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***Helcococcus kunzii* MLS<sub>B</sub> resistance methyltransferase *erm*(47) is induced by  
diverse ribosome-targeting antibiotics**

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**Running title:** Erm(47) is induced by multiple drug classes

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32 **Abstract**  
33

34 **Objectives:** To determine the mechanism of induction of *erm*(47) and its atypical expression in the  
35 Gram-positive opportunistic pathogen, *Helcococcus kunzii*, where it confers resistance to a subset of  
36 clinically important macrolide, lincosamide and streptogramin B (MLS<sub>B</sub>) antibiotics.

37 **Methods:** The resistant *H. kunzii* clinical isolate UCN99 was challenged with subinhibitory  
38 concentrations of a wide range of ribosome-targeting drugs. The methylation status of the *H. kunzii*  
39 ribosomal RNA at the MLS<sub>B</sub> binding site was then determined using a mass spectrometry approach,  
40 and was correlated with any increase in resistance to the drugs.

41 **Results:** The *H. kunzii erm*(47) gene encodes a monomethyltransferase. Expression is induced by  
42 sub-inhibitory concentrations of the macrolide erythromycin, as is common for many *erm* genes,  
43 and surprisingly also by 16-membered macrolide, lincosamide, streptogramin, ketolide,  
44 chloramphenicol and linezolid antibiotics, all of which target the 50S ribosomal subunit. No  
45 induction was detected with spectinomycin, which targets the 30S subunit.

46 **Conclusions:** The structure of the *erm*(47) leader sequence functions as a hair-trigger for the  
47 induction mechanism that expresses resistance. Consequently, translation of the *erm*(47) mRNA is  
48 tripped by MLS<sub>B</sub> compounds and also by drugs that target the 50S ribosomal subunit outside the  
49 MLS<sub>B</sub> site. Expression of *erm*(47) thus extends previous assumptions about how *erm* genes can  
50 be induced.

51

## Introduction

Infection with opportunistic bacterial pathogens presents a serious threat to immunocompromised individuals.<sup>1</sup> One such pathogen, *Helicobacter kunzii*, is a facultatively anaerobic Gram-positive coccus that can be found as an integral part of the skin microbiota of healthy individuals.<sup>2</sup> This species is now being increasingly detected in skin and soft tissue infections, such as diabetic foot ulcers,<sup>3</sup> often in mixed cultures with other Gram-positive cocci. *H. kunzii* is generally intrinsically susceptible to many antibiotics, although a disturbing increase in the incidence of resistance to MLS<sub>B</sub> drugs and to other clinically effective classes of antibiotics has been observed.<sup>4</sup> Recently, *H. kunzii* strain UCN99 was shown to have acquired a 115-kb island within its chromosome that encodes a range of antibiotic resistance determinants.<sup>5</sup> These include the previously uncharacterized gene *erm*(47), which confers resistance to a range of MLS<sub>B</sub> drugs.<sup>5</sup>

Phylogenetically, the *erm*(47) gene belongs to the large family of *erm* genes that are found in diverse pathogenic and drug-producing bacteria.<sup>6, 7</sup> Each *erm* gene encodes a methyltransferase that specifically targets the N<sup>6</sup>-position of nucleotide A2058 in 23S rRNA (*Escherichia coli* numbering),<sup>8</sup> which becomes modified by addition of either one or two methyl groups.<sup>7</sup> Type I Erm monomethyltransferases add a single methyl group to confer high resistance to lincosamides, low to moderate resistance to macrolide and streptogramin B antibiotics,<sup>9</sup> and no appreciable resistance to ketolides such as telithromycin<sup>10</sup> that are derivatives of the macrolide erythromycin.<sup>11</sup> Addition of two methyl groups by type II Erm dimethyltransferases confers high resistance to all MLS<sub>B</sub> antibiotics<sup>6, 7</sup> and also to ketolides.<sup>12</sup> Consistent with these resistance phenotypes, Erm monomethyltransferases are generally found in drug-producing actinobacterial species,<sup>9</sup> whereas pathogenic bacteria generally acquire the broader range of resistance conferred by Erm dimethyltransferases.<sup>6, 7</sup> However, exceptions do exist and *erm*(42) found in pathogenic Pasteurellaceae species has been shown to be a monomethyltransferase.<sup>13</sup>

77 The transfer of additional methyl groups to the rRNA has a measurable fitness cost for the  
78 bacterial cell, and thus expression of *erm* genes is often shut down in the absence of drugs.<sup>14</sup>  
79 Expression of *erm* can then be activated upon detection of a drug for which the resistance  
80 mechanism is required. Such induction is generally specified by a narrow range of drugs. For  
81 instance, the dimethyltransferase ErmC is induced by the macrolide erythromycin, which has a 14-  
82 membered lactone ring, but is not induced by 16-membered ring macrolides, lincosamides or  
83 streptogramin B drugs<sup>15</sup> despite the ability of ErmC to confer resistance to all of these compounds.<sup>7</sup>  
84 Furthermore, alteration of the erythromycin structure by removing its cladinose sugar results in loss  
85 of induction.<sup>16, 17</sup> This latter point has been central in the development of the ketolide derivatives of  
86 erythromycin where the cladinose moiety has been substituted with a keto group,<sup>18</sup> enabling  
87 ketolides to exert their inhibitory effects without tripping expression of MLS<sub>B</sub> resistance.<sup>11, 19, 20</sup>  
88 Other structurally distinct classes of drugs such as chloramphenicol and linezolid bind at ribosomal  
89 sites that overlap those of the MLS<sub>B</sub> antibiotics,<sup>21, 22</sup> but despite this, none of has previously been  
90 associated with induction of *erm*-mediated resistance.

91 Growth studies of *H. kunzii* strain UCN99 showed that *erm*(47) confers resistance to a range of  
92 MLS<sub>B</sub> antibiotics and that this phenotype is transferable to other bacterial species.<sup>5</sup> In the present  
93 study, we investigate the mechanism of *erm*(47) induction and determine whether the resultant  
94 rRNA modification falls into the type I or type II group of methyltransferases. We have applied  
95 Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry to  
96 determine the methylation status of *H. kunzii* rRNA after being challenged with a range of clinically  
97 relevant drugs that target the bacterial ribosome. We show that *H. kunzii* *erm*(47) is an inducible  
98 monomethyltransferase that contravenes many of the previous assumptions about how *erm* genes  
99 are induced. Although, as might have been expected, full induction is obtained in the presence of  
100 sub-inhibitory amounts of erythromycin, we show that *erm*(47) expression is also induced by a

101 range of compounds that include the 16-membered macrolide spiramycin, the lincosamide  
102 lincomycin, the type-B streptogramin quinupristin, the ketolide telithromycin, as well as the non-  
103 MLS<sub>B</sub> drugs chloramphenicol and linezolid.  
104  
105

## 106 **Materials and Methods**

### 108 ***Bacterial strains and growth conditions***

109 The susceptible strain *H. kunzii* ATCC51366 and the resistant strain UCN99 containing *erm*(47)  
110 were grown at 37 °C in cation-adjusted Mueller-Hinton broth (CA-MHB) supplemented with 5%  
111 lysed horse blood and 20 mg/L nicotinamide adenine dinucleotide.<sup>5</sup> Minimal inhibitory  
112 concentrations (MICs) of the antibiotics listed below were determined by the broth microdilution  
113 method according to EUCAST guidelines (<http://www.eucast.org/>). The UCN99 strain was grown  
114 with one-fourth the MICs of erythromycin (64 mg/L), spiramycin (64 mg/L), lincomycin (64  
115 mg/L), telithromycin (2 mg/L), dalfopristin/quinupristin (0.03 mg/L), chloramphenicol (1 mg/L),  
116 linezolid (0.25 mg/L) or spectinomycin (2 mg/L) to test the effects of these drugs on induction of  
117 the *erm*(47) gene.

### 119 ***RNA purification***

120 *H. kunzii* cells were grown to late log phase in 50 mL medium and were harvested by centrifugation  
121 at 10000 g for 20 min. Cells were washed by twice resuspending in 100 mL buffer A (50 mM Tris-  
122 Cl pH 7.2, 10 mM MgCl<sub>2</sub>, 100 mM NH<sub>4</sub>Cl) and pelleted by centrifugation. Cells were lysed by  
123 sonication at 4 °C in 10 mL buffer A. Cell debris containing the chromosomal DNA was removed  
124 by centrifugation at 15000 g for 10 min. and the supernatant was extracted with phenol/chloroform  
125 to remove proteins. Cellular RNA was recovered by ethanol precipitation, and was redissolved in  
126 100 µL H<sub>2</sub>O.

### 128 ***MALDI mass spectrometry analysis of rRNAs***

129 The 23S rRNA region around A2058 was isolated using a hybridization method.<sup>23, 24</sup> A mixture of  
130 150 pmol of *H. kunzii* rRNA and 500 pmol of an oligodeoxynucleotide complementary to the

131 nucleotide sequence G2032-G2084 (Fig. 1A) was heated for 5 min at 90 °C in 100 µl of 50 mM  
132 Tris.Cl pH 7.5 followed by slow cooling to 37 °C. The mixture was incubated for a further 60 min  
133 at 37 °C with 0.5 µg RNase A, and nuclease digestion was stopped by extracting with  
134 phenol/chloroform. The RNA-DNA hybrid was recovered by ethanol precipitation and run on a  
135 denaturing 13% polyacrylamide gel to release the rRNA fragment that had been protected from  
136 RNase A digestion. The rRNA fragment of approximately 57 nucleotides ran slightly slower on the  
137 gel than its complementary DNA oligonucleotide, and was excised and extracted.

138 RNA aliquots of 2.5 pmol in 1 µL were mixed with 0.5 µL 3-hydroxypicolinic acid (0.5 M in  
139 50% acetonitrile) and digested in a total volume of 3 µL with either RNase A or RNase T1 (Sigma-  
140 Aldrich) for 3 h at 37 °C. The resultant oligonucleotides were analysed in positive ion mode by  
141 MALDI mass spectrometry as previously described.<sup>23, 24</sup>

142

#### 143 ***Secondary structure calculations erm(47) mRNA stability***

144 Models for RNA folding based on minimum free energy structures were predicted using publically  
145 assessible online tools<sup>25</sup> and included mfold (<http://www.bioinfo.rpi.edu/applications/mfold/>). The  
146 free energies ( $\Delta G$ ) of structures were estimated from the stacking interaction contributions of  
147 different RNA basepairs determined experimentally at 25 °C.<sup>26</sup>

## 148 **Results** 149

### 150 ***Resistance phenotypes***

151 The MICs of a range of drugs targeting the ribosomal 50S subunit were determined for the  
152 susceptible and resistant *H. kunzii* strains (Table 1). The UCN99 strain exhibited appreciably higher  
153 resistance to the macrolides erythromycin and spiramycin and to the lincosamide lincomycin. No  
154 resistance was conferred to the ketolide telithromycin, the streptogramin A/B combination of  
155 dalfopristin/quinupristin, chloramphenicol nor to the oxazolidinone linezolid. As expected, there  
156 was no resistance to the 30S subunit-targeting drug spectinomycin (Table 1).

157

### 158 ***Erm(47) is a monomethyltransferase***

159 The MALDI mass spectrometry approach used here measures the masses of RNA oligonucleotides  
160 to within 0.2 Da.<sup>27</sup> Thus the addition of one methyl group (plus 14 Da) or two methyl groups (plus  
161 28 Da) is readily detected in rRNA fragments of known sequence. After RNase T1 digestion of  
162 rRNA from the susceptible strain, nucleotide A2058 of the macrolide/lincosamide binding site (in  
163 bold, Fig. 1A) resides in the fragment AAAGp with a mass/charge ( $m/z$ ) of 1351 (Fig. 1B).  
164 Digestion of the rRNA region with RNase A yields a larger fragment of GGAAAGACp ( $m/z$  2675)  
165 containing this nucleotide (Fig. 1C).

166 Sub-inhibitory amounts of erythromycin produced no change in the rRNA of the susceptible  
167 ATCC51366 strain (Fig. 2A), as would be predicted for a strain lacking resistance determinants.  
168 Equivalent treatment of the resistant UCN99 strain with erythromycin (addition of one-fourth MIC,  
169 Table 1) caused a shift in the AAAGp fragment from  $m/z$  1351 to  $m/z$  1365 (Fig. 2C),  
170 corresponding to the addition of a single methyl group. No peak was detected at  $m/z$  1379 under  
171 these (or any other) drug conditions, showing that no dimethylation of A2058 takes place and that  
172 Erm(47) is exclusively a monomethyltransferase. Under noninducing condition, a minor amount of

173 A2058 monomethylation was observed (Fig. 2B), indicating that expression of *erm*(47) in *H. kunzii*  
174 is not completely shut down in the absence of drug.

175

#### 176 ***Induction by other drugs***

177 Other drugs that target the 50S ribosomal subunit were tested for their ability to induce *erm*(47)  
178 expression. After establishing the MICs for telithromycin, lincomycin, spiramycin,  
179 dalfopristin/quinupristin, chloramphenicol and linezolid, one-fourth of the inhibitory concentrations  
180 added to cell cultures (Table 1). Telithromycin (Fig. 2D), lincomycin (Fig. 2E) and spiramycin  
181 induced *erm*(47) to a level that results in full monomethylation of nucleotide A2058. Lower, but  
182 nevertheless significant, levels of *erm*(47) expression were induced by chloramphenicol (Fig. 2F),  
183 the streptogramin A/B pair, dalfopristin/ quinupristin (Fig. 2G), and linezolid (Fig. 2H).

184 Comparable control studies were carried out with the 30S ribosomal subunit drug,  
185 spectinomycin. Methylation profiles remained identical to those from cells with no drug (Fig. 2B),  
186 showing that spectinomycin was not an inducer of *erm*(47).

187

## 188 Discussion

189 We show here that *erm*(47) encodes a methyltransferase that adds a single methyl group to 23S  
190 nucleotide A2058 of *H. kunzii*. This rRNA modification confers high resistance to lincosamide  
191 antibiotics<sup>9</sup> and moderate to high macrolide resistance, but no appreciable resistance to ketolides  
192 such as telithromycin.<sup>10</sup> Although the resistance observed here to the 16-membered ring macrolide  
193 spiramycin is somewhat higher than might have been expected, the resistance phenotype conferred  
194 by *Erm*(47) (Table 1) otherwise matches well with that seen for other *Erm* monomethyltransferases  
195 in a wide range of bacteria.<sup>6,7</sup> Methylation of rRNA by *Erm* variants, and ensuing effects on the  
196 translational machinery, incur a measurable cost to the host bacterium,<sup>14</sup> and thus expression of  
197 these enzymes is often shut down in the absence of drug. When antibiotic appears in the  
198 environment and the resistance mechanism is needed, expression of *erm* can then be rapidly  
199 induced at the translational level.<sup>28</sup>

200 The premise of such an induction mechanism is that it confers a selective advantage to the  
201 bacterium by preventing nonproductive gene expression in the absence of drug while facilitating  
202 rapid implementation of resistance upon drug detection.<sup>29</sup> Full induction of *erm*(47) is seen here  
203 with erythromycin and lincosamine (Fig. 2) conferring high level resistance to these drugs (Table  
204 1). Expression of *erm* is not normally initiated by 16-membered macrolides<sup>6,7</sup> and although  
205 induction of *erm*(47) by spiramycin was unexpected, the subsequent resistance conferred to this  
206 macrolide does offer a clear functional advantage to *H. kunzii* (Table 1). In the case of the  
207 dalbopristin/quinupristin compounds, the streptogramin B moiety quinupristin contacts nucleotide  
208 A2058,<sup>21,22</sup> and conceivably promotes *erm*(47) induction. Here, the accompanying streptogramin A  
209 component, dalbopristin, would act in synergy to aid quinupristin binding, and no resistance is  
210 conferred to this drug combination despite monomethylation of the rRNA (Table 1). No induction  
211 of *erm*(47) was seen with the 30S subunit-targeting drug spectinomycin, which is also consistent

212 with the view that drugs which bind to other ribosomal targets outside the MLS<sub>B</sub> site do not act as  
213 inducers of *erm* genes.

214 However, the other 50S-targeting drugs chloramphenicol and linezolid bind outside the MLS<sub>B</sub>  
215 site,<sup>21, 22</sup> but nevertheless induce a significant degree of *erm*(47) expression. Neither  
216 chloramphenicol nor linezolid has previously been linked with *erm* induction, and the expression of  
217 *erm*(47) seen here offers no apparent advantage to the bacterial cell. These observations challenge  
218 the classic model for induction and prompts the question of how *erm*(47) differs from other  
219 inducible *erm* genes.

220 At a first glance, the *erm*(47) operon is organized in a manner similar to other inducible *erm*  
221 genes, where translation occurs on a bicistronic mRNA together with an upstream leader  
222 sequence.<sup>28</sup> As in the classic model for other *erm* operons, the *erm*(47) mRNA can fold into a  
223 conformation (Fig. 3A), where the groundstate in the absence of antibiotic occludes the ribosome  
224 binding site and the start codon of the methyltransferase cistron. In the absence of antibiotics, the  
225 inherent helicase activity of the translating ribosome<sup>30</sup> would disrupt the 1:2 helix to read through  
226 the *erm* leader sequence and then dissociate from the mRNA after synthesizing the short leader  
227 peptide. Here, the ribosomal binding site and first codon of the *erm*(47) cistron would remain  
228 inaccessible within the 3:4 helix. However, when an antibiotic such as erythromycin causes the  
229 ribosome to stall on region 1 of the leader sequence, helix 1:2 would remain unraveled and enable  
230 mRNA refolding to expose the downstream start site where another ribosome would enter and  
231 initiate methyltransferase synthesis (Fig. 3B).

232 The propensity of ribosomes to stall on the *erm* leader is determined by both the type of antibiotic  
233 and by the encoded sequence of the leader peptide. The macrolides erythromycin and azithromycin  
234 stall ribosomes on *E. coli*<sup>31</sup> and *Staphylococcus aureus* mRNAs<sup>32</sup> by a similar mechanism that is  
235 accentuated by specific tripeptide sequences such as Arg-Leu-Arg. This sequence is present in the

236 *erm*(47) leader (Fig. 3) and several other *erm* leader sequences.<sup>33</sup> Stalling by telithromycin is also  
237 promoted at Arg-X-Arg triplets,<sup>31</sup> consistent with *erm*(47) induction by this drug. Furthermore, the  
238 *erm*(47) leader also contains runs of serine codons that have been shown to augment ribosome  
239 stalling in the presence of chloramphenicol or linezolid.<sup>34</sup> Pausing of the ribosome in at any of these  
240 sites in the leader could lead to rearrangement of the mRNA secondary structure as described above  
241 (Fig. 3B) and result in *erm*(47) expression.

242 Also particular to the *erm*(47) operon is the small but reproducible amount of *erm*(47) expression  
243 seen in drug-free cells (Fig. 2B). Generally in *erm* mRNAs, the overall stability of the occluded  
244 structure in the absence of drugs (Fig. 3A) has to be greater than that an alternative structure  
245 facilitated by ribosome stalling. However, free energy estimates suggest that the groundstate folding  
246 of the *erm*(47) operon might favor a conformation with helix 2:3 extending into a paired structure  
247 between region 1 and region 4 (Fig. 3C). Although such a configuration would occlude the  
248 initiation site of the *erm*(47) methyltransferase, each translational event on the leader sequence  
249 would briefly expose the downstream initiation site to leave a narrow window for expression of  
250 *erm*(47) even in the absence of drug.

251 A mechanism favoring the Fig. 3C structure, would explain the minor amount of *erm*(47)  
252 expression in drug-free cells. Furthermore, this leakiness could conceivably be exacerbated by  
253 drugs such as chloramphenicol and linezolid that do not normally induce *erm* genes but which, in  
254 the sub-inhibitory amounts used here, clearly provoke *erm*(47) expression. Although the mechanism  
255 for this remains unclear, the lack of induction by the 30S subunit inhibitor spectinomycin points  
256 towards a 50S-specific effect, and might be linked to the proximity of the chloramphenicol and  
257 linezolid binding sites to that of the MLS<sub>B</sub> compounds. Taking these points together, we conclude  
258 that the *erm*(47) operon is activated by a much wider range of drugs than previously have been  
259 associated with the induction of *erm*-encoded resistance.

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263

264 **Transparency declarations**

265 Nothing to declare.

266

267

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351

## 352 **Figure Legends**

353

354 **Figure 1** (A) Schematic of the 3'-half of *H. kunzii* 23S rRNA based on secondary structures at the  
355 Gutell lab comparative RNA site (<http://www.rna.icmb.utexas.edu>). The sequence around the Erm  
356 methyltransferase target at nucleotide A2058 (bold line within the boxed area of the schematic) was  
357 isolated for mass spectrometry analysis. The *E. coli* numbering system for rRNA nucleotides<sup>35, 36</sup> is  
358 used throughout. (B) MALDI mass spectrum of this rRNA sequence after digestion with RNase T1.  
359 The target nucleotide A2058 resides in the tetranucleotide AAAGp at  $m/z$  1351.2 prior to  
360 methylation. (C) Mass spectrum of the same rRNA sequence after RNase A digestion, where A2058  
361 is in the larger oligonucleotide GGAAAGACp at  $m/z$  2675.4. The calculated singly protonated  
362 masses of the oligonucleotides (inserted tables) match the experimentally measured  $m/z$  values  
363 (shown on the spectral peaks) to within 0.1 Da.

364

365 **Figure 2** Enlargement of RNase T1 spectral region containing nucleotide 2058 (boxed on spectrum  
366 in Fig. 1B). (A) RNA from the susceptible ATCC51366 strain without *erm*. Unmethylated A2058  
367 is in the AAAGp fragment at  $m/z$  1351; the neighboring fragment CUAGp at  $m/z$  1304 remains  
368 unchanged under all conditions and is shown for reference. (B) RNA prior to addition of antibiotic  
369 from the resistance UCN99 strain containing *erm*(47). (C – H) The UCN99 strain after *erm*(47)  
370 induction with one-fourth the MICs of the antibiotics indicated. After induction with erythromycin,  
371 lincosamine and telithromycin, the  $m/z$  1351 peak is shifted to  $m/z$  1365 indicating that nucleotide  
372 A2058 has been modified with a single methyl group. No spectral peak was evident at  $m/z$  1379  
373 showing that no dimethylation of this nucleotide takes place. Chloramphenicol,  
374 dalbopristin/quinupristin and linezolid also induce the expression of *erm*(47) to varying degrees.

375

376 **Figure 3** Putative secondary structures of the *erm*(47) leader mRNA. (A) The classic ground-state  
377 structure for inducible *erm* operons<sup>28</sup> in which the base-paired segments 1:2 and 3:4 are formed. In  
378 this conformation, translation of the methyltransferase would be prevented due to sequestering of

379 the ribosome binding site (Shine-Dalgarno, in blue) and the initiation codon (green) within the  
380 secondary structure formed by segments 3 and 4 (insert). (B) Drug-induced pausing of ribosomes  
381 around the series of hydrophobic residues (Arg-Leu-Arg, bold) encoded in region 1 of the leader  
382 sequence frees region 2 to form a stable helical structure with region 3. This in turn opens up the  
383 Shine-Dalgarno and start codon sequences in region 4 enabling translation of the *erm*(47)  
384 methyltransferase to be initiated. We note that the sequence at the 3'-end of *H. kunzii* 16S rRNA  
385 restricts its interaction to the AGGU (blue) nucleotides of the Shine-Dalgarno regions whereas in  
386 numerous other bacteria this interaction would be significantly more extensive and (for instance in  
387 *E. coli*) include five additional nucleotides upstream of the leader cistron (boxed in blue). (C) An  
388 alternative ground-state structure that also sequesters the translational start site of the *erm*(47)  
389 cistron. Free energy ( $\Delta G$ ) estimations indicate that this structure is more favorable than that in panel  
390 A. After drug-induced pausing of a ribosome in region 1, this structure requires no conformational  
391 rearrangement of regions 2 and 3 in order to free the methyltransferase initiation site.  
392

**Table 1.** Resistance phenotypes conferred by *erm(47)* in *H. kunzii*.

| <i>H. kunzii</i> strains    | Minimal inhibitory concentrations (mg/L) |                 |                    |                 |                               |                      |           |                    |
|-----------------------------|------------------------------------------|-----------------|--------------------|-----------------|-------------------------------|----------------------|-----------|--------------------|
|                             | erythro-<br>mycin                        | linco-<br>mycin | telithro-<br>mycin | spira-<br>mycin | dalfopristin<br>/quinupristin | chloram-<br>phenicol | linezolid | spectino-<br>mycin |
| ATCC 51366                  | 1                                        | 2               | 2                  | 4               | 0.25                          | 4                    | 1         | 8                  |
| UCN99<br>[ <i>erm(47)</i> ] | ≥256                                     | ≥256            | 1                  | ≥256            | 0.12                          | 4                    | 1         | 8                  |

**Table 1.** MICs for the susceptible ATCC 51366 strain and the resistant UCN99 strain of *H. kunzii* harbouring *erm(47)* were determined by broth microdilution. The MIC values were used to establish growth conditions for the UCN99 strain, where one-fourth of the MIC for each drug was used to test for *erm(47)* induction.

A

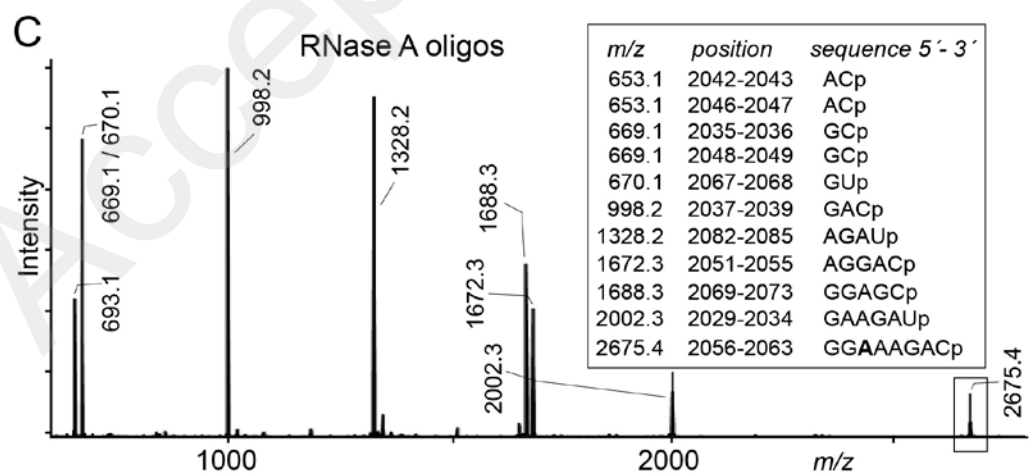
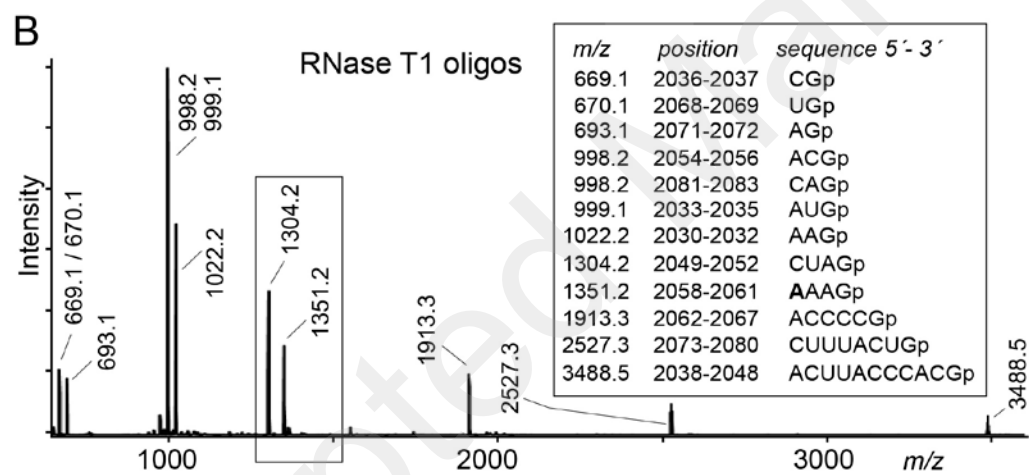
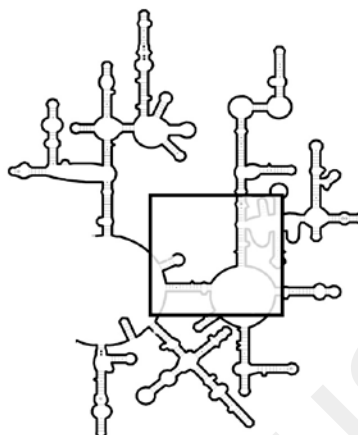
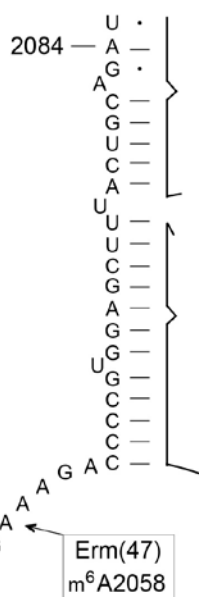
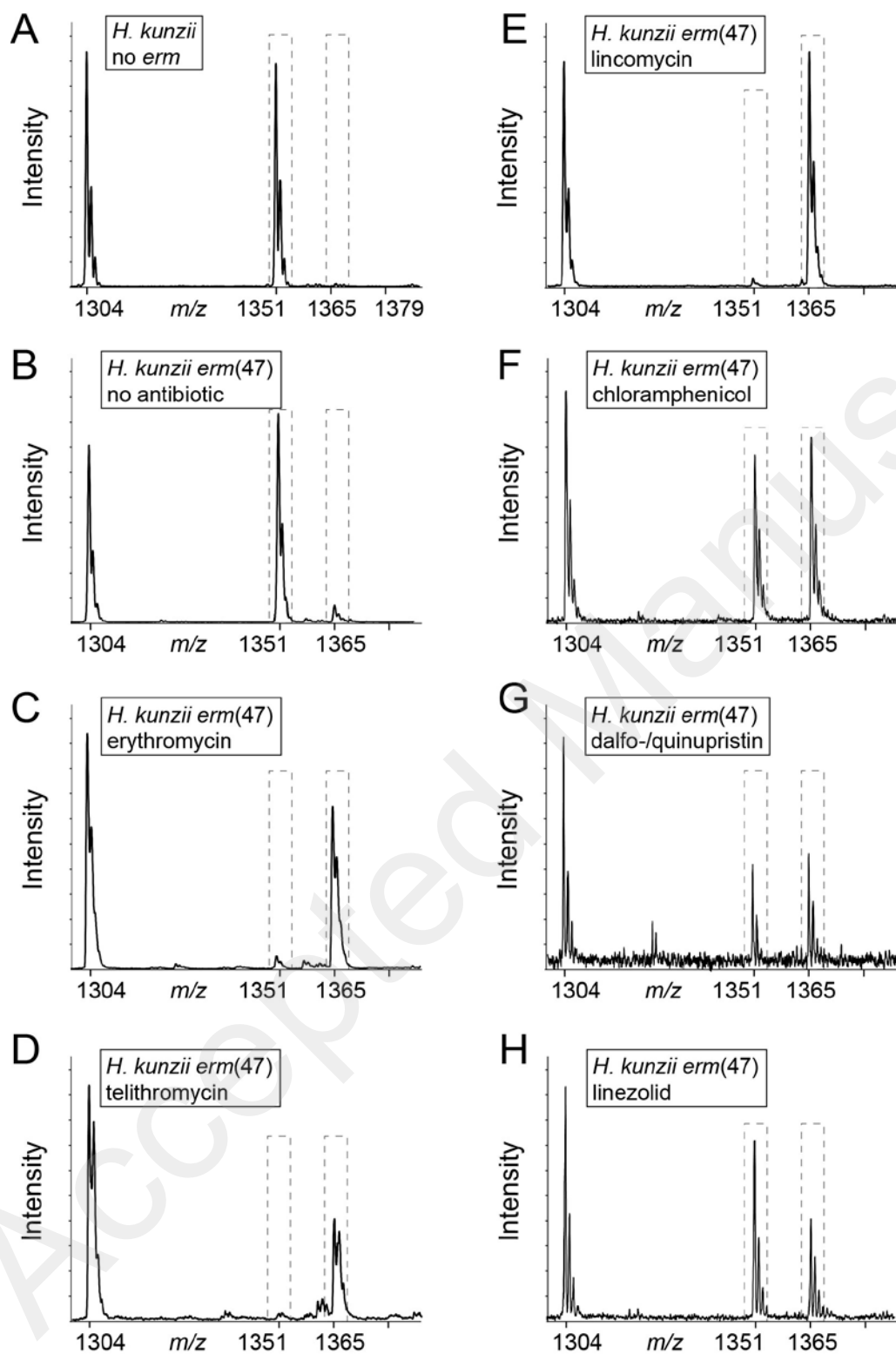


FIG 2



**FIG 3**

