

1 **Whole genome sequencing for predicting clarithromycin resistance in *Mycobacterium***
2 ***abscessus***

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4 Samuel Lipworth^{1*}, Natasha Hough^{1*}, Laura Leach², Marcus Morgan², Katie Jeffery², Monique
5 Andersson², Esther Robinson³, E. Grace Smith³, Derrick Crook¹, Tim Peto¹ and Timothy
6 Walker¹

7

8 *Contributed equally

9

10 ¹ Nuffield Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford, UK,
11 OX3 9DU

12 ² Oxford University Hospitals NHS Foundation Trust, John Radcliffe Hospital, Oxford, UK, OX3
13 9DU

14 ³ Public Health England Regional Mycobacterial Reference Laboratory, Birmingham Heartlands
15 Hospital, Birmingham, UK, B9 5SS

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17 **Running title:** WGS for *M. abscessus* clarithromycin susceptibility prediction

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19 Corresponding author:

20 Samuel Lipworth

21 Email: samuel.lipworth@medsci.ox.ac.uk,

22 Tel: 07743448071

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28 **Abstract**

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31 *Mycobacterium abscessus* is emerging as an important pathogen in chronic lung diseases with
32 concern regarding patient to patient transmission. The recent introduction of routine whole
33 genome sequencing (WGS) as a replacement for existing reference techniques in England
34 provides an opportunity to characterise the genetic determinants of resistance. We conducted a
35 systematic review to catalogue all known resistance determining mutations. This knowledge
36 was used to construct a predictive algorithm based on mutations in the *erm(41)* and *rrl* genes
37 which was tested on a collection of 203 sequentially acquired clinical isolates for which there
38 was paired genotype/phenotype data. A search for novel resistance determining mutations was
39 conducted using an heuristic algorithm.

40

41 The sensitivity of existing knowledge for predicting resistance in clarithromycin was 95% (95%
42 CI 89 - 98%) and the specificity was 66% (95% CI 54 – 76%). Subspecies alone was a poor
43 predictor of resistance to clarithromycin. Eight potential new resistance conferring SNPs were
44 identified. WGS demonstrates probable resistance determining SNPs in regions the NTM-DR
45 line probe cannot detect. These mutations are potentially clinically important as they all
46 occurred in samples predicted to be inducibly resistant, and for which a macrolide would
47 therefore currently be indicated. We were unable to explain all resistance, raising the possibility
48 of the involvement of other as yet unidentified genes.

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55 **Introduction**

56

57 The *Mycobacterium abscessus* complex (*M. abscessus*) are rapidly growing nontuberculous
58 mycobacterium (NTM) of increasing clinical concern with a rising burden of associated
59 pulmonary disease (Prevots et al. 2010). *M. abscessus* poses a significant problem, particularly
60 in patients with cystic fibrosis (CF), where infection is associated with a more rapid decline in
61 lung function and can be a barrier to transplantation(Esther et al. 2010). Of particular concern
62 are the findings from recent work that have suggested person-to-person transmission of virulent
63 clones amongst the CF population within a healthcare setting (Bryant et al. 2013, 2016),
64 although not all studies have supported this (Harris et al. 2014; Tortoli et al. 2017).

65

66 The taxonomy of *M. abscessus* is contentious. It is currently divided into three subspecies: *M.*
67 *abscessus* subspecies *abscessus* (*Mabs*), *M. abscessus* subspecies *massiliense* (*Mmas*), and
68 *M. abscessus* subspecies *bolletii* (*Mbol*)(Adekambi et al. 2017). The organism has intrinsic
69 resistance to multiple antibiotics including β -lactams, rifampicin and aminoglycosides due to the
70 synergistic action of the cell envelope and genetic factors (Nessar et al. 2012). Treatment
71 requires prolonged courses of multiple antibiotics, but outcomes are thought to vary across the
72 different subspecies. *Mmas* has been associated with clarithromycin susceptibility and
73 favourable treatment outcomes, whereas *Mabs* has been associated with inducible macrolide
74 resistance and poorer treatment outcomes (Koh et al. 2011).

75

76 Whole genome sequencing has been implemented in stages across England since December
77 2016, replacing existing reference techniques for mycobacterial identification. As a
78 consequence, there is now the opportunity to explore the molecular determinants of drug

79 resistance for all clinical NTM isolates. Macrolides are important agents in the management of
80 NTM infection, The American Thoracic Society/Infectious Diseases Society of America and
81 British Thoracic Society (ATS/IDSA and BTS) guidelines recommend including a macrolide in
82 treatment regimens where samples are either susceptible, or demonstrate inducible resistance
83 (Haworth et al. 2017; Griffith et al. 2007).. They act by binding to the 50S ribosomal subunit and
84 resistance in mycobacteria primarily occurs through target site modification for example by erm
85 methylases and point mutations (Nash, Brown-Elliott, and Wallace 2009). As there is a
86 particularly strong correlation between *in vitro* susceptibility and clinical response to macrolide
87 treatment of *M. abscessus* (Jeon et al. 2009; Choi et al. 2017), we have undertaken a study to
88 assess the feasibility of predicting clarithromycin susceptibility from whole genome sequencing
89 data for all three subspecies of *M. abscessus*.

90

91 **Methods**

92

93 **Literature search**

94

95 We first conducted a systematic review of the literature to search for known drug resistance
96 conferring mutations in *M. abscessus*. Pubmed was searched with the terms ‘*Mycobacterium*
97 *abscessus*’ AND ‘clarithromycin’ OR ‘macrolide’ OR ‘drug resistance’ OR ‘antibiotic resistance’,
98 looking for English language articles published up to April 2018. To be included in the final list,
99 articles had to contain genotyping of coding regions relevant to clarithromycin resistance in *M.*
100 *abscessus* in addition to paired drug susceptibility data. Studies looking at both clinical and
101 non-clinical samples were included. 298 abstracts were screened for relevance and 81 full text
102 articles were obtained of which 26 met the inclusion criteria (Figure 1).

103

104 **Sample selection and sequencing**

105

106 We next sought all available clinical isolates (N = 180) which had undergone whole genome
107 sequencing by the Public Health England (PHE) laboratory in Birmingham (UK) as part of the
108 routine diagnostic workflow, and for which paired phenotypic data were also available. We
109 supplemented this with 23 isolates for which the same data was available from a WGS archive
110 at the University of Oxford. Isolates were collected between May 2014 and January 2017 and
111 no prior selection according to site of isolation nor whether confirmed *M. abscessus* complex
112 disease by guidelines was made. Clinical samples were cultured in BD BACTEC™ MGIT™
113 liquid mycobacterial growth indicator tubes from which an aliquot was removed to be prepared
114 for WGS as previously described (Votintseva et al. 2015).

115

116 Libraries for Illumina MiSeq sequencing were prepared using the Illumina Nextera XT protocol
117 with manual library normalisation. Samples were batched 12 to 16 per flow cell and paired end
118 sequencing was performed with the MiSeq reagent kit V2. Bioinformatics was performed using
119 the PHE bioinformatics pipeline as previously described (Walker et al. 2015; Votintseva et al.
120 2015). Briefly, reads were mapped to the *Mabs* reference genome (NC_010397.1) with Stampy
121 v1.22 and variants called using Samtools v0.1.18 (Only variants with >= 5 high-quality reads,
122 mean quality per base >= 25 and > 90% high-quality bases were retained as variants;
123 heterozygous variants with >10% minor variant were not retained). A self-self blast approach
124 was used to mask repetitive regions. Sub-species were identified by computing maximum
125 likelihood (ML) phylogenetic trees incorporating published representative isolates from each
126 subspecies. A whole genome SNP alignment was used as input to IQ-TREE OMP version 1.5.5
127 using a generalised time reversible model. The *erm*(41) and *rplV* genes were manually
128 inspected for insertions/deletions from aligned fasta files using Seaview version 4.6.2. All newly
129 sequenced data has been uploaded to NCBI under project accession number PRJNA420644.

130

131 **Drug susceptibility testing**

132

133 Phenotypic drug susceptibility testing (DST) was performed at the PHE National Mycobacterial
134 reference service in London. DST was performed using the broth microdilution method with 96-
135 well RAPMYCO microtitre plates (Mueller Hinton medium with TES buffer, Thermo Fisher).

136 Plates were read at day three post-inoculation, and if poor growth again at day 5, according to
137 Clinical and Laboratory Standards Institute (CLSI) guidelines (Committee for Clinical Laboratory
138 Standards 2000). Isolates deemed susceptible or intermediate were re-incubated and read again at days
139 7, 14 and 21. Those found to be resistant (R MIC \geq 8 μ g/ml) at any of these time points are described as
140 phenotypically resistant. A call of phenotypically sensitive (S MIC \leq 2 μ g/ml) or intermediate (I MIC >
141 2 - $<$ 8 μ g/ml) was only made after the full 21 days of incubation. This study was an opportunistic
142 retrospective analysis of routinely collected clinical data and as such phenotypic testing was not repeated
143 on discordant isolates.

144

145 **Genotypic prediction of clarithromycin susceptibility**

146

147 We used BioPython software to extract base calls from whole genome sequence FASTA files,
148 comparing these to a list of genomic loci which our literature search indicated were associated
149 with clarithromycin resistance (table 2). We then predicted phenotypes using an hierarchical
150 algorithm (Figure 2). A resistant phenotype was predicted where any mutations were present at
151 *rrl* positions 2270 or 2271 (*E. coli* numbering 2058/2059), or where the less well characterized
152 *rrl*_A2269G or *rrl*_A2293C or *rrl*_G2281A mutations were seen. In the absence of these
153 mutations, susceptibility was predicted where an isolate had a truncated *erm*(41) gene or a C
154 nucleotide at position 28 in *erm*(41). Inducible resistance was predicted where a wild type call
155 (T) was present at position 28 in *erm*41. However, if an *erm*41_C19T mutation was also
156 present, susceptibility was predicted instead of inducible resistance. In cases where there was a

157 null call at *rrl* 2270/2271, we subsequently attempted local assembly of the *rrl* gene using Ariba
158 (Hunt et al. 2017), followed by comparison by alignment against the reference. Where this was
159 not possible due to low coverage in this region, no prediction was made. Statistics quoted were
160 calculated using R Studio v1.1.383.

161

162 **Search for novel resistance conferring mutations**

163

164 We attempted to characterise new resistance mutations within genes linked to drug resistance
165 from the literature search. To maximise power for discovering new potential resistance
166 mutations, we included all genomes available to us. All variants in these genes or their
167 promoter regions were extracted using Python software from variant call files. Phylogenetic
168 SNPs were identified by considering each subspecies in turn, assumed to be benign and
169 excluded from further analysis.

170

171 We considered variants at the level of SNPs in promoter regions or rRNA and amino acid
172 changes in coding regions. A mutation (a variant with an observable phenotype) was
173 characterised as causing resistance if it occurred as the only variant in the relevant region in a
174 resistant isolate or if it was always associated with resistance when it was seen and did not co-
175 occur with any other mutations known to cause resistance. Variants were characterised as
176 consistent with susceptibility ('benign') if all isolates were susceptible when it occurred alone or
177 if it occurred only in susceptible isolates. We assumed no prior knowledge in this section of the
178 analysis and the identification of known resistance SNPs was used as an internal validation of
179 our approach.

180

181 **Results**

182

183 We studied 143 *Mabs*, 20 *Mbol* and 40 *Mmas* genomes. Genotypic predictions were made on
184 the basis of mutations identified by the literature search. All relevant mutations identified were
185 contained in the genes *rrl* and *erm(41)* (figure 2 and table 2). The genes *rplV*, *whiB7* and *rplD*
186 were also considered of potential interest and were additionally searched for variants.

187

188 **Genotypic predictions**

189

190 Inducible resistance was predicted in 101 isolates, of which, 74/101 (73%) were reported as
191 phenotypically resistant. After excluding isolates for which no prediction could be made due to
192 missing data in key genomic loci (n = 20) as well as those with an intermediate phenotype (n =
193 4), the sensitivity was 95/100 (95%, 95% CI 89 - 98%) and specificity was 52/79 (66%, 95% CI
194 54 - 76%) . The very major error rate (phenotype resistant, WGS prediction sensitive) was 5/100
195 5% (95% CI% 1 – 9%) and the major error rate (phenotype susceptible, WGS prediction
196 resistant) was 27/79, 34% (95% CI 24 – 44%). Positive predictive value was 95/122, 78% (95%
197 CI 69 - 85%) and the negative predictive value was 52/57, 91% (95% CI 81 - 97%) (Table 3). The
198 F score for WGS predictions was 0.86. When isolates with a prediction of inducible resistance
199 were further excluded, the specificity of a resistance prediction was 21/21 (100%, 95% CI 93 -
200 100%) and the sensitivity was 21/26 (81%, 95% CI 61 - 93%).

201

202 **Clarithromycin resistance in the subspecies**

203

204 81/143 *Mabs* were resistant, 58 sensitive and 4 intermediate. For *Mbol* 18/20 were resistant
205 and for *Mmas* 19/40 were resistant (table 1). There was one *Mmas* isolate carrying a full length
206 *erm(41)* gene which was phenotypically resistant to clarithromycin. This was not unexpected
207 from a genotypic perspective as it harboured a wild type thymine nucleotide at position 28
208 *erm(41)*, associated with inducible resistance.

209

210 **Mechanisms of resistance**

211

212 The negative predictive value of a truncated *erm(41)* gene for clarithromycin susceptibility was
213 53% (21/39 - there was one *Mmas* isolate with a full length *erm(41)*). In 11/18 instances,
214 resistance in the presence of a truncated *erm(41)* could be explained by a mutation in position
215 2270 or 2271 in *rrl*. No coverage at all was seen at these positions for 4/18 isolates. No genomic
216 explanation could be identified for the remaining three discordant isolates (table 1).

217

218 All isolates which had any mutation of positions 2269, 2270 or 2271 (E. Coli numbering 2057,
219 2058, 2059) in *rrl* were resistant to clarithromycin (21/203 (10%)). Such a mutation was found in
220 3 *Mbol*, 11 *Mmas* and 7 *Mabs* isolates. We did not observe any isolates with an *rrl* mutation
221 which also harboured a T28C mutation in *erm(41)*. Where this occurred in isolates reported in
222 the literature, they were always resistant (Kehrmann et al. 2016; Rubio et al. 2015).

223

224 Of 37 isolates with a T28C mutation in *erm(41)* and no other relevant mutations, 84% (31/37)
225 were susceptible to clarithromycin, 11% (4/31) had intermediate susceptibility and 5% (2/31)
226 were resistant. This mutation was exclusively found in *Mabs* isolates. We did not identify any
227 drug resistance associated mutations in any of these intermediate or resistant isolates. Across
228 all three subspecies, of 101 isolates with the T28_erm41 call associated with inducible
229 resistance (and no other relevant mutation), 73% (74/101) were resistant and 27% (27/101)
230 susceptible at the final day 21 reading.

231

232 **De novo search for resistance determining mutations**

233

234 The search for potential novel resistance determining mutations for clarithromycin revealed 13
235 SNPs of interest (table 4). Of these, five have previously been described in the literature.
236 There were additionally four SNPs (*rrl*_A2746T, *rrl*_G836A, *rrl*_T2674G and *rrl*_T636C) which
237 were only ever seen in resistant isolates but always co-occurred with known resistance
238 determining SNPs. There was one phenotypically resistant isolate which harboured 18 novel
239 SNPs. On performing a nucleotide BLAST of a 120 base region encompassing all of these
240 SNPs, there was a 99% (E 2 x10⁻⁵³) match with *Streptococcus* species. This therefore likely
241 represents sample contamination with flora from the nasopharynx. No new resistance
242 associated variants were discovered in *rplV*, *rpld* or *whiB7*.

243

244 **Discussion**

245

246 We conducted a systematic review of drug resistance determining mutations for clarithromycin
247 in *M. abscessus* and used the results to make genotypic predictions. The sensitivity of this
248 approach was 95% (95% CI 89 – 98%) and the positive predictive value 78% (95% CI 69 –
249 85%). The prevalence of resistance amongst our collection of isolates was high compared to
250 that which has been reported elsewhere (Koh et al. 2011; Hatakeyama et al. 2017; Li et al.
251 2016; Cowman et al. 2016).

252

253 These results show that for clarithromycin, drug resistance can be predicted from WGS data as
254 it has been previously through targeted PCR and line probe assays such as the Hain GenoType
255 NTM-DR. Assessment of the genotype of *erm(41)* with molecular diagnostics allows prediction
256 of its functional status which has been thought to correlate to treatment outcome (Haworth et al.
257 2017). Similarly, as the absence of a functional *erm(41)* gene has been associated with good
258 therapeutic outcomes its molecular detection ought to be beneficial to patients (Koh et al. 2011),
259 although in our study this alone was not an adequate predictor of in vitro resistance. A

260 genotypic prediction of inducible resistance produced a variable phenotype in our study (27/101
261 sensitive). Discriminating such isolates predicted to be inducibly resistant which are
262 unexpectedly sensitive after prolonged incubation with clarithromycin or show early time point
263 high level resistance may help to identify additional genotypic markers to better identify patients
264 more likely to benefit from the use of macrolides...

265

266 In addition to mutations identified in the literature search, we also managed to identify variants
267 that may plausibly be new resistance determining mutations. However, these will require
268 validation against an independent data set. Using routinely collected diagnostic data to improve
269 our understanding of the molecular determinants of drug resistance is a key advantage WGS
270 has over line-probe assays or PCR. The eight previously undescribed mutations we report in
271 this work could be of clinical importance because they all occur in samples which the existing
272 literature predicts to be inducibly resistant. As BTS guidelines recommend that patients with
273 such isolates should be given a macrolide, it is important to determine further whether these
274 SNPs are true resistance-determinants, and whether macrolide therapy should be avoided in
275 their presence.

276

277 Previous authors have suggested that it is clinically useful to discriminate between subspecies,
278 (Koh et al. 2011) as *Mmas* is typically associated with durable susceptibility to clarithromycin
279 and *Mbol* and *Mabs* with inducible resistance (unless the T28C mutation is present). We found
280 identifying sub-species alone to be an inadequate predictor of *in vitro* clarithromycin phenotype.
281 There were three *Mmas* isolates in our collection that were resistant to clarithromycin and had
282 no mutations known to be relevant. Mougari and colleagues found that in 39/40 *Mmas* selected
283 for clarithromycin resistance, this could be explained by an *rml* mutation at positions 2270/2271
284 with a further sample containing an *rplV* insertion (Mougari et al. 2017). All of our isolates

285 contained this 'insertion' (also present in the NC_010397.1 reference) which was associated with
286 susceptibility to clarithromycin except in the presence of a relevant *rri* mutation.

287

288 In keeping with previous reports, we identified an isolate of *Mmas* with a full length *erm*(41) and
289 a thymine nucleotide at position 28(Shallom et al. 2015). This likely represents recombination
290 between the subspecies. A recent study showed the Hain GenoType NTM-DR line probe assay
291 incorrectly predicted subspecies in 8% of samples, presumably because it lacks the whole
292 genome resolution provided by sequencing and is vulnerable to between species recombination
293 (Kehrmann et al. 2016).

294

295 Despite analysing all mutations occurring in *erm*(41) and *rri* for the full collection of genomes,
296 we were unable to predict all clarithromycin resistance. This may be because there are other
297 genes implicated or due to unreliable DST results. Future work should aim to select discordant
298 genotypes and identify additional infrequently occurring genetic loci implicated in clarithromycin
299 resistance, for example by using genome wide association (GWAS) approaches. All of the new
300 clarithromycin resistance mutations we discovered occurred in isolates which we originally
301 predicted to be inducibly resistant. Although *M. abscessus* is primarily thought to be an
302 environmental organism, these patients may be colonised for long periods with subsequent
303 potential exposure to multiple courses of macrolides. An alternative hypothesis may therefore
304 be that some or all of these SNPs are compensatory mutations which act to reduce a fitness
305 cost of the expression of *erm*, which has been experimentally demonstrated in other bacteria
306 (Gupta et al. 2013). There were four SNPs which only occurred in resistant samples but were
307 always seen with a known drug resistance causing SNP, possibly also representing
308 compensatory mutations.

309

310 Key weaknesses of our study include that we were unable establish a temporal relationship
311 between antibiotic prescribing and inducible phenotypic resistance as we did not have the
312 relevant ethics approval to link to patient records. If for example, any SNPs on our list of novel
313 mutations were observed in isolates from patients who had never previously had macrolide
314 therapy, it would be much more likely that they were genuine resistance conferring rather than
315 compensatory mutations. In addition it is possible that some of the genomes were same patient
316 replicates over a number of months/years, although this may have also diversified the range of
317 mutations observed. We chose to include all available samples to maximise detection of low
318 frequency resistance determining SNPs meaning there was no validation set available. Our list
319 of novel resistance determining SNPs will therefore require validation on an independent
320 dataset before being applied to the clinical setting. We chose to target a select list of genes with
321 known SNPs identified in the literature search; other approaches such as GWAS will likely be
322 additive to the knowledge base we present here.

323
324 In summary, WGS allows identification of known resistance conferring mutations as well as
325 demonstrating probable novel resistance determining SNPs in regions the Hain NTM-DR line
326 probe cannot detect which if further validated may change management. Identification of
327 subspecies alone inadequately predicts macrolide resistance in *M. abscessus*. Our data does
328 not support the replacement of phenotypic tests at this point in time; as more paired
329 genome/DST data becomes available in the near future, and we learn more about the molecular
330 determinants of drug resistance, it is likely that sensitivity and specificity of WGS resistance
331 prediction will improve. Given that WGS data is already being produced in the UK for the
332 purposes of molecular epidemiology, it would now be possible to phase out existing molecular
333 tests and replicate their results *in silico* at no additional cost.

334

335 **Transparency declaration**

336 The authors have no conflicts of interest to declare.

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340

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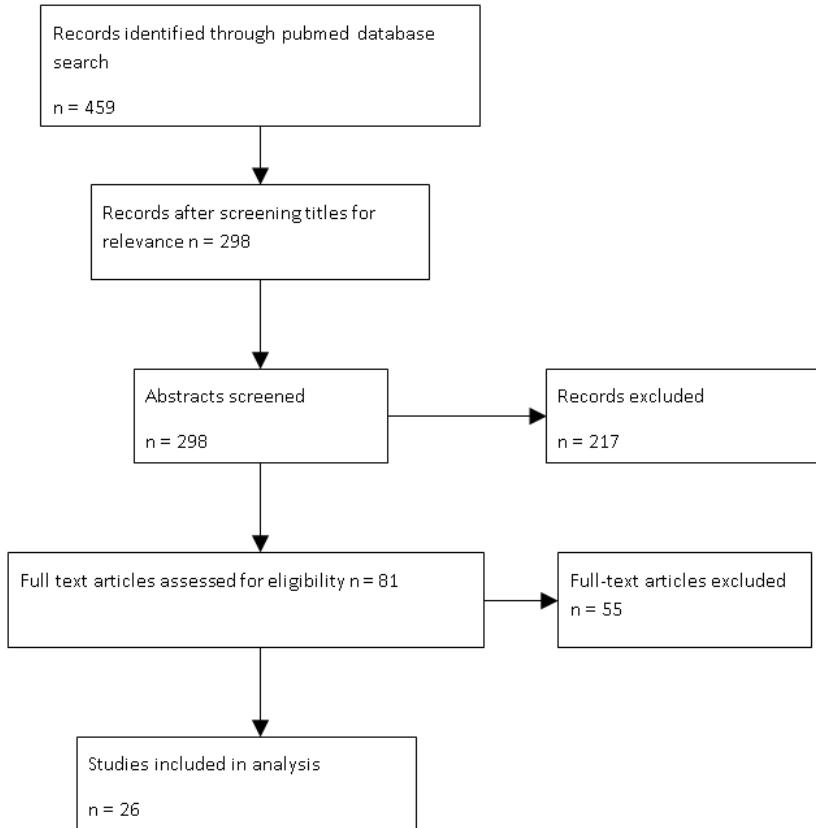
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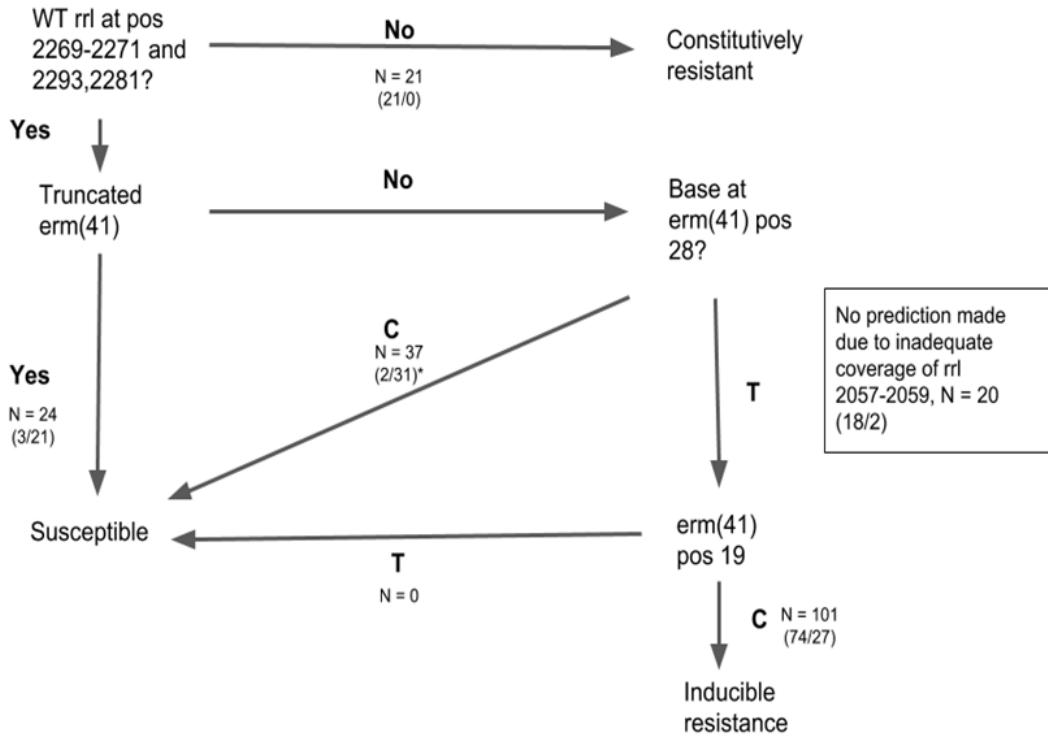
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503 **Figure 1:** Flow diagram showing stages of the systematic literature search



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505 **Figure 2:** decision algorithm for predicting drug resistance in *M. abscessus* based on the
506 literature search with numbers of isolates meeting each predictive criterion shown. Bracketed
507 numbers represent (N resistant / N sensitive). * 4 isolates had intermediate susceptibility

	erm41 pos. 28	erm41 length	erm41 pos. 19	rrl. pos 2269	rrl pos. 2270	rrl pos. 2271	N	Phenotype	Predictio
Mabs	T	full	C	A	C	A	5	5R	R
	T	full	C	A	T	A	2	2R	R
	C	full	C	A	A	A	37	4I, 2R, 31S	S
	T	full	C	A	A	A	87	62R, 25S	R
	Excluded due to inadequate coverage over rrl 2270-2271						12	10R 2S	
Mbol	T	full	C	G	A	A	1	1R	R
	T	full	C	A	G	A	2	2R	R
	T	full	C	A	A	A	13	11R, 2S	R
	Excluded due to inadequate coverage over rrl 2270-2271						4	4R	
Mmas	T	truncated	C	A	C	A	3	3R	R
	T	truncated	C	A	G	A	3	3R	R
	T	truncated	C	A	A	G	5	5R	R
	T	truncated	C	A	A	A	24	21S, 3R	S
	T	full	C	A	A	A	1	1R	R
	Excluded due to inadequate coverage over rrl 2270-2271						4	4R	

Table 1 - summary of genotypes and corresponding clarithromycin phenotypes for the 203 isolates. Pos - *M. abscessus* numbering position in gene, prediction - genotypic prediction using the algorithm shown in Figure 2, Mabs - *M abscessus abscessus*, Mbol - *M. abscessus bolletii*, Mmas - *M. abscessus massiliense*, N – total number of isolates with genotype, S – Sensitive, R – Resistant, IR – Inducible Resistance

<i>erm(41)</i> length	<i>erm(41)</i> position 28	<i>rrl</i> position 2270 (2058)	<i>rrl</i> position 2271 (2059)	Other	Phenotype
Full	T	A	A		Inducible resistance(Ba stian et al. 2011; Maurer et al. 2014; Hanson et al. 2014; Brown- Elliott et al. 2015; Nie et al. 2015; Shallom et al. 2015; Ramírez, de Waard, and Araque 2015; Rubio et al. 2015; Chua et al. 2015; J. Kim et al. 2016; Kehrmann et

					al. 2016; Mougari et al. 2017; Jeong et al. 2017; Bryant et al. 2013; Carvalho et al. 2018; Chew et al. (2017) Sensitive(Ch ua et al. (2015)
Truncated		A	A		Sensitive(Koh et al. 2011; Hanson et al. 2014; Shallom et al. 2015; Ramírez, de Waard, and Araque 2015; Chua et al. (2015; J. Kim

					et al. 2016; Kehrmann et al. 2016; Mougari et al. 2017; Koh et al. 2017; Jeong et al. 2017; Carvalho et al. 2018; Chew et al. (2017)
Full	C	A	A		Sensitive(Jeo ng et al. 2017; Mougari et al. 2017; Kehrmann et al. 2016; Chua et al. 2015; Zhu et al. 2015; Ramírez, de Waard, and

					Araque 2015; Shallom et al. 2015; Nie et al. 2015; Brown-Elliott et al. 2015; Hanson et al. 2014; Maurer et al. 2014; Rubio et al. 2015; Carvalho et al. 2018; Chew et al. (2017)
Full or Truncated	C or T	G	A		Resistant(Mo ugari et al. 2017; Koh et al. 2017; Kehrmann et al. 2016; Li et al. 2016; Rubio et al. 2015; Zhu et

					al. 2015; Shallom et al. 2015; Maurer et al. 2014; Wallace et al. 1996; Bastian et al. 2011; Bryant et al. 2013; Carvalho et al. 2018; Liu et al. 2017; Chew et al. 2017)
Full or truncated	C or T	C	A		Resistant(Mo ugari et al. 2017; Koh et al. 2017; Kehrmann et al. 2016; Shallom et al. 2015; Maurer et al. 2014; Bryant et al.

					2013; Liu et al. 2017)
Full or truncated	T	T	A		Resistant(Moungari et al. 2017; Kehrmann et al. 2016; Bastian et al. 2011)
Full		A	C		Resistant(Moungari et al. 2017; Wallace et al. 1996; Bastian et al. 2011; Liu et al. 2017)
Full or truncated	T or C	A	G		Resistant(Moungari et al. 2017; Kehrmann et al. 2016; Li et al. 2016;

					Rubio et al. 2015; Shallom et al. 2015; Maurer et al. 2014; Wallace et al. 1996; Chew et al. 2017)
Truncated		A	T		Resistant(Ba stian et al. 2011)
Full	T	A	A	C19T <i>erm(41)</i>	Sensitive(S.- Y. Kim et al. 2016)
Truncated		A	A	A2269G <i>rrl</i> (2057)	Resistant(Ru bio et al. 2015)
Full	Unknown	Unknown	Unknown	A2293C <i>rrl</i> (2082) + G2281C <i>rrl</i> (2069)	Resistant(Liu et al. 2017)

Table 2: Resistance determining mutations for clarithromycin identified in the literature search.

M. abscessus numbering is used with *E. coli* numbering in brackets.

Genomic Prediction	In vitro phenotype		
	Sensitive	Resistant	Intermediate
No Prediction*	2	18	0
Inducible resistance	27	74	0
Resistant	0	21	0
Sensitive	52	5	4
Sensitivity	95% (95% CI 89 - 98%)		
Specificity	66% (95% CI 54 - 76)%		
Positive predictive value	78% (95% CI 69 - 85%)		
Negative predictive value	91% (95% CI 81.0 - 97%)		

Table 3 - WGS Predictions vs DST phenotype for clarithromycin.

Sensitivity/Specificity/PPV/NPV are calculated excluding isolates with intermediate phenotype and those where no prediction was made due to inadequate coverage at key positions.

Position	Nucleotide/Amino acid change	Rule met

rrl 2039	A > G	1
rrl 1401	T > C	2
rrl 371	T > C	2
rrl 795	G > A	1
rrl 2270*	A > C	1
rrl 2270*	A > G	2
rrl 2271*	A > G	2
rrl 2270 *	A > T	2
erm(41) 131	A > V	2
rrl 2279	G > A	2
rrl 2269*	A > G	2
erm (41) -31**	A > T	2
rrl 1932	A > G	2

Table 4: Mutations (both novel and previously described) detected during *de novo* search for resistance determining SNPs. Rule 1 = occurs as only SNP in relevant regions in resistant isolate, rule 2 = all samples resistant when SNP occurs, never seen in sensitive isolate. All numbering is relative to *M. abscessus*. * mutation already described in literature - *M. abscessus rrl* numbering 2270/2271 is *E. coli* numbering 2058/2059. ** mutation in *erm(41)* promoter region, 31 bases upstream of start of coding region.