

1 **Improved performance predicting clarithromycin resistance in *Mycobacterium abscessus***  
2 **on an independent dataset**

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26 In our recent study of 203 sequential isolates evaluating the ability of whole genome sequencing  
27 (WGS) to predict clarithromycin resistance in *Mycobacterium abscessus* (*M. abscessus*)(1), we  
28 demonstrated high sensitivity but poor specificity of mutations identified in a literature search.  
29 Most of the discrepancies occurred in isolates predicted to be inducibly resistant (they showed  
30 early time point susceptibility but became resistant after prolonged incubation).

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32 WGS for non-tuberculous mycobacteria has continued to be rolled out across England with all  
33 isolates from London included from January 2018, affording us the opportunity to examine the  
34 performance of the algorithm we described previously on an independent dataset (none of the  
35 isolates reported here were included in our previous study). We prepared a customised Ariba(2)  
36 library to implement the algorithm outlined in our recent publication(1), extended to include the  
37 novel mutations which we described. Using this we predicted clarithromycin susceptibility for  
38 259 isolates (10 with intermediate susceptibility were excluded) and compared these results to  
39 those obtained from DST using RAPMYCO (Thermo Fisher) plates as previously described.  
40 Isolates were classified as resistant if they showed early or late time-point (inducible) resistance.

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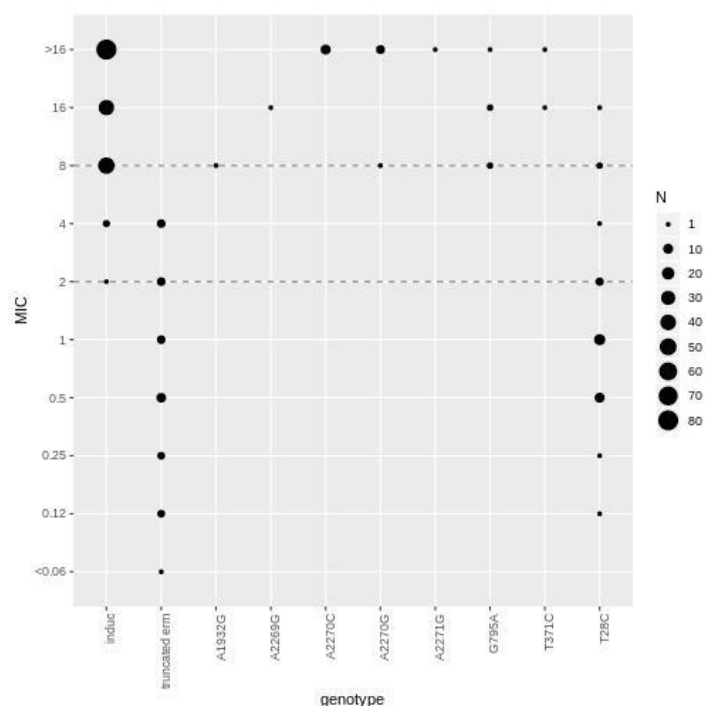
42 This yielded a sensitivity of 194/197 (98%, 95% CI 96-100%) and a specificity of 61/62 (98%,  
43 95% CI 91-100%) (Figure 1). For comparison our previously reported values were sensitivity  
44 95/100 (95%; 95% CI 89-98%) and specificity 52/79 (66%; 95% CI, 54-76%). The very major  
45 error rate on this new dataset was 3/194 (2%, 95% CI 0-3%) and the major error rate was 1/62  
46 (2%, -2 - 5%)(3). Of the novel *rrl* mutations which we described previously, T371C, G795A and  
47 A1932G were also seen in this dataset and again always associated with resistance. As in our  
48 original study, these occurred only in samples otherwise predicted inducibly resistant; the  
49 overall sensitivity/specificity was therefore the same regardless of whether they were included.  
50 For all isolates with discordant genotypic predictions or intermediate phenotypes we considered  
51 the possibility of mixed infection or within patient sub-clones(4), using a previously described

52 probabilistic method(5). This demonstrated that one such isolate predicted sensitive (due to  
53 being subspecies massiliense) but with a resistant phenotype was likely a mixed  
54 massiliense/abscessus infection. Two further isolates with intermediate phenotypes, one  
55 predicted sensitive and the other resistant, were found with high posterior probability (>99%) to  
56 have sub-clones with mutations at positions which confer macrolide resistance in the *rrl* gene.

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58 The improved performance of our algorithm may reflect the increased experience which has  
59 been gained in the RAPMYCO sensititre method by laboratory staff over time. The performance  
60 of WGS for predicting clarithromycin susceptibility in *M. abscessus* is now approaching FDA  
61 standards; providing genotypic information alongside DST to clinicians is likely to be desirable.  
62 Considering mixed populations may add value to resistance prediction in *M. abscessus*. The  
63 sequencing data from this second dataset is available under project accession number  
64 PRJNA420644 and our ariba library [github.com/samlipworth/Mab\\_ariba](https://github.com/samlipworth/Mab_ariba) is freely available which  
65 will allow others to easily apply our algorithm to their own data.

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68 Figure 1: Minimum Inhibitory Concentration (MIC, unit is µg/ml) frequency by genotype for 269  
69 *M. abscessus* isolates (including those with intermediate phenotypes). Dotted lines represent  
70 the Clinical Laboratory Standards Institute (CLSI) breakpoints for clarithromycin ( $\leq 2$  µg/ml  
71 sensitive,  $>2 - < 8$  µg/ml intermediate,  $\geq 8$  µg/ml resistant). Induc - Inducible resistance, isolate  
72 is subspecies *bolletii/abscessus* with no other relevant mutation and thus genotypically  
73 predicted to show inducible resistance. Truncated erm - massiliense isolate with truncated  
74 erm(41) gene and no other relevant mutations. All other mutations except T28C which is a  
75 mutation in erm(41) represent nucleotide mutations in the *rrl* gene.

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