

Figure S1 – BDQ MIC for all isolates

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

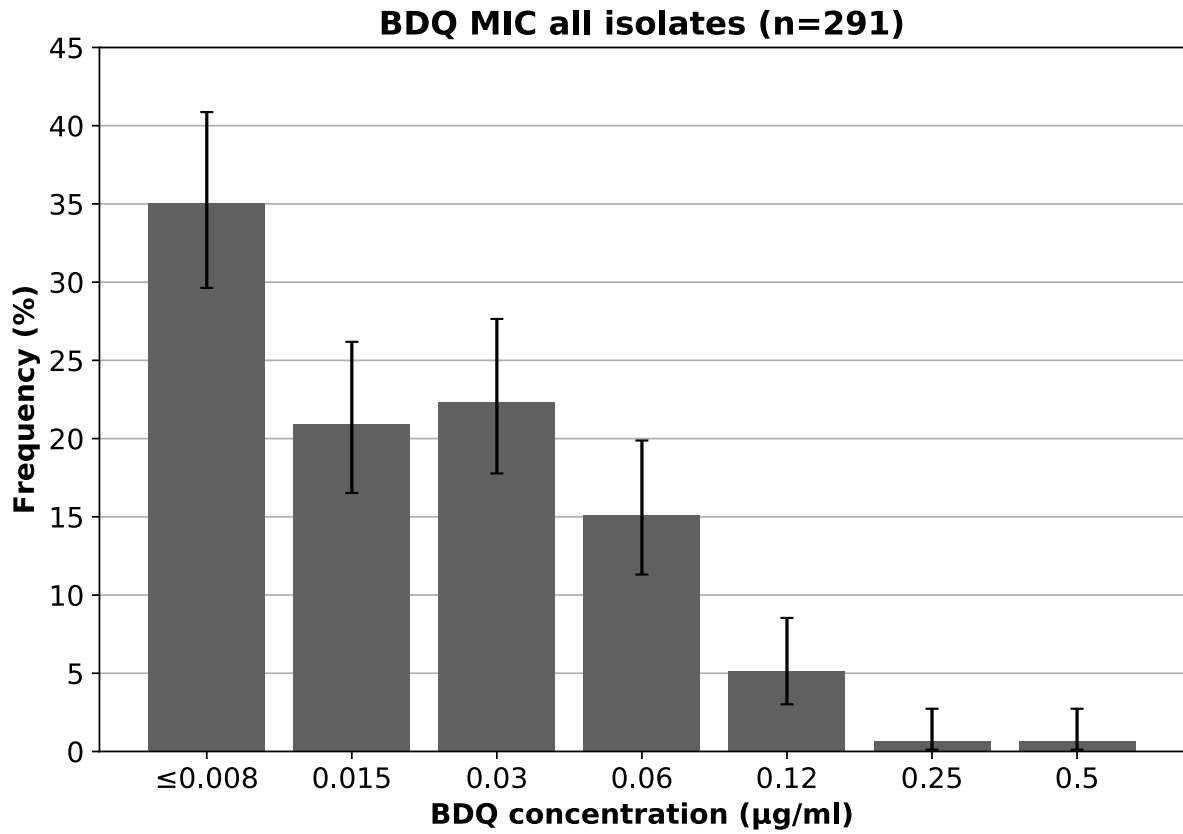


Figure S2 – BDQ MIC for individual mutations

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

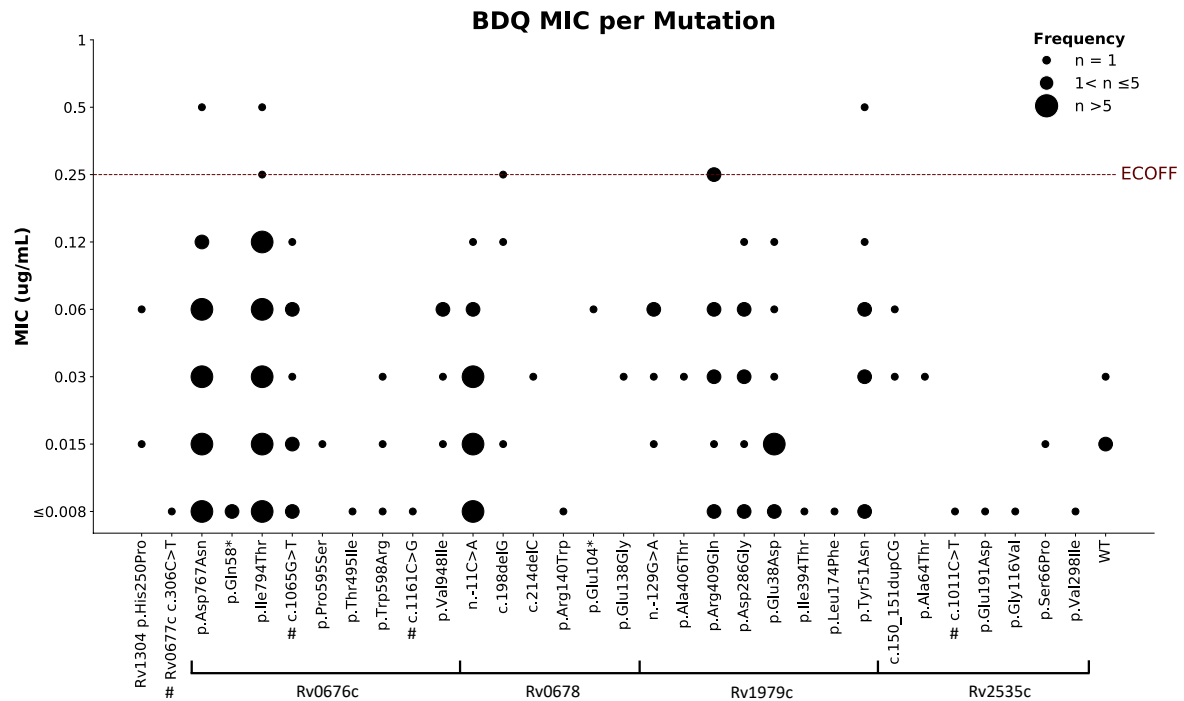


Figure S4: Sensitivity analysis – BDQ MIC for individual mutations
 Observed BDQ MIC values for each mutation in the dataset for which phenotypic data was available. MIC reading was done after two weeks of incubation.

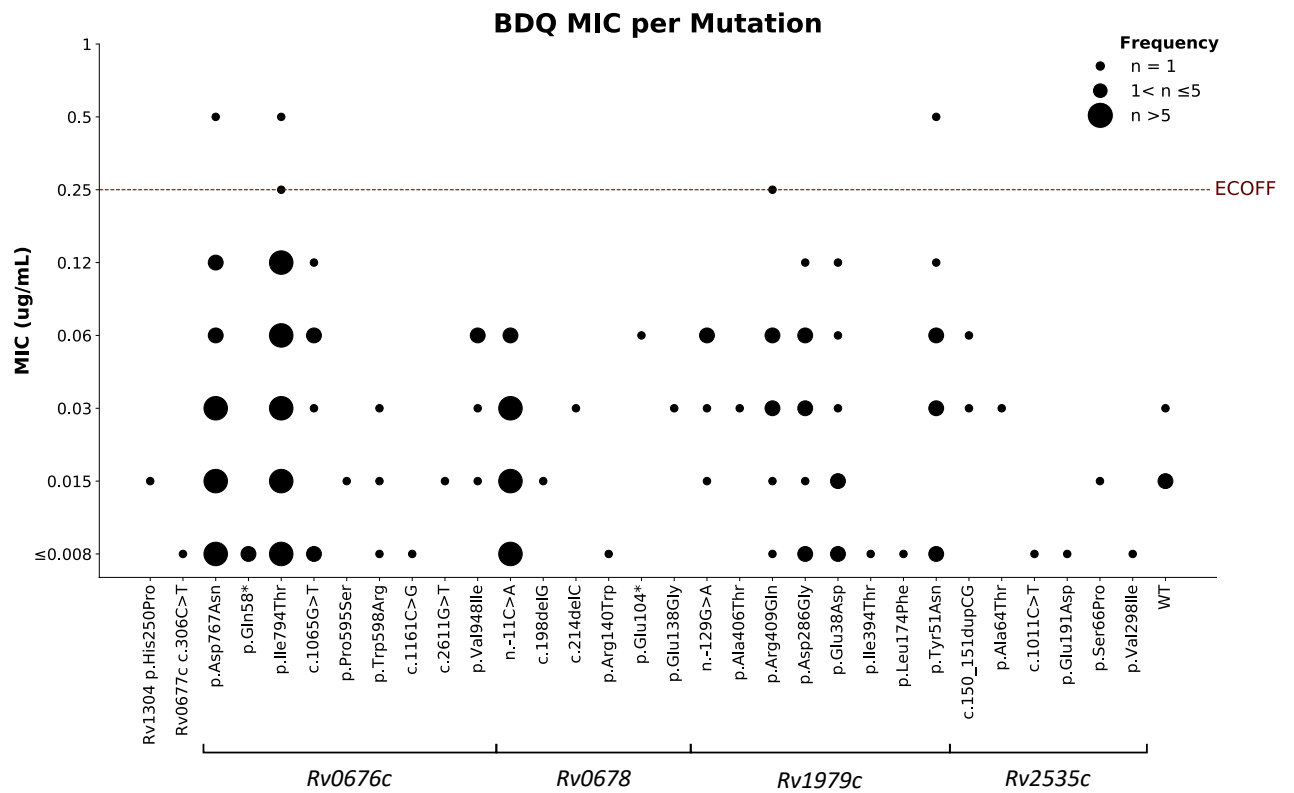


Figure S5: Sensitivity analysis – BDQ MIC per lineage

(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.

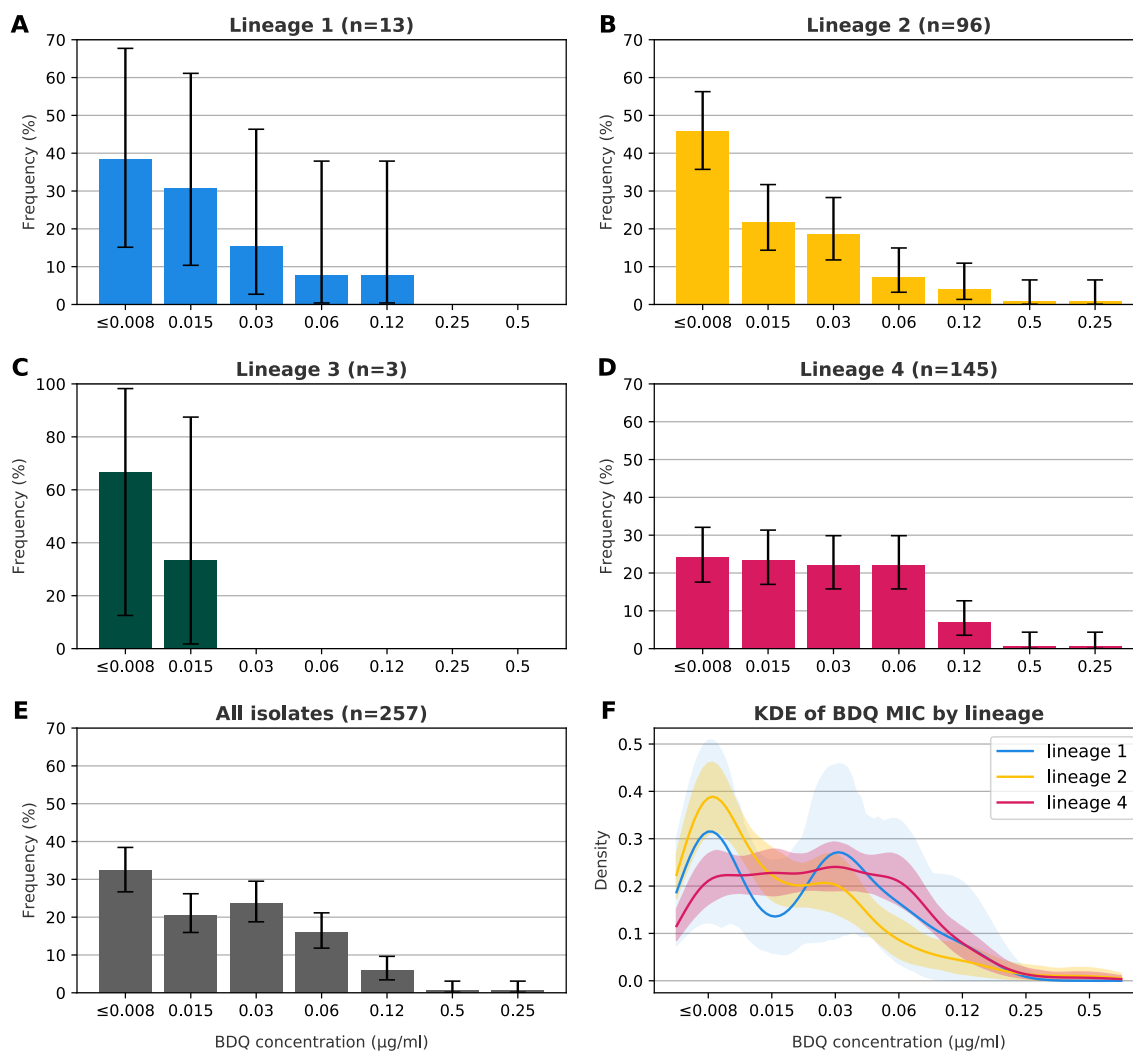


Table S1 – Genomic regions investigated

Gene	Region	H37Rv Coordinates
Rv1305 (atpE)	coding	1461045 - 1461290
	upstream	1460895 - 1461044
Rv0678	coding	778990 - 779487
	upstream	778840 - 778989
Rv2535c (pepQ)	coding	2859300 - 2860418
	upstream	2860419 - 2860569
Rv1979c	coding	2221719 - 2223165
	upstream	2223164 - 2223315
Rv0676c	coding	775586 - 778481
	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)
<i>Rv1304</i> (<i>atpB</i>)	749A>C	His250Pro	1	2012	0.015
<i>Rv0678</i> (<i>mmpR</i>)	310G>T	Glu104*	1	2011	0.06
	198delG	Ile67fs	1	2011	0.015
	-11C>A	NA	73	2003	≤0.008 – 0.06
	214delC	Arg72fs	1	2010	0.03
	413A>G	Glu138Gly	1	2013	0.03
	418C>T	Arg140Trp	1	2013	≤0.008
<i>Rv2535c</i> (<i>pepQ</i>)	150_151dupCG	Asp51fs	2	2012	0.03 – 0.06
	573G>C	Glu191Asp	2	2010	≤0.008
	347G>T	Gly116Val	1	2013	NA
	-118A>G	NA	1	2012	NA
	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
<i>Rv1979c</i>	-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
	1226G>A	Arg409Gln	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	Ile394Thr	1	2012	≤0.008
<i>Rv0676c</i> (<i>mmpL5</i>)	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
	1065G>T	Pro355Pro	23	2007	≤0.008 – 0.12
	2299G>A	Asp767Asn	147	2003	≤0.008 – 0.5
	2381T>C	Ile794Thr	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
<i>Rv0677c</i> (<i>mmpS5</i>)	306C>T	Asn102Asn	1	2012	≤0.008

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

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