Paucity of adaptive selection in PmrAB two-component system may resist emergence of colistin resistance in *Acinetobacter baumannii*

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Running title: Paucity of adaptive selection to sustain colistin resistance in *Acinetobacter baumannii*

Keywords: Colistin resistance; PmrAB; Point mutation; Selection analysis; Purifying selection; *Acinetobacter baumannii*

Abstract

Investigations on the selection pressure acting on point mutations in PmrAB two-component system may provide insights into the future of colistin therapy in *Acinetobacter baumannii*, since mutations in *pmrAB* are implicated in colistin resistance. We performed adaptive selection analysis of *pmrAB* and compared with the available data on colistin resistant strains. We analysed PmrAB sequences in 3113 draft genomes of *A. baumannii* obtained from RefSeq database. Adaptive selection analysis was performed by two widely used programs namely, HyPhy and PAML. In addition, to examine the reliability of the approach, the same analysis was performed on *gyrA* of *Escherichia coli* and *Salmonella enterica*, since adaptive mutations on *gyrA* confer quinolone resistance. Mutations that had caused colistin resistance were found to be neither adaptive nor polymorphic, rather they occur at sites that are either under neutral or purifying selection. Strong negative evolutionary selection pressure is also observed at sites throughout both PmrA and PmrB. Sites with high levels of polymorphisms in PmrAB were found to be under neutral selection. Notably, there was no sign of positive selection. Some of them are rather deleterious. These conditions might be maintaining the incidence of colistin resistance in *A. baumannii* under check. Therefore, in the context of colistin resistance, natural selection plays only a minor role and we assert that in future, *A. baumannii* may not be able to sustain and successfully disseminate colistin resistance. Therefore, at present the concerns raised about continuing the usage of colistin for the treatment against *A. baumannii* infections appears to be unnecessary.

Introduction

Single nucleotide polymorphisms (SNPs) contribute greatly towards the microevolution of a bacterium (Chouard, 2010; Reznick & Ricklefs, 2009). Consequently, these bacteria are able

53 to adapt to their environment comparatively faster without any significant associated costs.
54 These mutations also have a profound effect on drug resistance and pathogenesis. In recent
55 years, colistin resistance has been found to be due to SNPs in the genes encoding the two-
56 component system (TCS) PmrAB, as well as in the *lpx* operon, that are responsible for the
57 production and regulation of lipopolysaccharide (LPS) in bacterial cell walls (Moffatt et al.,
58 2010; Snitkin et al., 2013; Thi Khanh Nhu et al., 2016).

59 PmrAB is thought to regulate lipid-A biosynthesis of gram negative outer membranes (Boll et
60 al., 2016). Lipid-A is a constituent of the outer membrane LPS, which is the target of colistin.
61 Colistin resistant strains of *A. baumannii* have modifications and/or loss of LPS from their
62 outer membrane, with concomitant mutations in the *pmrCAB* and/or *lpx* operon (Boll et al.,
63 2016; Carretero-Ledesma et al., 2018). Emergence of such resistance is worrisome, since
64 colistin is the last-choice drug against multidrug resistant *A. baumannii*. The objective of this
65 study was therefore to identify the hyper-variable regions of the TCS PmrAB in *A. baumannii*
66 to gain insights into their contribution towards the structure and function of this TCS. Since
67 point mutations in PmrAB have been implicated in resistance to colistin, sequence analysis of
68 PmrAB in the context of the evolutionary selection pressure acting on these mutations may
69 provide insights into the fate of colistin resistance in *A. baumannii*. We report that, both
70 PmrA and PmrB are under strong purifying evolutionary selection pressure. In addition, there
71 was no sign of adaptive evolution. Mutations that cause colistin resistance are neither
72 adaptive nor polymorphic. Some of them are rather deleterious. Therefore, it is highly
73 unlikely that these mutations will sustain in the long term and hence, *A. baumannii* may not
74 be able to emerge as a colistin-resistant organism.

75 **Methods**

76 Minimum inhibitory concentration (MIC) for colistin were performed according to European
77 Committee on Antimicrobial Susceptibility Testing guidelines (EUCAST, 2017). *pmrA*
78 coding sequences were amplified by *in house* designed primers and custom sequenced
79 (**Supplementary methods**). For selection analysis, 3113 genomes were retrieved from
80 RefSeq from which, *pmrA* and *pmrB* sequences were extracted. *pmrAB* sequences originated
81 from colistin resistant strains that appeared in the literature were also included. Selection
82 analysis was performed with Single Likelihood Ancestor Counting method of the HyPhy
83 package, and CodeML of the PAML package. All methods in detail have been provided in
84 **Supplementary methods**.

85 **Results**

86 ***Mutation hotspots in PmrAB are not responsible for colistin resistance***

87 In our observations, two of our clinical isolates, PKAB15 and PKAB19 were identified to be
88 colistin resistant (**Table 1**). We amplified and performed sequencing of the *pmrA* genes from
89 these two isolates. One non-synonymous mutation in each of these isolates was identified in
90 *pmrA*, when compared to the reference strain *A. baumannii* ATCC17978, both of which
91 (D10N in PKAB15, and R212L in PKAB19) have never been reported hitherto. Both these
92 mutations change charged amino acids with uncharged ones which, we believe might have
93 significant alterations on the structure of the protein to bring about colistin resistance.

94 Across all the 3113 genomes investigated in the study, 43 sites with non-synonymous
95 mutations were identified in *pmrA* (**Table 2**). Of these, five were highly polymorphic sites.
96 Most of the mutations occur in the signal receiver domain and the region connecting the
97 receiver domain to the DNA-binding domain (**Figure 1**). The DNA binding domain recorded
98 as few as seven sites with non-synonymous mutations, four of which had rare mutations
99 while one was among the five most polymorphic sites. A well-conserved DNA binding

100 domain emphasizes the importance of genes that are regulated by PmrAB, and therefore, it
101 cannot afford many mutations in this region.

102 One hundred fifty sites with amino acid substitutions in the PmrB protein sequence were
103 found in our study, of which, 64 sites had rare mutations, each occurring in ≤0.1% of the
104 genomes analysed (**Table 3**). Eighteen of the sites were highly polymorphic (**Table 3 &**
105 **Figure 2, middle panel**). Most of these belong to the two trans-membrane regions and the
106 intracellular domain connecting them. The HATPase_c domain, which binds the ATP moiety
107 required for auto-phosphorylation of the sensor kinase, is the most conserved domain. In
108 particular, with the exception of a few rare mutations, the ATP binding domain, Mg^{2+} binding
109 domain and the G-X-G motif were well conserved (**Figure 2, upper & middle panel**). The
110 Mg^{2+} metal cofactor and the G-X-G motif line the ATP binding pocket and are therefore
111 crucial for the function of PmrB. Clearly, mutations in these sites can severely compromise
112 its function.

113 Most of the amino acid substitutions previously reported in PmrAB in isolates of *A.*
114 *baumannii* that are resistant to colistin were found to be at non-polymorphic sites in our
115 analysis. To date, four sites in *pmrA* and 26 sites in *pmrB* have been described as having non-
116 synonymous mutations in the isolates resistant to colistin (**Supplementary Table S1**). Only
117 one of the four resulting amino acid substitutions in PmrA and only three of the 26 in PmrB
118 belong to polymorphic sites. In PmrA, the only substitution (S119T) at a polymorphic site,
119 which has been shown to correlate with colistin resistance, occurs in the region that connects
120 the domain of the signal receptor to the DNA binding domain (**Figure 1, top & middle**
121 **panel**). In the case of PmrB, two of the three polymorphic sites in PmrB of colistin-resistant
122 isolates have frequent substitutions A142V and P170Q; both sites fall into the intracellular
123 domain of the protein. P360Q mutation occurs in the third polymorphic site, which lies in the

124 ATPase domain of PmrB (**Figure 2, top & middle panel, Supplementary table S1**). Since
125 the amino acid substitutions previously described in colistin resistant isolates of *A. baumannii*
126 are sporadic observations, we understand that both PmrA and PmrB are constrained when
127 accommodating non-synonymous SNPs. Therefore colistin resistance has not been an
128 emerging phenomenon in the case of *A. baumannii*, unlike resistance to other antibiotics. We
129 therefore examined the selection pressure at each amino acid site of the TCS, to see if there is
130 negative selection acting on them, which could be the cause of the underlying mutation
131 constraints.

132 ***Both PmrA and PmrB are subject to negative selection pressure***

133 To estimate the strength of natural selection pressure acting on each amino acid site in PmrA
134 and PmrB, we performed codon based selection analysis. We observed significant negative
135 selection (also known as 'purifying selection') all through both the protein sequences. In
136 PmrA, 43 sites out of 224 are subjected to a strong negative selection pressure. As a result,
137 these sites and the regions around them remain conserved (Figure 1, bottom panel).
138 Functionally important sites, both in the signal receiver domain and in the DNA binding
139 domain, exhibit a much more negative selection pressure than the rest of the sequence. While
140 no signs of positive selection were found in PmrA, sites with high levels of polymorphism
141 were found to be under neutral selection instead. Three of the four mutated sites of PmrA
142 (M12K/M12I, P102H, S119T and L206P) that are reported in colistin-resistant isolates of *A.*
143 *baumannii* belong to these highly polymorphic regions, which are under neutral selection
144 (**Supplementary Table S1 & Figure 1, middle panel**).

145 Similarly, 52 out of 444 sites are under significant negative selection in PmrB (Table 3 &
146 Figure 2, bottom panel). Sites under negative selection were well distributed throughout the
147 protein sequence. As in the case of PmrA, these sites and regions around them are fairly

148 conserved in PmrB. In particular, the extracellular region, which spans the functional sites of
149 the HATPase_c, HisKA and HAMP domains, has far fewer mutations than the rest of the
150 molecule, due to the presence of the majority of the sites under negative selection therein
151 (Figure 2, top & middle panel). Notably, though most of these sites are under purifying
152 selection, the functional domains managed to acquire amino acid substitutions (Figure 2,
153 middle & bottom panel). This can explain the loss of fitness associated with colistin
154 resistance in previous reports. As many as 23 out of the 26 amino acid substitutions in PmrB
155 that occur in colistin-resistant isolates fall in functional domains (**Supplementary Table S1**
156 **& Figure 2, top panel**). However, these mutations occur mainly on sites that are under
157 neutral selection (**Figure 3A**), each of them being delimited by the sites under negative
158 selection in the functional domains (**Figure 2, top & bottom panels**). However, there exists
159 a site at 153 of PmrB that is under significant positive selection (**Table 3 & Figure 3B**).

160 The SLAC method is equivalent to the site model of the equally well accepted program
161 CodeML of the PAML package, but both employ different sets of procedures and algorithms
162 (Yang, 2007). To examine the consistency of the obtained results, we performed the same
163 selection analysis for PmrA and PmrB with CodeML. CodeML produced similar results to
164 that we obtained with SLAC of HyPhy; it did not find any sign of significant positive
165 selection in any of the sites of PmrA and PmrB (**Supplementary file S2**). This increases the
166 reliability of the study and reinforces our results on the lack of adaptive selection on PmrAB.

167 Taken together, mutations in PmrAB TCS that are held responsible for colistin resistance in
168 *A. baumannii* are neither adaptive nor polymorphic (Figure 3A). Rarher, the PmrAB TCS is
169 under strong purifying selection to sustain colistin resistance in the long term. Therefore, it is
170 plausible to believe that resistance to colistin, when occurred due to mutations in PmrAB, is
171 not disseminated owing to the underlying purifying selection that causes the loss of fitness.

172 ***Selection analysis of gyrA of E. coli and S. enterica was able to detect signatures of***
173 ***adaptive evolution that validates our approach***

174 To validate this approach of selection analysis and increase reliability of the results, we
175 employed the same methodology to detect positively selected sites in well-known antibiotic
176 targets that cause antibiotic resistance. We considered DNA gyrase A (*gyrA*) to be the most
177 appropriate for this purpose, as there is abundant of literature that share common findings
178 about mutations in this gene in multiple organisms that lead to quinolone resistance.
179 Mutations at Ser-83 and Asp-87 in GyrA are the most frequently reported sites to be
180 responsible for quinolone resistance in Enteric bacteria (Domínguez et al., 2002; Everett et
181 al., 1996; Lindgren et al., 2003; Vila et al., 2001, 1994). Therefore, our results of selection
182 analysis can be qualified as valid if these two sites are detected under the same selection
183 analysis framework applied to *pmrAB*. Interestingly, selection analysis of *gyrA* successfully
184 detected these two sites (Ser-83 and Asp-87) to be under adaptive evolution in both *E. coli* as
185 well as *S. enterica* (**Figure 3B & Supplementary file S2**). This not only substantiated the
186 reliability of the methodology, but also validated our results obtained for *pmrAB*. In addition,
187 this also provided evidences that adaptive evolution had an important role to play behind the
188 emergence of quinolone resistance in *E. coli* and *S. enterica*, while it has little role to play in
189 colistin resistance in *A. baumannii*.

190 **Discussion**

191 The emergence and spread of multidrug-resistant ESKAPE group of pathogens including *A.*
192 *baumannii* have prompted many health professionals to assume that we are on the verge of a
193 post-antibiotic era. Colistin and to some extent, tigecycline appear to be the only treatment
194 options for treating infections caused by multidrug-resistant *A. baumannii*. In addition, the
195 notable efficacy of colistin against tigecycline-resistant *A. baumannii* advocates colistin as

196 the last option against the organism. However, reports of colistin resistance in *A. baumannii*
197 have raised unprecedented concerns, as it is thought that resistance to colistin may become
198 widespread in the future if not used judiciously. Contrary to this belief, we have shown in this
199 work that resistance to colistin may not become a general phenomenon and these concerns
200 are therefore unwarranted. Similar opinions were also put forwarded by Lo´pez-Rojas *et al.*
201 based on the observed reduced fitness and reduced virulence of experimentally induced
202 colistin-resistant *A. baumannii* isolates (Lo´pez-Rojas et al., 2011). However, they also
203 cautioned that, though the synonymous mutations are initially not adaptive, compensatory
204 mutations may attenuate the reduced fitness. In contrast, using selection analysis, our
205 investigation provided more direct evidence that mutations that cause colistin resistance are
206 neither adaptive nor polymorphic and therefore, may not take over the global population.

207 In *A. baumannii*, resistance to colistin occurs in two mechanisms: enzymatic modification of
208 lipid A moieties of the bacterial cell wall, either by the addition of phosphoethanolamine or
209 galactosamine, or hyperacylation, and complete loss of cell wall lipo-polysaccharide (LPS)
210 due to inactivation of lipid A biosynthesis (Beceiro et al., 2011; Boll et al., 2015; Henry et al.,
211 2011; Moffatt et al., 2011, 2010; Pelletier et al., 2013). Colistin resistance by both
212 mechanisms is the result of deletions and/or substitutions in the PmrAB two-component
213 system (Adams et al., 2009; Arroyo et al., 2011; Beceiro et al., 2011). Resistance to colistin
214 due to mutations in PmrAB is the most common mode of colistin resistance.

215 PmrAB mutation analysis, in conjunction with domain analysis suggests that the signal
216 receptor domain and the DNA binding domain, as well as the functional sites therein are
217 ideally conserved in PmrA. This indicates that PmrA transduces a very specific signal, input
218 from PmrB and in turn, regulates a limited and non-degenerate set of genes. On the other
219 hand, the transmembrane domains as well as the intracellular domain of PmrB are highly

220 variable. Other domains such as the kinase and ATP-binding domain are comparatively
221 conserved and contain more sites with purifying selection. Since there is only one
222 intracellular domain delimited by transmembrane α-helices, this domain must be recruiting
223 the response regulator(s). Since this domain is variable, we can state that it recruits more than
224 one response regulators and/or transcription factors, resulting in cross-talks between parallel
225 signal transduction pathways, one of which is the cognate PmrA-mediated signalling. More
226 detailed investigations at the molecular level are needed to corroborate this hypothesis.

227 In this study, we collected 3113 draft genomes from the RefSeq database to evaluate the
228 degree of natural selection acting on each site of PmrAB two-component system. Initially, the
229 study considered *pmrAB* sequences only related to colistin resistant strains. This comprise of
230 44 *pmrA* and 39 *pmrB* sequences that were collected from the whole genomes of colistin
231 resistant strains as well as from the full length coding sequences associated with colistin
232 resistance that appear in the literature. Selection analysis with this dataset showed just one
233 site with purifying selection and no adaptive selection in PmrA. Similarly, it showed neither
234 positive nor negative selection in PmrB (**Supplementary file S2**). However, when all the
235 *pmrAB* sequences from the 3113 genomes were considered, it was found that the PmrAB
236 TCS is under strong purifying selection. This dramatic change in the results can be attributed
237 to the homogeneity of the initial sequence data that contains sequences associated with only
238 colistin resistant strains. In cases where the sequences belong to a homogenous population
239 exposed to similar environmental conditions, most of the mutations are transient mutations,
240 which underestimates any real purifying as well as adaptive selection (Kryazhimskiy &
241 Plotkin, 2008). A similar scenario exists when the strains undergo convergent and parallel
242 evolution resulting from colistin exposure (Crandall et al., 1999). The larger dataset allowed
243 the inclusion of genomes reported worldwide over a wide range of time scale that ensures a
244 heterogeneous set of samples/populations for analysis.

245 To evaluate long term emergence of colistin resistance, we need to examine the scenario
246 post-exposure to colistin. In addition, if any site is under adaptive selection, selection analysis
247 should identify signatures of adaptive selection from a sufficiently large dataset, even in the
248 absence of colistin exposure. We could then qualify these mutations are stable enough to
249 sustain long term resistance to colistin. Most of the amino acid substitutions in PmrAB
250 reported to date are either from longitudinal isolates of patients or induced in laboratory
251 conditions. Reports of clinical isolates bearing colistin resistance are scant. In addition, the
252 colistin exposure to *A. baumannii* lasts as long as the treatment of the patient, and the
253 resistance (if arises) reverts back to susceptible phenotype on withdrawal of colistin. (Adams
254 et al., 2009; Cheah et al., 2016; Snitkin et al., 2013). Therefore, it is plausible to believe that
255 mutations that cause colistin resistance are transient mutations owing to the short exposure to
256 the antibiotic as clinicians prefer to give short term colistin therapy due to its association with
257 nephrotoxicity. Further, with no evidence of compensatory mutations linked to colistin
258 resistance identified till date, which strengthens our supposition that colistin resistant
259 mutations may never get fixed in the global population of *A. baumannii* (Lesho et al., 2013;
260 Lo´pez-Rojas et al., 2011; Rolain et al., 2011). This observation may not apply to the
261 populations in micro-environments, such as a laboratory cultures.

262 Apparently, resistance to colistin is achieved by a cost of fitness. Studies in the past have
263 shown reduced fitness in colistin resistant isolates, both in terms of growth rate under
264 laboratory conditions and reduced virulence (Beceiro et al., 2014; Lo´pez-Rojas et al., 2011;
265 López-Rojas et al., 2013). In addition, to compensate for the loss/modification of the LPS, the
266 transcriptome and the corresponding proteome are largely shifted towards expression of
267 genes responsible for membrane integrity and biogenesis (Boll et al., 2016; Cheah et al.,
268 2016; Henry et al., 2011). A recent work confirms that surface lipoproteins are overexpressed
269 and localized profusely on the cell surface, which could compensate for the loss of membrane

270 integrity. These studies highlight the excessive cost of colistin resistance, and therefore,
271 mutations in PmrAB may be deleterious and subject to strong purifying selection.

272 A significant number of sites in both PmrA and PmrB are under purifying selection. In
273 addition, we also identified mutation hotspots, sites that are exceptionally polymorphic.
274 Interestingly, sites with mutations that cause colistin resistance are not polymorphic sites.
275 Rather, colistin resistance mutations are largely under neutral selection. In addition, a few of
276 colistin resistance mutations are also under purifying selection (**Figure 3A & B**). We suggest
277 that these conditions maintain the incidence of colistin resistance under check. Further, loss
278 of fitness due to colistin resistance resulting from the combined effects of neutral colistin
279 resistance mutations and the purifying selection acting on the overall protein sequences
280 ensure that the colistin resistance remains uncommon. The reasons for this loss of fitness
281 include physiological costs in terms of slow growth rate, loss of membrane integrity and its
282 compensation, and reduced virulence. In addition to this, because of the high selection
283 pressure exerted by the host's immune system and the presence of drugs, the short exposure
284 of the bacterium to colistin in the host, it is highly unlikely that random genetic drift can fix
285 these mutations. These observations explain us why reports of colistin-resistant isolates of *A.*
286 *baumannii* have been sporadic and they might remain so. In the near future, *A. baumannii*
287 may not be able to emerge as a colistin-resistant organism, unless other mechanisms such as
288 plasmid-mediated (*e. g.*, *mcr*-1) resistance emerge.

289 The other mechanism of development of Colistin resistance is due to mutations in *lpxACD* of
290 *A. baumannii*. However, unlike mutations in *pmrAB*, where virtually all mutations that cause
291 Colistin resistance are due to substitutions, mutations reported in *lpxACD* are due to large
292 indels, non-sense mutations and insertion sequence-mediated gene disruptions that cause
293 complete loss of cell wall lipopolysaccharide (Carretero-Ledesma et al., 2018; Girardello et

294 al., 2017; Moffatt et al., 2010). There is no direct method to analyse signatures of selection
295 pressure from such mutations. However, it has already been reported through growth curve
296 and virulence assays that fitness costs associated with *lpx* mutations are even higher than
297 those associated with *pmr* mutations (Beceiro et al., 2014) and therefore, *lpxACD* mutants are
298 unsustainable in the long run.

299 One site at 153 of PmrB appears to be under adaptive selection. This site has $dN - dS$ value of
300 9.48851 ($p = 0.013$). Surprisingly, this site has never been reported to be mutated in colistin
301 resistant strains of *A. baumannii*. This site belongs to one of the transmembrane domains of
302 PmrB. The fact that it has so far never caused colistin resistance indicates that the positive
303 selection gain may not overcome the cost of colistin resistance. Since the site belongs to a
304 transmembrane domain, it is also possible that the mutations that occur at this site might
305 serve an advantage in terms of structural stability, rather than having a functional advantage.
306 Further, studies employing site directed mutagenesis experiments could throw additional
307 information about this site.

308 The study attempted to assert the reliability of the methodology by applying it to *gyrA* on the
309 basis of whether it can identify sites that are expected to be under adaptive selection in
310 multiple organisms. The computational method of selection analysis has precisely detected
311 the sites under adaptive evolution in GyrA, an observation which is supported by a wealth of
312 previous literature about quinolone resistance in bacteria. In case of *E. coli*, resistance to
313 quinolones requires multiple mutations in different genes including *gyrA* to achieve a
314 clinically relevant level. The requirement for multiple mutations suggests that there is strong
315 selection for resistance mutations invariably associated with unusually high mutation rates
316 occurring due to antibiotic exposure. Quinolones are known to interact with the DNA-GyrA
317 complex, rather than with the enzyme alone. One study correlated that *gyrA* mutations lead to

318 reduction of drug binding to the protein-DNA complex to 60-fold less than that of wild-type
319 GyrA (Willmott & Maxwell, 1993). In this respect, identification of the sites that are most
320 pronounced to be associated with quinolone resistance by adaptive selection analysis
321 provides sufficient grounds to emphasize the power of the methodology to detect adaptive
322 evolution in protein coding sequences.

323 **Conclusion**

324 The anticipation and management of natural selection is an important aspect of the fight
325 against multidrug resistance (Lesho et al., 2013). This was the central theme of this work in
326 the context of the degree of natural selection acting on PmrAB TCS, which has been
327 implicated in resistance to colistin. We showed that in case of resistance to colistin, natural
328 selection plays only a minor role, if at all, and therefore, continuing use of colistin could still
329 be a safe option. Therefore, the concerns raised by healthcare professionals regarding rapid
330 emergence of colistin resistance and about continuing the usage of colistin for the treatment
331 against *A. baumannii* infections are unwarranted.

332 **Acknowledgements**

333 We duly thank and acknowledge the remote access to supercomputing provided by
334 **Bioinformatics Resources and Applications Facility (BRAF), C-DAC, Pune** for the
335 computationally intensive analyses.

336 **Funding**

337 This work was supported by University Grants Commission, Government of India [Grant
338 Ref. No. F. 30-70/2016 (SA-II)].

339 **Transparency declarations**

340 None to declare.

Author contributions: SP: Literature search, Figures, Data collection, Data analysis, Bench works, Writing of the article; **RS, AR**: Data collection, Bench works, Data analysis; **AT**: Critical revision of the article, administrative, technical, or logistic support; **KPA**: Conception and design, analysis and interpretation, writing of the article, provision of materials, patients, and resources; **PK**: Supervision of the entire work, Conceptual design of the experiment, analysis and interpretation, writing of the article, provision of materials, patients, resources and funding.

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465 ## Figure Legends

466

467 **Figure 1. Mutation analysis of PmrA of *A. baumannii*.**

468 **Top panel** – The domain analysis (predicted by HMMER)(Finn et al., 2011). The secondary structure
469 was predicted by Phyre2 web server (Kelly et al., 2015). The black triangles show functional sites of
470 the protein (predicted by the Conserved Domain Database) (Marchler-Bauer et al., 2011). A-Active
471 site, P-Phosphorylation site, D-Dimerization interface, R-Intermolecular recognition site. Barrels and
472 arrows represent α-helices and β-sheets, respectively. Numbers in brackets denote the start-end
473 positions of the corresponding domains.

474 **Middle panel** – Positions and numbers of non-synonymous mutations across all the genomes under
475 the analysis.

476 **Bottom panel** – Selection along the protein sequence expressed in terms of normalized *dN – dS*
477 (calculated by Datamonkey web server) (Kosakovsky Pond & Frost, 2005). Asterisks indicate sites
478 under negative selection with $p \leq 0{\cdot}05$. Triangles show the sites identified in colistin resistant isolates
479 in previous literatures.

480 This figure appears in colour in the online version of *JAC* and in black and white in the print version
481 of *JAC*.

482

483 **Figure 2. Mutation analysis of PmrB of *A. baumannii.***

484 **Top panel** – The domain analysis (predicted by HMMER)(Finn et al., 2011). The secondary structure
485 was predicted by Phyre2 server and trans-membrane (yellow barrels) by TMHMM (Kelly et al., 2015;
486 Sonnhammer et al., 1998). The black triangles show functional sites of the protein (predicted by the
487 Conserved Domain Database) (Marchler-Bauer et al., 2011). AB-ATP binding site, M-Mg^{2+} binding
488 site, G-GXG motif. Barrels and arrows represent α-helices and β-sheets, respectively. Yellow
489 coloured regions of the α-helices denote transmembrane domains. Numbers in brackets denote the
490 start-end positions of the corresponding domains.

491 **Middle panel** – Positions and numbers of non-synonymous mutations across all the genomes under
492 the analysis.

493 **Bottom panel** – Selection along the protein sequence in terms of normalized *dN – dS* (calculated by
494 Datamonkey web server) (Kosakovsky Pond & Frost, 2005). Asterisks indicate sites under negative
495 selection with $p \leq 0{\cdot}05$, green asterisk representing a site with positive selection. Triangles show the
496 sites identified in colistin resistant isolates in previous literatures.

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498 of *JAC*.

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500 **Figure 3. A) Venn diagram depicting the amino acid substitutions resulting from non-**
501 **synonymous mutations in** *pmrA* and *pmrB*, which are responsible for resistance to colistin in *A.*
502 *baumannii* are under neutral selection, most of which are non-polymorphic sites. Numbers in each set
503 indicate the number of sites falling under the corresponding category. **B)** Volcano plot of the *dN – dS*
504 data for *pmrAB* and *gyrA* plotted against $-\text{Log}_{10}$ of the *p*-value. The sites under adaptive selection in
505 PmrB and GyrA have been indicated.

506 This figure appears in colour in the online version of *JAC* and in black and white in the print version
507 of *JAC*.

Table 1. Molecular characters and other clinical details of isolates PKAB15 and PKAB19

Resistant Determinants

	bla_{IMP-1}	bla_{OXA-23}	bla_{OXA-51}	bla_{OXA-58}	bla_{NDM-1}	bla_{PER-1}	bla_{ADC}	*adeB*	*aac(3)-I*	*aac(6)-Ib*	*aph(3)-I*	*ant(3)-I*	*rmtC*	*armA*
PKAB15	+	+	+	-	-	+	-	+	-	-	-	-	-	-
PKAB19	-	+	+	-	-	-	-	+	-	-	+	-	-	-

Biofilm production and Antimicrobial susceptibility [a]

	MEM (mg/L)	CAZ (mg/L)	CRO (mg/L)	AMK (mg/L)	GEN (mg/L)	TOB (mg/L)	TGC (mg/L)	CST (mg/L)	Biofilm[b]
PKAB15	>256(R)	>256(R)	>256(R)	>256(R)	>256(R)	>256(R)	0.75(S)	3(R)	+
PKAB19	32(R)	32(R)	>256(R)	8(S)	256(R)	<2(S)	0.38(S)	3(R)	++

[a]R – Resistant, I – Intermediate, S – Sensitive, according to EUCAST Guidelines(EUCAST, 2017)
[b]+ – Weakly positive ($OD_{Control} < OD < 2xOD_{Control}$), ++ – Strongly positive ($OD > 3xOD_{Control}$)
MEM – Meropenem, CAZ – Ceftazidime, CRO – Ceftriaxone, AMK – Amikacin, GEN – Gentamicin, TOB – Tobramycin, TGC – Tigecyclin, CST – Colistin

Nucleotide sequence accession numbers

Nucleotide sequences of *pmrAB* from *Acinetobacter baumannii* strains PKAB19 and PKAB15 have been deposited in GenBank under accession numbers MH925082 and MH925083, respectively.

526 **Table 2**. Codon-wise mutation and selection analysis and the corresponding *p*-values for
527 *pmrA*. Only values for codons with non-zero normalized *dN* – *dS* values are shown. Sites
528 reported to be associated with colistin resistant strains are shown in bold. *p*-values >0.05 are
529 shaded red, <0.05 shaded green, intermediate color is yellow. Normalized *dN* – *d*S values >0
530 are shaded red, <0 shaded green, intermediate color is yellow.

Codon	*p*-value	Normalized *dN*-*dS*	#Non-synonymous mutations
3	0.264358	1.58451	2
4	0.296151	-3.71198	0
5	0.539133	-2.125	0
6	0.00820638	2.208	4
8	**0.450377**	**3.08981**	**2**
9	0.264775	-4.15186	0
10	0.459423	2.99064	6
12	**0.00515319**	**3.30361**	**7**
14	0.259259	-4.94689	1
15	0.453651	-2.84841	3
16	0.588192	3.67763	18
17	0.037037	-9.89377	0
18	0.0366219	-20.2495	88
19	0.037037	-9.89377	0
20	0.538401	2.4815	3
21	0.126661	-7.37897	0
25	0.337556	-4.42948	0
27	0.261537	-4.38016	0
29	0.0676334	-8.80995	0
31	0.555372	-1.65067	1
33	0.264775	-4.15186	0
34	0.333333	-3.29792	0
35	0.55381	-2.12147	0
36	0.459444	-2.65666	2
37	0.333333	-3.29792	0
38	0.174609	-6.07459	0
39	0.0123457	-13.1917	0
40	0.0394633	-13.1752	0
41	0.291797	-4.25337	0
42	0.111111	-8.24481	1
43	0.266116	-4.38016	0
44	0.51388	3.15339	101
45	0.0694484	-8.84626	0
46	0.264775	-4.15186	0
48	0.188558	-6.38329	0
51	0.0342525	-14.541	2
52	0.0185623	-12.4556	0
53	0.168915	-6.35978	0
54	**0.00115942**	**-24.6886**	**1**
55	0.314606	-4.02386	0
56	0.0123457	-13.1917	0
59	0.264775	-4.15186	0
60	0.0123457	-13.1917	0

Codon	*p*-value	Normalized *dN-dS*	#Non-synonymous mutations
62	0.119672	-10.7783	1
63	0.333333	-3.29792	0
64	0.0150859	-14.9871	0
66	0.0397356	-13.145	0
67	0.00264645	-18.0118	0
68	0.111111	-6.59585	0
70	0.498667	3.29154	8
71	0.333333	-3.29792	0
72	0.333333	-3.29792	0
73	0.037037	-9.89377	0
74	0.00411523	-16.4896	0
75	0.0123457	-13.1917	0
76	0.303064	-4.28582	0
77	0.305164	-3.60236	0
78	0.305164	-3.60236	0
79	0.338828	-3.29792	0
80	0.0009653	-24.7344	5
81	0.481296	-2.97962	0
82	0.708465	0.342856	10
83	0.33968	-4.4018	0
84	0.172438	-6.30536	0
85	0.337556	-4.42948	0
86	0.173193	-6.80852	3
88	0.037037	-9.89377	0
90	0.0123457	-13.1917	0
91	0.166357	-6.41356	0
92	0.0701058	-8.30372	0
93	0.796512	3.35393	26
94	0.112114	-6.59585	0
97	0.264775	-4.15186	0
98	0.284264	-4.15186	0
99	0.546441	-2.13659	0
100	0.305164	-3.60236	0
101	0.263682	-4.42059	0
102	**0.0123457**	**-13.1917**	**0**
103	0.284264	-4.15186	0
104	0.00478642	-17.4213	0
105	0.265431	1.49679	1
107	0.00474956	-17.455	0
108	0.161317	-6.4	0
109	0.037037	-9.89377	0
110	0.0178326	-14.8407	1
111	0.406641	-3.30926	3
112	0.291608	-3.76981	0
113	0.0701058	-8.30372	0
114	5.65E-06	-36.2772	0
115	0.568532	-2.02235	0
116	0.109719	-7.8853	0
117	0.333333	-3.29792	0
118	0.333198	1.64879	1
119	**0.609372**	**4.51149**	**199**
120	0.00463535	-16.4896	0
122	0.260794	-4.39264	0
123	0.333333	-3.29792	0
124	0.33968	-4.4018	0

Codon	*p*-value	Normalized *dN-dS*	#Non-synonymous mutations
125	0.552136	3.28531	3
126	0.0123457	-13.1917	0
127	0.119708	-9.46518	27
128	0.306009	1.90736	25
129	0.458626	3.0322	26
130	0.114213	-8.84854	0
131	0.352489	-3.71363	0
132	0.17445	-6.26275	0
133	0.811993	2.6014	63
134	0.173347	-6.80603	1
135	0.00411523	-16.4896	0
136	0.265113	1.49589	1
137	0.597828	-1.8392	0
139	0.550199	-2.14243	0
141	0.333195	1.64862	1
142	0.258655	-4.42898	0
143	0.116565	-8.75883	0
144	0.00130132	-20.7593	0
146	0.00411523	-16.4896	0
147	0.000152416	-26.3834	0
148	0.0701058	-8.30372	0
149	0.263321	-4.42665	0
150	0.00147606	-19.7875	0
151	0.33968	-4.4018	0
152	0.333333	-3.29792	0
155	0.0296732	-12.4095	0
156	0.120704	-6.59585	0
158	0.333198	1.64879	1
159	0.00448563	-17.7063	0
161	0.111111	-6.59585	0
162	0.305164	-3.60236	0
163	0.159045	-6.34428	0
165	0.260128	-4.93241	1
166	0.649555	-0.85846	0
168	0.0841187	-9.40117	103
169	0.0185623	-12.4556	0
173	0.517064	-2.02165	5
174	0.264775	-4.15186	0
175	0.33606	-3.29792	0
177	0.037037	-9.89377	0
178	0.0701058	-8.30372	0
179	0.45178	-2.74555	0
180	0.258614	1.54525	3
185	0.264775	-4.15186	0
186	0.0701058	-8.30372	0
190	0.111111	-6.59585	0
191	0.037037	-9.89377	0
192	0.264775	-4.15186	0
193	0.0701058	-8.30372	0
194	0.00137174	-19.7875	0
196	0.259123	-4.42098	0
199	0.000152416	-26.3834	0
200	0.0701058	-8.30372	0
201	0.264775	-4.15186	0
202	0.0984731	-8.25712	0

Codon	*p*-value	Normalized *dN-dS*	#Non-synonymous mutations
203	0.091224	-7.77091	0
204	0.037037	-9.89377	0
205	0.266116	-4.38016	0
206	**0.0330216**	**-13.4333**	**1**
207	0.333333	-3.29792	0
209	0.0701058	-8.30372	0
211	1.39E-11	-75.919	0
212	0.505965	-2.94466	0
213	0.00411523	-16.4896	0
214	0.305164	-3.60236	0
215	0.505964	-2.94466	0
217	0.333102	-3.86639	0
218	0.111111	-6.59585	0
219	0.0808058	-8.30372	0
220	0.333165	1.64855	1
221	0.295666	-4.26472	0
222	0.555472	-1.65007	2
223	0.337557	-4.42948	0
224	0.354016	-3.29792	0

533 **Table 3**. Codon-wise mutation and selection analysis and the corresponding *p*-values for
534 *pmrB*. Only values for codons with non-zero normalized *dN* – *dS* values are shown. Sites
535 reported to be associated with colistin resistant strains are shown in bold. *p*-values >0.05 are
536 shaded red, <0.05 shaded green, intermediate color is yellow. Normalized *dN* – *d*S values >0
537 are shaded red, <0 shaded green, intermediate color is yellow.

Codon	*p*-value	Normalized *dN-dS*	#Non-synonymous mutations
5	0.75505	0.02736	5
6	0.23262	0.86962	1
8	0.00182	-14.404	0
9	0.58075	-1.0608	0
13	**0.55552**	**1.84816**	**4**
14	**0.74388**	**3.18365**	**6**
16	0.24758	0.81882	2
17	**0.67942**	**-0.0326**	**5**
19	0.08941	-4.1207	0
21	0.33333	0.92411	8
23	0.27049	-2.2776	0
25	0.75816	3.51406	57
27	0.25223	0.82388	3
29	0.07326	-4.9802	0
30	0.05913	-5.4891	0
31	0.80176	3.69314	8
33	0.58791	-0.7609	2
34	0.28581	-2.8642	0
36	0.33333	-1.8482	0
37	0.24741	-2.4901	0
40	0.3244	-2.1937	0
42	0.33333	-1.8482	0
43	0.28627	1.14615	4
45	0.59629	0.97052	15
46	0.27067	-2.4901	0
47	0.5053	-1.331	0
48	0.33333	-1.8482	0
49	0.22739	-2.8632	0
50	0.02931	-6.6678	0
51	0.33333	-1.8482	0
53	0.23685	-2.7488	0
54	0.15783	-4.1439	35
55	0.25926	-2.7723	2
56	0.5046	-1.3278	0
58	0.03704	-5.5447	0
60	0.236	-4.0995	2
61	0.57394	2.45633	4
62	0.56844	2.64003	7
63	7.39E-13	-49.802	0
64	**0.67964**	**3.27573**	**20**
65	0.44231	-1.6114	28
67	0.41978	-0.0866	28
69	0.27067	-2.4901	0

Codon	*p*-value	Normalized *dN-dS*	#Non-synonymous mutations
70	0.24741	-2.4901	0
74	0.19416	-3.1985	0
76	0.1748	-4.2132	35
78	1.37E-08	-30.905	0
79	0.0036	2.24631	2
80	**0.33333**	**-1.8482**	**0**
82	0.24247	-2.7524	0
83	0.06121	-4.9802	0
84	0.29742	-2.7524	0
85	0.03704	-5.5447	0
86	0.24741	0.81861	28
87	**0.50023**	**-1.3073**	**0**
88	0.26749	-2.7265	2
89	0.43388	-1.6695	3
91	0.45724	-1.5569	0
92	0.24741	-2.4901	0
93	0.74348	-0.0338	28
94	0.07402	-6.2937	2
96	0.11111	-3.6965	0
99	0.82493	1.76685	35
101	0.33333	-1.8482	0
102	0.12153	-3.6965	0
103	0.06121	-4.9802	0
104	0.27067	-2.4901	0
105	0.00051	-12.938	0
106	0.24743	0.81867	1
109	0.5373	1.81139	7
110	0.72533	0.40327	35
111	0.11122	-3.6965	0
112	0.3285	1.52432	35
113	0.32791	0.91666	7
114	0.08543	-4.5292	0
115	0.11109	-4.621	2
116	**0.27065**	**0.92403**	**1**
118	0.25475	-2.6568	0
119	0.40741	-1.8482	4
120	0.25007	-2.6131	0
121	**0.24438**	**-2.7308**	**0**
122	0.24741	-2.4901	0
123	0.01983	-7.4702	0
124	0.34697	-3.1326	2
126	0.30008	1.16939	1
127	0.5483	-0.9621	7
128	8.59E-07	-24.901	0
129	0.29979	-2.7306	0
131	0.05609	-5.4979	0
132	0.04702	-7.673	8
133	0.55556	-0.9241	4
134	**0.33321**	**0.92395**	**2**
135	0.22887	-2.8447	0
137	0.5741	-0.8505	2
138	0.97399	5.54467	375
140	0.23685	-2.7488	0
141	0.33331	0.92408	2
143	0.03704	-5.5447	0

Codon	*p*-value	Normalized *dN-dS*	#Non-synonymous mutations
144	0.15348	-4.159	1
145	**0.00882**	**2.47068**	**23**
146	0.06121	-4.9802	0
148	0.00015	-14.786	0
150	0.5046	-1.3278	0
152	0.29902	-2.0603	0
153	0.01367	9.48851	11
154	0.25926	-2.7723	3
155	0.24759	0.8188	4
156	0.03704	-5.5447	0
157	0.14703	-4.19	0
159	0.00013	-17.562	1
160	0.50737	-1.3069	14
161	0.25926	-2.7723	1
162	0.55556	-0.9241	5
163	0.75834	3.51471	7
164	0.65538	2.63617	9
165	0.5529	-0.9379	27
166	0.27169	-2.6968	3
167	0.11111	-3.6965	0
168	0.51381	-1.2995	0
169	0.2346	-2.8447	0
170	**0.91393**	**2.80089**	**39**
171	0.01864	-9.0238	0
172	0.24741	-2.4901	0
173	0.57387	-0.852	8
176	0.00375	-9.9603	0
177	0.05605	-5.5	0
178	0.13718	-3.8698	0
179	0.35446	-2.7808	34
180	0.54467	-1.1199	7
181	0.21056	-3.6934	5
182	0.06121	-4.9802	0
183	**0.11419**	**-3.6965**	**0**
184	**0.22729**	**-2.8644**	**0**
185	0.13346	-4.8381	1
186	0.14473	-4.0495	0
187	2.85E-06	-28.648	4
188	0.11111	-3.6965	0
190	**0.22729**	**-2.8644**	**0**
191	0.03704	-5.5447	0
192	**0.78192**	**1.79977**	**7**
194	**0.27072**	**0.92411**	**1**
197	0.23685	-2.7488	0
198	0.13218	-3.4066	0
199	0.2602	-2.6265	0
200	0.33333	-1.8482	0
201	0.11111	-3.6965	0
203	0.06121	-4.9802	0
206	0.00093	-12.45	0
207	0.5736	-0.853	1
208	**0.67468**	**0.21796**	**21**
209	0.24741	-2.4901	0
210	0.23891	0.8543	27
211	1.29E-06	-23.529	0

Codon	*p*-value	Normalized *dN-dS*	#Non-synonymous mutations
212	0.02674	-6.181	0
214	0.23391	-2.8531	0
215	6.27E-07	-24.027	0
217	0.24741	-2.4901	0
218	0.40536	-2.0004	1
220	0.24279	-2.7488	0
221	0.28777	-2.8447	0
222	0.15337	-4.1615	1
223	0.29864	-2.0629	0
224	0.11111	-3.6965	0
226	**0.86831**	**1.84822**	**21**
229	0.53891	-1.1768	2
230	0.00046	-14.352	0
231	0.483	-1.7181	1
232	**0.42867**	**-1.8548**	**19**
233	**0.80247**	**3.69645**	**15**
234	**0.11111**	**-3.6965**	**0**
235	**0.80225**	**0.92229**	**6**
236	0.25926	-2.7723	3
237	0.03526	-6.2927	0
238	0.00023	-14.941	0
239	0.75517	2.72389	4
240	0.2854	1.14537	3
241	0.11111	-3.6965	0
242	0.05965	-5.465	0
243	0.29898	0.87883	2
244	0.25012	-2.6126	0
245	0.55144	-0.985	7
246	0.00023	-14.941	0
247	0.03024	-9.7957	1
248	0.05025	-6.6009	2
249	0.00137	-11.089	0
250	0.05688	-5.4596	0
251	0.44218	1.7085	5
252	0.22712	-2.8665	0
253	0.59374	-0.7965	4
254	0.03541	-6.2853	0
256	**0.24741**	**-2.4901**	**0**
257	0.50503	-1.3142	4
258	0.24741	-2.4901	0
259	0.2374	-2.8111	0
260	0.55929	-0.9126	1
261	0.50023	-1.3073	0
262	**0.04518**	**-6.4739**	**1**
263	**0.86792**	**4.61876**	**7**
265	0.30006	-2.7282	0
266	0.15337	-4.1615	1
267	0.80615	1.23405	102
269	0.03704	-5.5447	0
270	0.51364	2.34224	9
271	0.55531	1.84772	6
272	**0.50568**	**-1.3328**	**0**
273	0.5391	-0.9241	6
274	0.67737	0.17619	7
275	0.00686	-10.165	1

Codon	*p*-value	Normalized *dN-dS*	#Non-synonymous mutations
277	0.28777	-2.8447	0
279	0.11111	-3.6965	0
280	0.33333	0.92411	7
282	0.06121	-4.9802	0
283	0.05268	0.65033	7
284	0.69625	-0.0465	8
285	0.01324	-8.2554	0
286	0.03704	-5.5447	0
287	0.11111	-3.6965	0
288	0.0025	-12.454	7
289	0.6288	2.772	7
290	0.24741	-2.4901	0
291	0.28816	-2.8408	0
292	0.11121	-4.6185	1
294	0.25765	-3.3392	3
295	0.33333	-1.8482	0
297	0.25475	-2.6568	0
298	0.24748	0.81869	1
299	0.26088	-2.4901	0
300	0.11111	-3.6965	0
301	0.23981	-3.7899	2
302	0.29971	-2.7314	0
303	0.45781	-1.5103	0
304	0.00015	-14.786	0
305	0.24741	-2.4901	0
306	0.12624	-3.9035	0
307	0.33333	-1.8482	0
308	0.00193	1.2334	2
311	0.22725	-2.865	0
312	0.29902	-2.0603	0
313	0.24741	-2.4901	0
314	0.5046	-1.3278	0
315	**0.21062**	**-3.6929**	**3**
318	0.32788	-2.7169	1
319	0.15334	-4.162	2
320	0.22712	-3.9869	32
321	0.00686	-10.165	1
322	0.03073	-7.3382	28
323	0.22731	-2.8642	0
325	0.24741	-2.4901	0
327	0.00048	-15.303	7
328	0.01195	-8.5438	0
329	0.33333	-1.8482	0
330	0.11111	-3.6965	0
331	0.33333	-1.8482	0
332	0.43366	1.63747	2
333	0.58216	-0.837	3
334	0.29902	-2.0603	0
336	0.24752	0.81872	4
337	0.57377	-0.8527	3
338	0.50539	1.36257	1
341	0.06121	-4.9802	0
342	0.33333	-1.8482	0
343	0.08941	-4.1207	0
344	0.24232	-2.7541	0

Codon	*p*-value	Normalized *dN-dS*	#Non-synonymous mutations
345	0.00537	-9.9603	0
346	0.01783	-8.317	1
347	0.00015	-14.786	0
349	0.19913	-4.3902	7
350	0.33333	-1.8482	0
351	0.0321	-8.5923	7
353	**0.43361**	**-1.6715**	**1**
354	0.29902	-2.0603	0
355	0.36127	-1.8482	0
357	0.46228	-1.5844	7
358	0.01235	-7.3929	0
359	0.45386	-1.5269	7
360	**0.55404**	**1.85946**	**217**
362	0.77995	0.9784	36
363	0.01701	-10.645	35
364	0.03704	-5.5447	0
365	0.26091	0.87901	1
366	0.46786	-1.4329	1
368	0.29902	-2.0603	0
371	0.06121	-4.9802	0
372	0.2592	-2.773	1
373	0.33333	-1.8482	0
374	0.01481	-7.3929	0
375	0.00318	-12.57	0
376	0.00375	-9.9603	0
377	**0.33333**	**-1.8482**	**0**
379	0.24741	0.81861	7
380	0.07326	-4.9802	0
381	0.24741	-2.4901	0
382	0.05423	-5.7317	0
383	0.00137	-11.089	0
384	0.1351	-3.3685	0
385	0.0145	-8.2104	0
386	0.33495	-1.8393	0
387	**0.24743**	**0.81867**	**1**
389	0.01261	-7.3777	0
390	**0.01235**	**-7.3929**	**0**
391	0.00093	-12.45	0
392	0.01514	-7.4702	0
393	0.43363	-1.6714	1
395	0.01325	-8.2534	0
397	0.12189	-3.6965	0
398	0.00023	-14.941	0
399	1.88E-06	-22.179	0
400	0.01645	-7.8731	0
402	0.51847	-1.2809	0
403	**0.04535**	**-7.3997**	**4**
405	0.33333	-1.8482	0
406	0.24741	-2.4901	0
407	0.35538	-1.8041	0
408	0.55546	1.8481	2
411	0.47862	-1.3634	0
412	0.58857	-0.7681	0
414	0.33329	0.92405	1
416	0.25964	-2.6216	0

Codon	*p*-value	Normalized *dN-dS*	#Non-synonymous mutations
417	0.33333	-1.8482	0
418	0.60593	-0.6736	1
419	0.24741	-2.4901	0
420	0.16811	-4.2674	7
421	0.24741	-2.4901	0
422	0.50567	-1.3328	0
423	2.45E-06	-24.624	0
424	0.33489	-1.8397	0
425	0.33333	-1.8482	0
426	0.33619	-1.8482	0
428	0.11301	-3.6965	0
429	0.03704	-5.5447	0
430	0.25462	-2.6556	0
431	5.08E-05	-16.634	0
433	0.02621	-8.4657	0
434	0.33333	-1.8482	0
435	0.2346	-2.8447	0
436	0.25206	-2.8409	7
438	0.24743	0.81867	1
439	0.7257	1.20481	7
440	0.57416	-0.8516	63
442	0.11111	-3.6965	0
444	**0.42936**	**-3.1198**	**883**

Figure 1

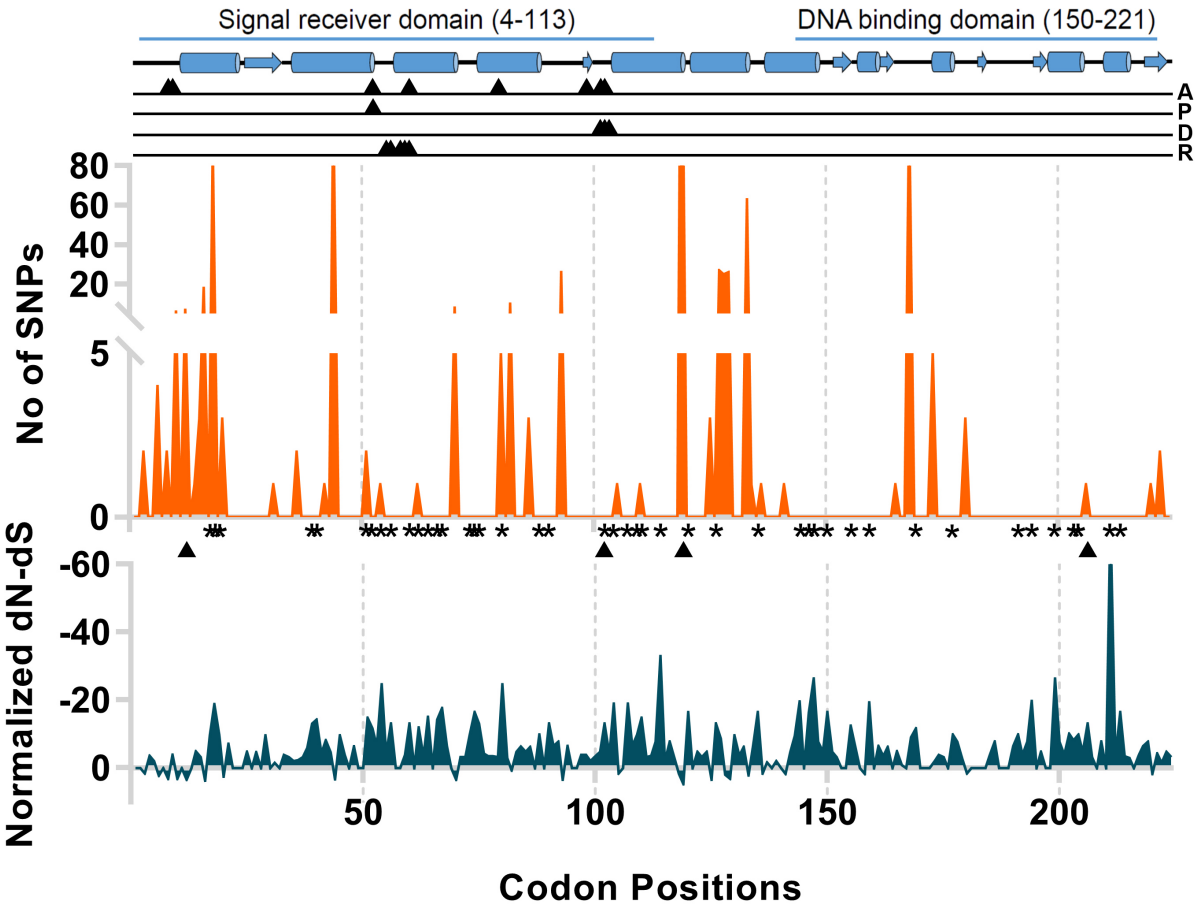

Signal receiver domain (4-113)
DNA binding domain (150-221)
A
P
D
R
No of SNPs
80
60
40
20
5
0
Normalized dN-dS
-60
-40
-20
0
50
100
150
200
Codon Positions

Figure 2

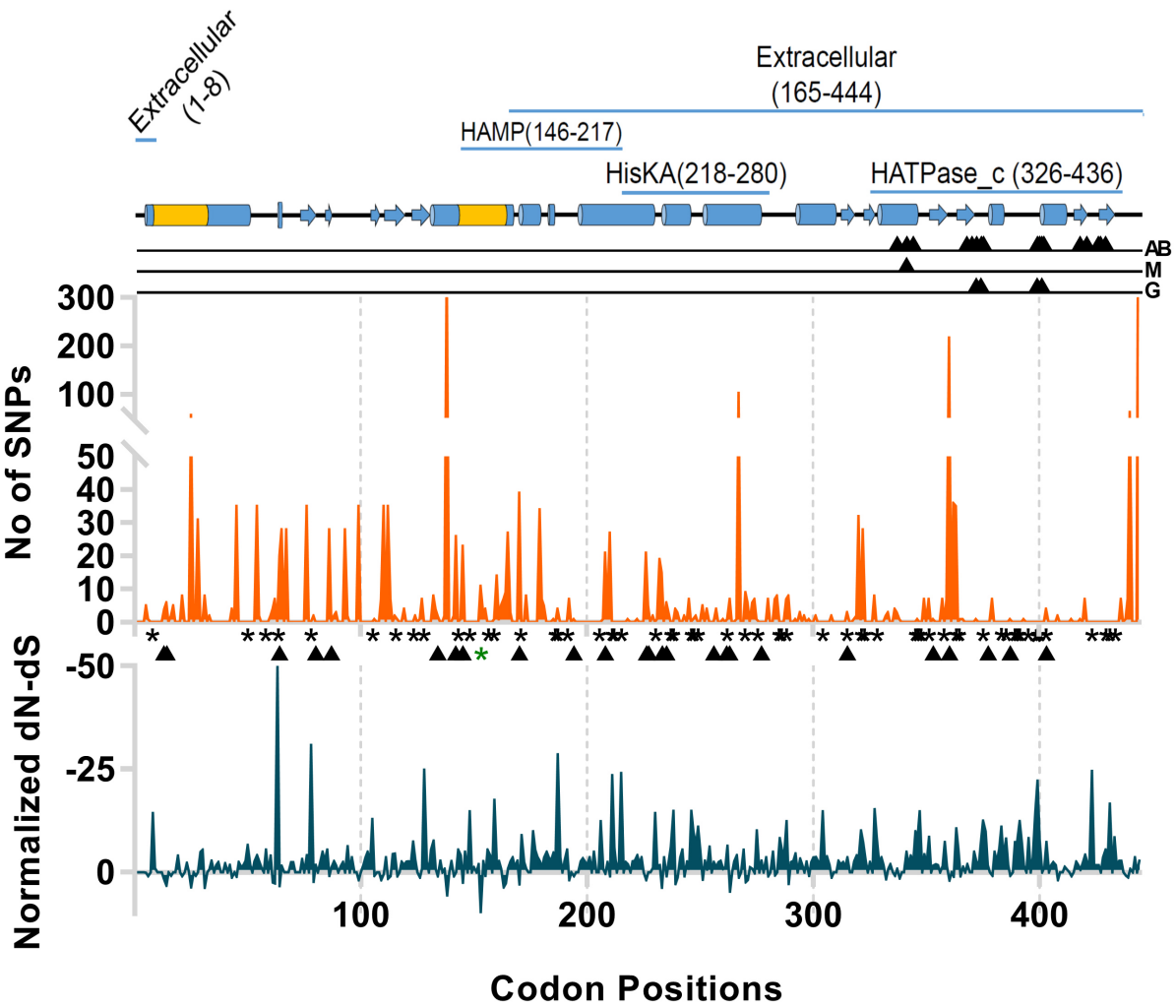
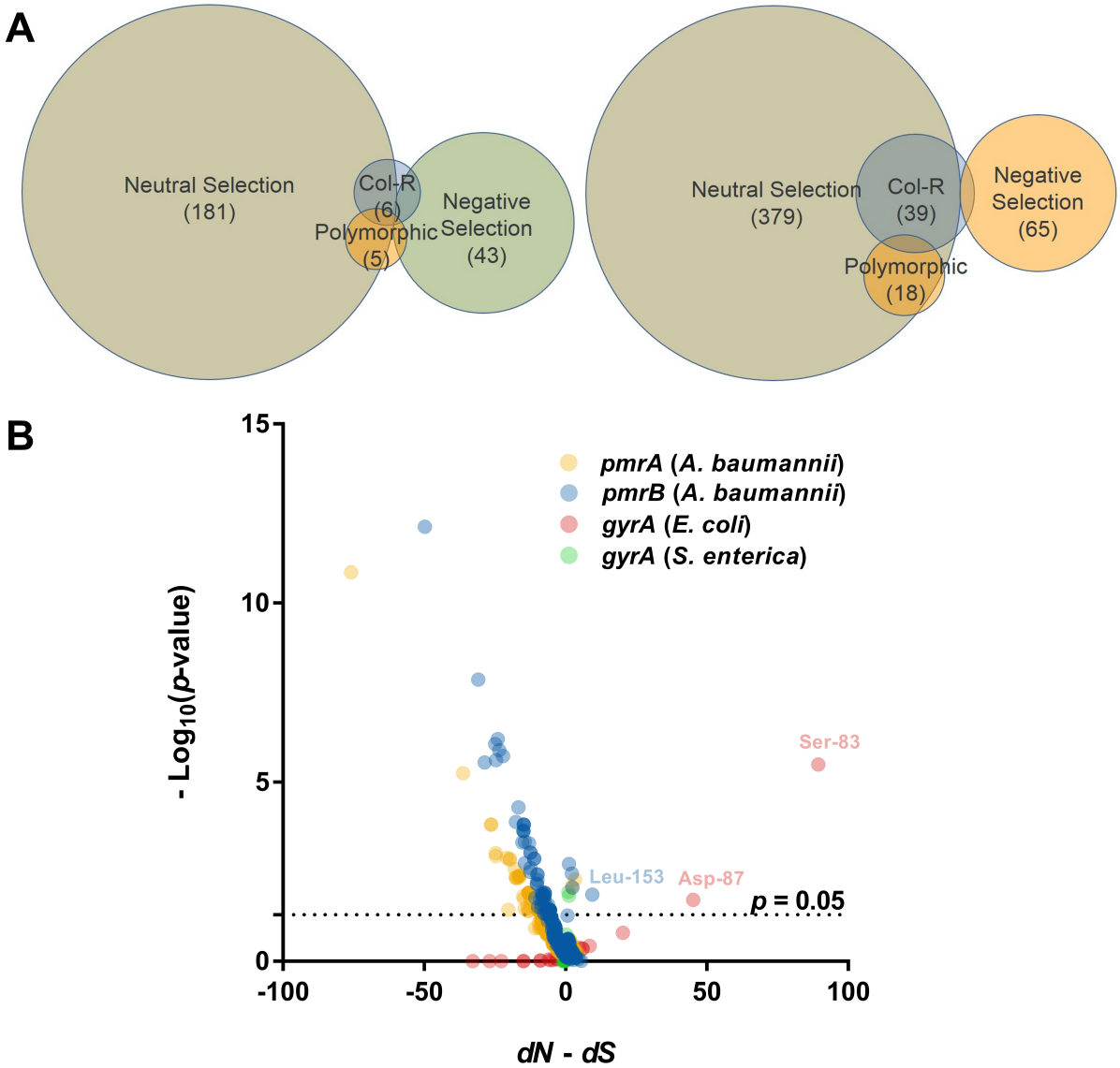

Extracellular
(1-8)
Extracellular
(165-444)
HAMP(146-217)
HisKA(218-280)
HATPase_c (326-436)
AB
M
G
No of SNPs
300
200
100
50
40
30
20
10
0
Normalized dN-dS
-50
-25
0
100
200
300
400
Codon Positions

Figure 3


A
Neutral Selection
(181)
Col-R
(6)
Polymorphic
(5)
Negative
Selection
(43)
Neutral Selection
(379)
Col-R
(39)
Polymorphic
(18)
Negative
Selection
(65)
B
pmrA (A. baumannii)
pmrB (A. baumannii)
gyrA (E. coli)
gyrA (S. enterica)
Ser-83
Leu-153
Asp-87
p = 0.05
- Log10(p-value)
dN - dS
15
10
5
0
-100
-50
0
50
100