Rv1305 (atpE)	upstream	1460895 - 1461044		
Rv0678	coding	778990 - 779487		
KVU078	upstream	778840 - 778989		
	coding	2859300 - 2860418		
Rv2535c (pepQ)	upstream	2860419 - 2860569		
Rv1979c	coding	2221719 - 2223165		
KV1373C	upstream	2223164 - 2223315		
Rv0676c	coding	775586 - 778481		
KV0070C	upstream	NA		
Rv0677c	coding	778477 - 778906		
	upstream	778907 - 779056		

Genomic regions that were investigated for mutations in the samples in the dataset. No upstream region for Rv0676c is defined as it would overlap with the Rv0677c gene.

Sensitivity analysis

Of the 509 isolates included in the main analysis, 58 originated from patients who were unconfirmed BDQ naïve. In order to investigate whether inclusion of these 58 mutations biased our analysis and results, we repeated the analysis, leaving out all data on these 58 isolates.

Table S2: Sensitivity analysis – Observed mutations

Observed baseline mutations						
Gene	DNA mutation	Protein Mutation Frequency		Oldest sampling year	BDQ MIC range (µg/ml)	
Rv1304 (atpB)	749A>C	His250Pro	1	2012	0.015	
Rv0678	310G>T	Glu104*	1	2011	0.06	
	198delG	lle67fs	1	2011	0.015	
	-11C>A	NA	73	2003	≤0.008 – 0.06	
(mmpR)	214delC	Arg72fs	1	2010	0.03	
	413A>G	Glu138Gly	1	2013	0.03	
	418C>T	Arg140Trp	1	2013	≤0.008	
	150_151dupCG	Asp51fs	2	2012	0.03 - 0.06	
	573G>C	Glu191Asp	2	2010	≤0.008	
	347G>T	Gly116Val	1	2013	NA	
Rv2535c	-118A>G	NA	1	2012	NA	
(pepQ)	190G>A	Ala64Thr	1	2013	0.03	
	196T>C	Ser66Pro	1	2005	0.015	
	892G>A	Val298Ile	1	2011	≤0.008	
	1011C>T	Arg337Arg	1	2015	≤0.008	
	-129G>A	NA	4	2012	0.015 - 0.06	
	114G>C	Glu38Asp	12	2006	≤0.008 – 0.12	
	857A>G	Asp286Gly	21	1998	≤0.008 - 0.12	
	151T>A	Tyr51Asn	24	2007	≤0.008 – 0.5	
Rv1979c	1226G>A	Arg409GIn	51	2010	≤0.008 – 0.25	
	1216G>A	Ala406Thr	1	2010	0.03	
	-147G>A	NA	1	2012	NA	
	520C>T	Leu174Phe	1	2013	≤0.008	
	1181T>C	lle394Thr	1	2012	≤0.008	
	172>T	Gln58*	2	2000	≤0.008	
	1792T>A	Trp598Arg	3	2012	≤0.008 – 0.03	
	740T>C	Val247Ala	3	2012	NA	
	2842G>A ^{\$}	Val948Ile ^{\$}	5	NA	0.015 - 0.06	
Rv0676c	1065G>T	Pro355Pro	23	2007	≤0.008 – 0.12	
(mmpL5)	2299G>A	Asp767Asn	147	2003	≤0.008 – 0.5	
	2381T>C	lle794Thr	225	2000	≤0.008 – 0.5	
	1161C>G	Val387Val	1	2012	≤0.008	
	1783C>T	Pro595Ser	1	2013	0.015	
	2661G>T	Val887Val	1	2012	0.015	
Rv0677c (mmpS5)	306C>T	Asn102Asn	1	2012	≤0.008	

Table S3: Sensitivity analysis – Proportion of samples with mutated BDQ resistance gene

	Proportion of samples with mutated BDQ resistance gene							
Lineage	Rv1304	Rv0678	Rv2535c	Rv1979c	Rv0676c	Rv0677c		
1	0%	4.8%	0%	100%	14.3%	0%		
	(0/21)	(1/21)	(0/21)	(21/21)	(3/21)	(0/21)		
2	0%	37.9%	1.0%	38.9%	78.0%	0%		
	(0/198)	(75/198)	(2/198)	(77/198)	(147/198)	(0/236)		
3	0%	0%	0%	0%	0%	0%		
	(0/7)	(0/7)	(0/7)	(0/7)	(0/7)	(0/7)		
4	0.4%	0.4%	3.6%	7.6%	100%	0.4%		
	(1/225)	(1/225)	(8/225)	(17/225)	(225/225)	(1/245)		