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1 **New insights concerning *Acinetobacter* genomic island-related elements**

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23

24 **Abstract**

25 The objective of this study was to mobilize the *Acinetobacter* genomic island 1-A
26 (AGI1-A) from *Enterobacter hormaechei* EclCSP2185 (*E. cloacae* complex) and to search
27 for the distribution and structure of AGI1-related elements in the NCBI database. AGI1-A
28 was transferred to *Escherichia coli*. Analysis of the attachment (*att*) sites could locate the
29 possible recombination crossover in the *att* sequences at position 10-11 (GG) in the last 18 bp
30 of *trmE*. *In silico* detection of AGI backbones in the wgs database identified AGI variants in
31 *Salmonella enterica* (83 strains), *Vibrio cholerae* (33), *E. hormaechei* (12), *Acinetobacter*
32 *baumannii* (2), most belonging to prevalent clones (ST40, ST69, ST114 and ST25
33 respectively), but also in *Escherichia coli* (1) and *Klebsiella pneumoniae* (1). Two groups of
34 backbone were identified: one similar to AGI1, the other with a short segment from a
35 *Shewanella* element upstream of ORF A022. The MDR regions were inserted by transposition
36 at the *res* site in four different positions: ATAGG (*A. baumannii*), CATAG (*S. enterica* and *V.*
37 *cholerae*), TAGGT (*S. enterica* and *K. pneumoniae*), and TGCAC (*S. enterica*) representing
38 four different lineages. In some *V. cholerae*, *E. hormaechei* and *E. coli*, deletion events
39 occurred that eliminated part of the backbone at the left junction. Analysis of the right
40 junction identified a fifth lineage in *V. cholerae* and *E. hormaechei* (CCATA). In conclusion,
41 based on the position of the MDR region, AGI-related elements belonged to five groups of
42 closely related genomic islands (AGI1-AGI5), with differences in the backbones that evolved
43 independently over time.

44

45 **Keywords:** antimicrobial resistance, AGI1, mobilization, integron

46

47 **1. Introduction**

48 Among the genomic islands (GIs), *Salmonella* genomic island 1 (SGI1) is one of the
49 largest family which contributes to the spread of antimicrobial resistance genes (ARGs). SGI1
50 was identified in multidrug-resistant (MDR) *Salmonella* Typhimurium DT104 that has
51 emerged in many countries. SGI1 is inserted into the 3'-end of the chromosomal *trmE* gene
52 and ARGs are clustered into a complex class 1 integron [1]. Many SGI1 variants resulting
53 from deletion/insertion in the backbone and the MDR region were described mostly in
54 *Salmonella* and *Proteus mirabilis* [2,3]. Other related GIs have been reported: SGI2, PGI1,
55 PGI2 and AGI1 [4,5,6,7]. AGI1 initially described in an MDR *Acinetobacter baumannii* ST25
56 was recently identified in the epidemic MDR *Enterobacter hormaechei* strain EclC2185
57 belonging to the ST114 high-risk clone [8]. To date four variants have been described: AGI1,
58 AGI1-A, -B and -C [7,8].

59 SGI1 and PGI1 are integrative elements that are mobilized by IncC conjugative
60 plasmids suggesting that these GIs might potentially spread among Enterobacteriales and
61 contribute to dissemination of ARGs [9,10]. AGI1 transfer has not been described to date.
62 Given that AGI1 has been detected in the non-phylogenetically related species *A. baumannii*
63 and *E. hormaechei*, we hypothesize that AGI1 is also a mobilizable element. In this work, we
64 have tested this hypothesis and searched for genes of the AGI1 backbone in the whole-
65 genome shotgun contigs database to evaluate the distribution and structure of AGI1-related
66 elements.

67

68

69 2. Materials and methods

70 2.1. Mobilization assays of AGII-A

71 Two methods of transfer were used for this purpose: one with the IncC helper plasmid
72 in the donor and one in the recipient, as previously described [9,10]. For the first method, the
73 IncC plasmid pEA409TEM24 (MG764534) carrying *bla*_{TEM-24}, *aacA4*, *aadA1* and *dfrA1*
74 conferring resistance to ticarcillin, cefalotin, ceftazidime, kanamycin, tobramycin,
75 sulfamethoxazole and trimethoprim was introduced into *E. hormaechei* EclCSP2185 that
76 harbours AGII-A (carrying *aadA1* and *dfrA1*) and is resistant to streptomycin, spectinomycin,
77 sulfamethoxazole, trimethoprim, cefalotin and nalidixic acid (Table 1). We mixed *Escherichia*
78 *coli* K-12 C600 (rifampicin resistant) and *E. hormaechei* EclCSP2185 harbouring
79 pEA409TEM24 in Trypticase soy broth and after overnight incubation at 37°C, the bacterial
80 cells were plated on selective Drigalski agar supplemented with rifampicin (60 mg/L) and
81 streptomycin (30 mg/L). The second method consisted in mixing *E. hormaechei* EclCSP2185
82 and *E. coli* K-12 C600 pEA409TEM24 with selection of transconjugants as above. Three
83 independent experimental repeats were performed.

84

85 2.2. Detection of AGII-A in the transconjugants

86 PCR mapping of AGII-A and its chromosomal location were carried out on *E. coli* K-
87 12 transconjugants (Table S1) [8]. The boundaries of AGII-A with the chromosome were
88 sequenced. The circular extrachromosomal form of AGII-A was assessed by two-step DNA
89 amplification and sequenced.

90 Insertion site of AGII-A in *E. hormaechei* was determined in AGII-A-free clones
91 obtained from *E. hormaechei* EclCSP2185 pEA409TEM24 plated on Drigalski agar
92 supplemented with nalidixic acid (20 mg/L) and ceftazidime (4 mg/L). The intact *trmE* in
93 streptomycin-sensitive clones was assessed by PCR and sequencing.

94

95 *2.3. In silico detection of AGI1-related elements in the NCBI wgs contigs database*

96 The backbone of AGI1 consists of two parts (a large one with 27 ORFs and the other
97 with only ORF A028) separated by a MDR region. AGI1-related elements were searched in
98 the National Center for Biotechnology Information (NCBI) database using the nucleotide
99 sequences of the AGI1-specific integrase (*int*_{AGI}) and ORF A028 for the right-hand side.
100 BLAST was performed against the wgs database [Gammaproteobacteria (taxid:1236)]
101 available until December 15, 2018 (<https://blast.ncbi.nlm.nih.gov/Blast.cgi#>). The AGI-
102 related elements detected were compared with those previously described. Multilocus
103 sequence typing (MLST) allele sequences were extracted from GenBank using BLAST and
104 the sequence type (ST) profiles were determined with the MLST Typer web service at the
105 Danish Center for Genomic Epidemiology (<https://cge.cbs.dtu.dk/services/MLST/>).

106

107

108 **3. Results and discussion**

109 *3.1. Mobilization assays of AGI1-A*

110 The transfer of AGI1-A to *E. coli* occurred at the rate of 10^{-7} to 10^{-8} per donor by both
111 methods. This rate is rather low when compared to that of transfer of other GIs. Nevertheless
112 heavy bacterial inoculums are encountered in the human or animal gut microbiota and waste
113 water suggesting that AGI1-A transfer might occur in natural environments.

114 The antibiotic resistance profile conferred by AGI1-A was observed in the
115 transconjugants (resistance to streptomycin, spectinomycin, sulfamethoxazole and
116 trimethoprim) (Table 1). The intact AGI1-A was integrated into the specific *attB* site at the 3'-
117 end of the *E. coli* chromosomal *trmE* gene by site-specific recombination between the *attB*
118 site of the recipient strain and the *attP* site of AGI1-A. Our selective media confirmed that the
119 cohabitation of AGI1-A and the IncC helper plasmid was not stable, as demonstrated with the
120 GIs of the SGI1 family [11,12]. The plasmid was absent in *E. coli* transconjugants selected
121 with streptomycin (antibiotic resistance marker of AGI1-A) in both transfer methods and
122 AGI1-A was lost in *E. hormaechei* pEA409TEM24 plated on agar with ceftazidime (IncC
123 resistance marker).

124

125 *3.2. Analysis of the chromosomal junctions of AGI1-A*

126 As for SGI1 and PGI1, the integrated form of AGI1-A was flanked by imperfect 18 bp
127 direct repeats. These sequences of 18-bp were found at the end of *trmE* and in the circular
128 extrachromosomal form of the GI. The left and right junctions (*attL* and *attR*) of AGI1-A and
129 the potential attachment site (*attP*) in the circular extrachromosomal form of AGI1-A in *E.*
130 *coli* were sequenced and analyzed. The sequence at the end of the *trmE* gene (*attB*) from
131 AGI1-A negative *E. hormaechei* was also determined.

132 The *attP* site sequences of the extrachromosomal circular form of AGI1-A in *E.*
133 *hormaechei* and in *E. coli* were identical but more divergent from the *attB* site sequences of *E.*
134 *coli* (3 nucleotide (nt) changes at position 9, 12 and 17) and *E. hormaechei* (4 nt changes at
135 position 6, 9, 12 and 17) (Figure 1). In addition, the *attP* and *attB* site sequences also diverged
136 slightly from their corresponding *attL* and *attR* site sequences in the AGI1-A integrated form
137 in *E. coli* and *E. hormaechei* (1 nt change at position 9 and 2 nt changes at position 6 and 9,
138 respectively). In both strains, only the 9 bp at position 10-18 were highly conserved for
139 *attP/attL* as for *attR/attB* site sequences. Interestingly the analysis of the *attR/attB* and
140 *attP/attL* sites of the SGI1, SGI1-V and PGI1 transconjugants obtained in our previous study
141 also revealed that the last 9 bp of the *attP/attL* and *attR/attB* sites were conserved in *Proteus*
142 *vulgaris* and *Enterobacter aerogenes* (Figure S1) [10]. These findings differed from those of
143 Doublet *et al* on SGI1 transfer in *E. coli* and in *Salmonella Agona*: the 18 bp sequences of the
144 *attP* and *attB* sites were almost identical (one nt change at position 12) [13]. In the integrated
145 form they noticed that the *attL* and *attR* were identical to *attP* and *attB*, respectively, in both
146 strains.

147 Examination of the last 18 bp sequence at the end of *trmE* of numerous bacterial
148 species in the Genbank database showed that the 2 nt at position 10 and 11 (GG) were highly
149 conserved for both *attB* and *attP* site sequences [14]. Consequently, as the chromosomal
150 integration of the GIs occurred via a conservative site-specific recombination between *attP*
151 and *attB*, the possible clivage site in the *att* sequences might be located in the GG at position
152 10-11 in the 18 bp sites.

153

154 3.3. Detection and characterization of the AGI1-related backbones in the NCBI wgs database

155 Blast searches in the NCBI wgs database detected 132 backbones of AGI-like
156 elements. AGI1 variants were found in pathogenic species: 83 *Salmonella enterica* out of

157 14188 genome assemblies (0.6%) and 33 *Vibrio cholerae* out of 1006 (3.3%); but also, in
158 opportunistic pathogens: *E. hormaechei* from *E. cloacae* complex (12/1365: 0.9%), *A.*
159 *baumannii* (2/3562: 0.06%), *E. coli* (1/15618: 0.006%) and *K. pneumoniae* (1/6956: 0.01%).
160 It is noteworthy that most of these strains belonged to clones of medical (or veterinary)
161 interest widely distributed worldwide: *S. enterica* ST40, *V. cholerae* ST69, *E. hormaechei*
162 ST114 and *A. baumannii* ST25. Complete AGI1 variants (including backbone and MDR
163 region) were found on a single contig in 40 *S. enterica*, 15 *V. cholerae* and 1 *A. baumannii*.
164 The MDR region and the backbone also were split in most cases for 43 *S. enterica*, 18 *V.*
165 *cholerae*, 12 *E. hormaechei*, 1 *A. baumannii*, *E. coli* and *K. pneumoniae*. Therefore, only the
166 two ends of the main backbone were analyzed and only one representative backbone was
167 described for each new variant found (Table S2). Surprisingly no AGI1 was detected among
168 the 115 genome assemblies of *P. mirabilis* whereas many variants of SG11 and PG11 have
169 been reported among this species [3,5].

170 The AGI1-related backbones could be divided into two main groups. The first group
171 included backbones similar to that of AGI1 (KP054476) found in *A. baumannii* (backbone 1)
172 with some minor variations for those in 31 *V. cholerae* and the 12 *E. hormaechei*: 216 nt
173 deletion in ORF A008; part of ORF A025 (258 or 390 nt) and ORF A026-*resG* missing
174 probably due to two independent deletion events after insertion of the MDR region at the
175 intact *res* site (backbone 1del) (Figure 2A, Table 2). They were respectively identical to the
176 backbones of AGI1, AGI1-A and AGI1-B described previously in *A. baumannii* D4
177 (KP054476), *E. hormaechei* EclC2185 (MH545561) and *V. cholerae* V060002 (AP018677)
178 [7,8].

179 In the second group of backbones ORFs A023 and A024 were replaced by a
180 nucleotide sequence similar (98.7%) to part of a GI-related element from *Shewanella* sp. W3-
181 18-1 including locus Sputw3181_4076 (CP000503) (Figure 2B) [15]. This sequence was 918

182 nt long in backbone 2A (70 *S. enterica*, 1 *V. cholerae* and in *K. pneumoniae*) and only 252 nt
183 in backbone 2Adel (*E. coli* and 3 *S. enterica*) in which ORFs A023-*res_G* were missing due to
184 genetic rearrangement following MDR region incorporation. In backbone 2B, a segment of
185 about 1141 nt similar to P012-P014 of PGI1 (96.0% nt identity) was found between A014 and
186 A016 in 5 *S. enterica*. In backbone 2C (1 *V. cholerae*), the region between A014 and A020
187 was replaced by a 3.1 kb complex structure composed of: 597 nt similar to a nucleotide
188 sequence found in *Vibrio campbellii* strain 20130629003S01 (CP020076) including locus
189 Vc3S01_0342 (93.0% identity), P013-P016 (96.0% nt identity), a nucleotide sequence
190 encoding a permease of *S. enterica* (100% amino acid identity) and P017 (95.6% nt identity)
191 (Figure 2C). Finally, five AGIs from *S. enterica* were unclassified, due to a very large
192 deletion resulting from the insertion of the MDR region flanked by insertion sequence (IS)
193 elements (Figure 2D). All these findings demonstrated the complexity of the genetic
194 exchanges generating the numerous variants of AGIs. Nevertheless the role of *Shewanella* has
195 to be pointed out. Indeed several backbones contained a *Shewanella* segment and noteworthy
196 the backbone of AGI1 is highly similar to a GI-related element from *Shewanella* (Figure S2).
197

198 3.4. Characterization of the MDR regions of the AGII-related backbones detected in silico

199 The MDR region was located (with some exceptions) downstream of *res_G*, as usually in GIs.
200 It was inserted by transposition in four neighbouring positions: ATAGG (*A. baumannii*),
201 CATAG (*S. enterica* and a strain of *V. cholerae*), TAGGT (*K. pneumoniae* and 2 strains of *S.*
202 *enterica*) and TGCAC (5 strains of *S. enterica*) as indicated by 5 bp duplication 71, 70, 72 and
203 153 bp upstream of *res_G*, respectively (Table 2). These observations suggested that these
204 MDR regions were acquired independently and that consequently these AGIs belonged to
205 four different lineages. No site duplication was detected in *V. cholerae* ST69, *E. hormaechei*,
206 *E. coli* and some *S. enterica*, because the *res_G* at the left junction of the MDR region was

207 deleted. However, examination of the 5 bp at the right junction of the MDR region suggested
208 that the AGIs in *V. cholerae* ST69, *E. coli* and some *S. enterica* were of the same lineage as
209 AGI1 (ATAGG); whereas those in *V. cholerae* ST155, *E. hormaechei* and in the epidemic *E.*
210 *hormaechei* EclC2185 recently published represented a fifth lineage (CCATA) [8]. Thus, the
211 integron has been acquired more than once and therefore the lineages different from that of
212 AGI1 should not be named as derivatives of AGI1. We suggest the following classification
213 for AGI-related elements based on the position of the MDR region in the backbone. For the
214 AGI1 group, the MDR region is inserted at the ATAGG site; the AGI2, AGI3, AGI4 and
215 AGI5 groups were assigned to its location at the CCATA, CATAG, TAGGT and TGCAC
216 sites, respectively. Consequently, AGI1-A and AGI1-C must be renamed AGI2 and AGI3
217 respectively [8]. Differences in the backbone sequences indicate that they had evolved
218 independently over time. For comparison with other GIs, we examined the insertion site of the
219 MDR region in SGIs and PGIs deposited in the nucleotide and the wgs databases. SGI1 was
220 detected among 431 *S. enterica*, 48 *P. mirabilis*, 4 *V. cholerae*, 1 *A. baumannii*, 1 *E. coli* and
221 1 *Morganella morganii*. They were inserted at the ACTTG site indicating that they belong to
222 the same lineage, except for SGI1-Pm2CHAMA (AACTT, GenBank accession no.
223 MF372716) and SGI1-V carrying *bla*_{VEB-6} (AAATT, HQ888851). SGI2 (AY963803) and
224 SGI1-J3 (EU924797) belonged to the same lineage: their MDR region was inserted within
225 S023 (site CCATG). Finally PGI1 was detected among 13 *S. enterica* and 8 *P. mirabilis*.
226 Their MDR region was inserted at the same site (AAATT). These findings indicated that the
227 insertion site of the MDR region is much more conserved among SGIs and PGIs than among
228 AGIs.

229 Concerning the analysis of the structure of the MDR regions, we excluded those for
230 which nucleotide sequences remained as numerous separated fragments and could not be
231 assembled, mainly due to the presence of repeated elements. A complete MDR region was

232 identified in 56 AGIs. Its length varied from 6 to 29.2 kb. An AGI found in a strain of *Vibrio*
233 was MDR-free. In most cases, the MDR region harboured a class 1 integron containing
234 classic ARGs at its 5'-end (i. e. *aadA1*, *aadA2*, *aadA7*, *aadB*, *bla_{PSE-1}*, *dfrA1*, *dfrA5*, *dfrA15*).
235 Notably *bla_{PER}* conferring resistance to penicillins and third generation cephalosporins was
236 detected in a complex class 1 integron in AGI1 from *A. baumannii*. Apart from the class 1
237 integron diverse transposons (or parts) were detected: (i) *mer* genes of Tn21 and *tetA(A)* of
238 Tn1721; (ii) *mer* and *tni* modules of Tn5053/Tn502 (8.4 kb in *V. cholerae* and *E. hormaechei*,
239 identical to that found in PGI1 (KJ411925)); (iii) Tn501; (iv) Tn2 (*bla_{TEM-1b}*); and (v) Tn5393
240 (*strA*, *strB*). Interestingly these structures also belong to the mosaic MDR regions of the PGI1
241 family and of SGI1-K subgroups. Other resistance genes previously detected on plasmids
242 were found in two strains. Firstly, the macrolide inactivation gene cluster *mphA-mrx-mphR*
243 (3.2 kb) within a 5.6 kb genetic element was adjacent to the 3'-end of a class 1 integron in *K.*
244 *pneumoniae* (Figure 2B). This cluster conferring resistance to erythromycin has been also
245 identified in SGI1-B2 in *P. mirabilis* (KP116299) [16]. Secondly the heavy metal resistance
246 gene *chrA* on a 1.7 kb element, encoding a chromate ion transport protein was inserted
247 between *sull* and IS6100 of the class 1 integron in a strain of *V. cholerae* (Figure 2C). This
248 configuration was also found in SGI1-B2.

249

250 **Conclusions**

251 AGI1-A is an integrative mobilizable element for which the possible recombination
252 crossover was predicted by examination of the *att* sites in AGI1-A transconjugants. *In silico*
253 detection of AGI backbones in the wgs database identified AGI variants mostly among
254 important pathogenic bacteria (*S. enterica* and *V. cholerae*) but rarely in opportunistic
255 organisms (*A. baumannii*, *K. pneumoniae* and *E. coli*) and none in *P. mirabilis* in which many
256 SGI1 and PGI1 variants have been reported. Two backbone groups have been identified and

257 five distinct insertion sites of the MDR region were detected. These observations suggested
258 that AGIs are not derivatives of a single ancestor that each acquired a class 1 integron but are
259 a set of quite closely related genomic islands.

260

261 **Declarations**

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264 **Competing Interests:** None declared.

265 **Ethical Approval:** Not required

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335

336 **Figure legends**

337 **Figure 1.** Alignment of the *attP* site in the circular form of AGI1-A, the *attL* and *attR* sites in
338 the integrated form of AGI1-A in *E. coli* K12 C600 and *E. hormaechei* EclCSP2185, the *attB*
339 site at the 3'-end of *trmE* in *E. coli* K12 C600 and AGI1-negative *E. hormaechei*
340 EclCSP2185. Identical nucleotides are indicated by an asterisk. Nucleotides in boldface letters
341 represent substitutions between *attP* and *attB* sites. Underlined nucleotides in boldface letters
342 represent differences in *attP/attL* sites and *attR/attB* sites. In *attL* and *attR*, nucleotide
343 identical to *attP* and *attB* are in red and blue letters, respectively.

344

345 **Figure 2.** Variations in the backbone of AGI1-related elements. Arrows indicate ORFs and
346 genes, and their transcriptional orientation. Black arrows represent chromosomal genes, white
347 arrows and rectangles the GI backbone, grey arrows ORF from *Shewanella* and dotted arrows
348 ORF from other genetic elements. *attL* and *attR* sites are represented. Dashes indicate the
349 location of the MDR region and target site duplications are represented. The MDR regions are
350 boxed. The 5'-CS and 3'-CS of the integrons are shown as filled arrows and the IRs (IRi and
351 IRt) are indicated. Target site duplications are represented. Transposons and IS elements are
352 shown as grey and hatched arrows, respectively, IRs are indicated by vertical bars. Antibiotic
353 resistance genes are shown as dotted arrows.

354

355 **Figure 1.**

356

357 Circular AGI1-A (*attP*) in *E. coli* K12 C600

TTCTGTATCGGGAAGTGA

358 Left junction in the integrated form (*attL*)

TTCTGTATTGGGAAGTGA

359 Right junction in the integrated form (*attR*)

TTCTGTATCGGTAAGTAA

360 3'-end of *trmE* in *E. coli* K12 C600 (*attB*)

TTCTGTATTGGTAAGTAA

361

***** ** ***** *

362

363 Circular AGI1-A (*attP*) in *E. hormaechei* EclCSP2185

TTCTGTATCGGGAAGTGA

364 Left junction in the integrated form (*attL*)

TTCTGCATTGGGAAGTGA

365 Right junction in the integrated form (*attR*)

TTCTGTATCGGTAAGTAA

366 3'-end of *trmE* in AGI-free *E. hormaechei* EclCSP2185

TTCTGCATTGGTAAGTAA

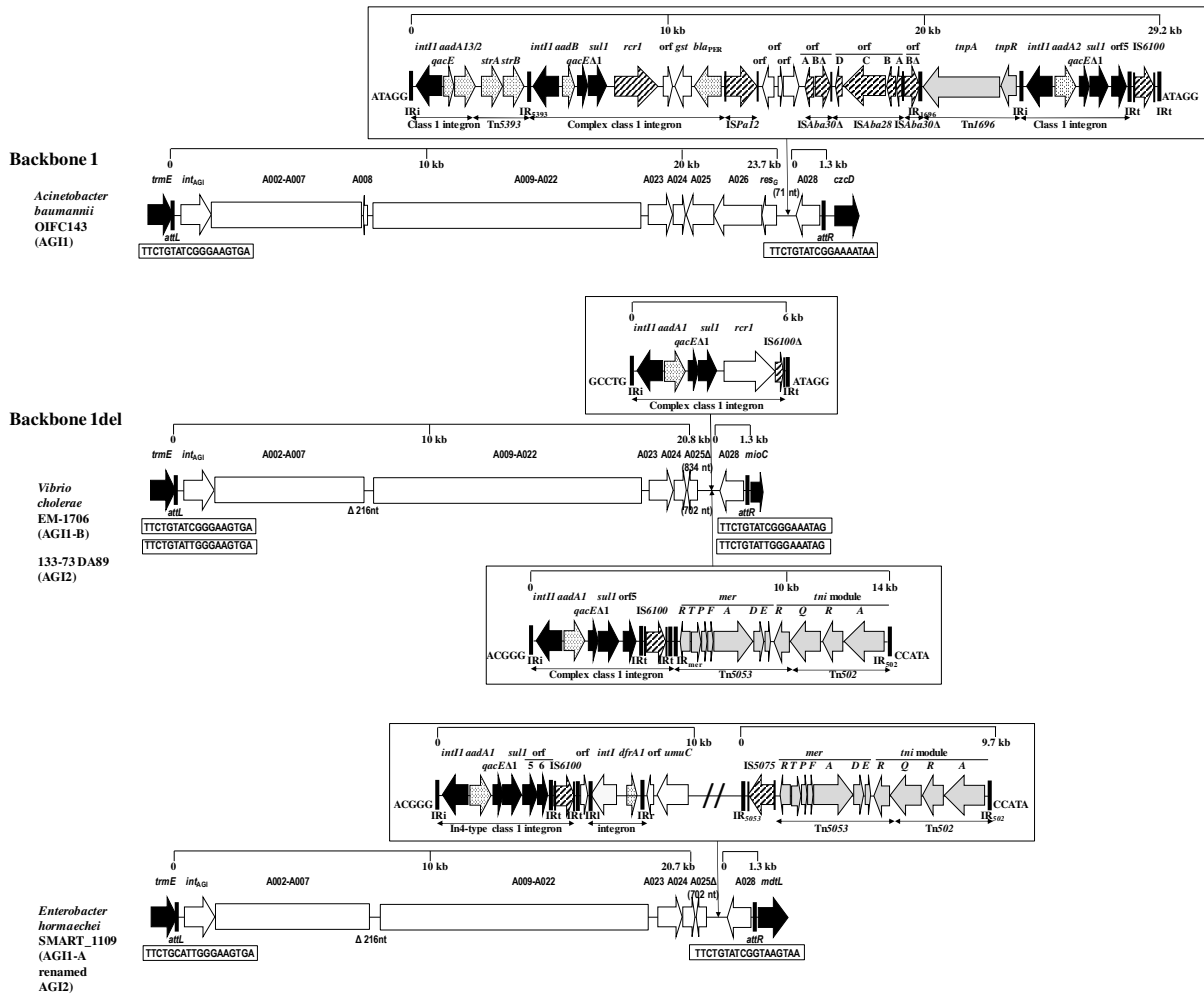
367

***** ** ** ***** *

368

369 **Figure 2.**

370 A. AGIs with backbone like AGI1 (backbone 1).

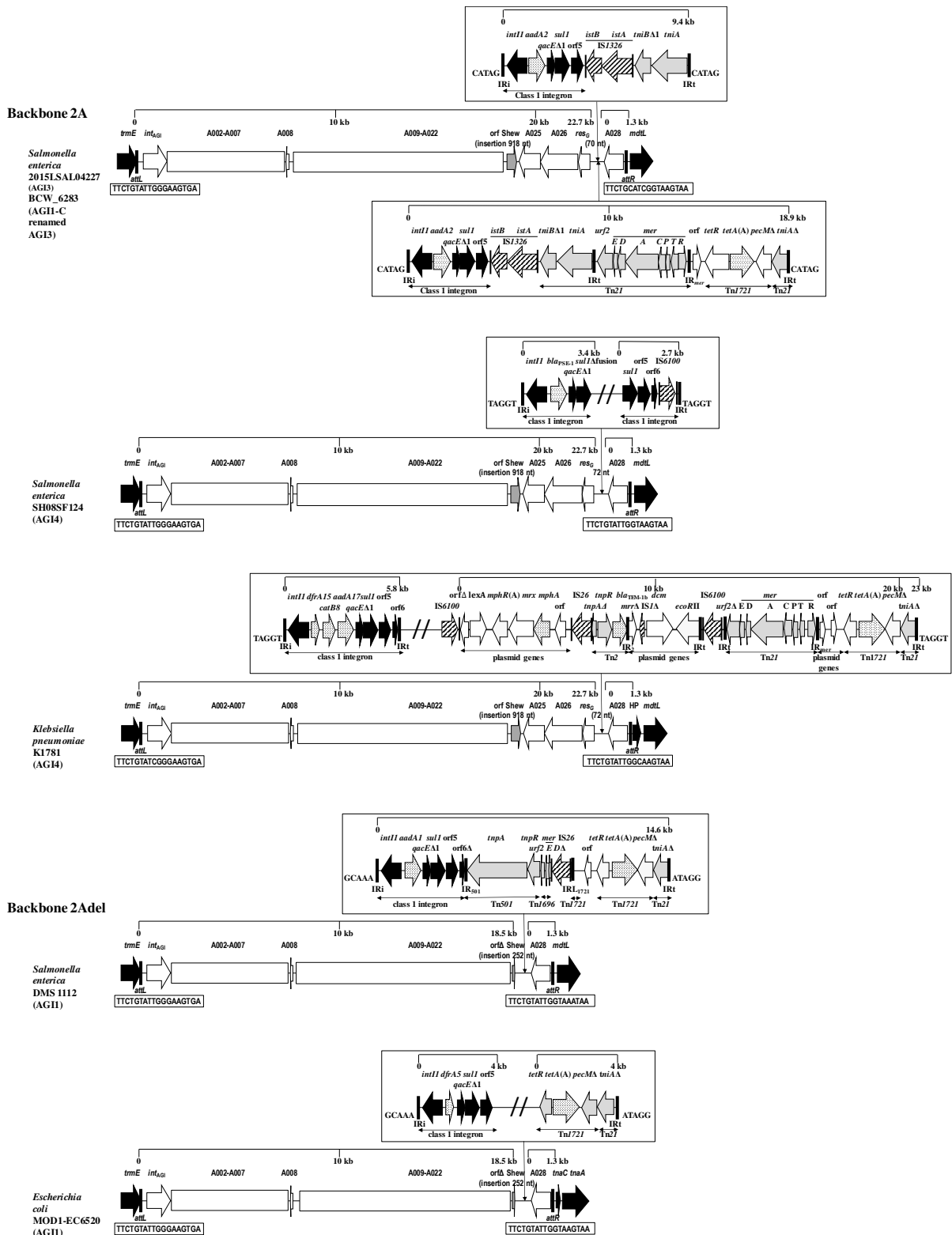


371

372

373

374 B. AGIs with the insertion of a short segment from a *Shewanella* element in the backbone
 375 (backbone 2).

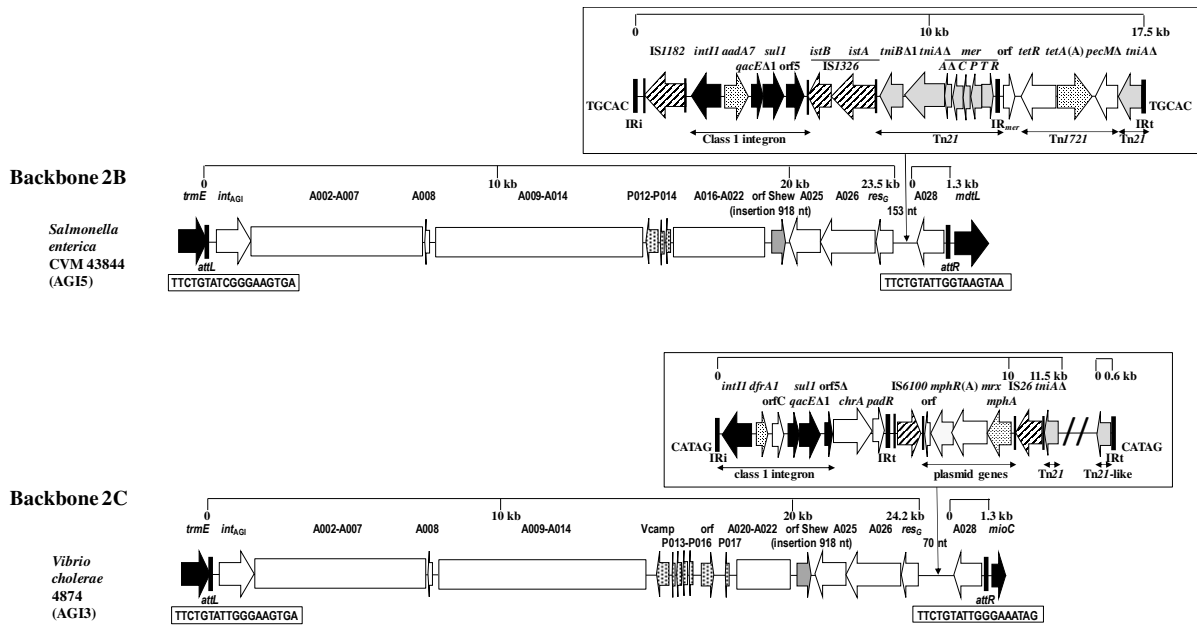


376

377

378

379 C. AGIs with backbone 2 and insertion of parts of other genetic elements.



380

381

382

383

384 D. AGIs with deleted backbone.

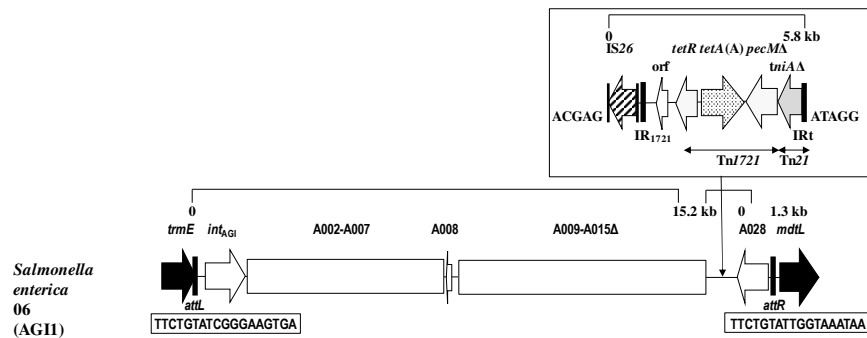
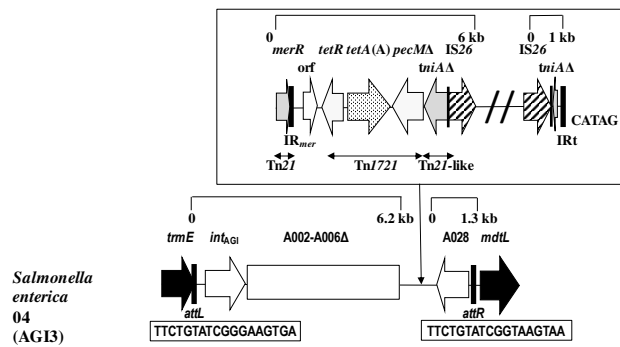
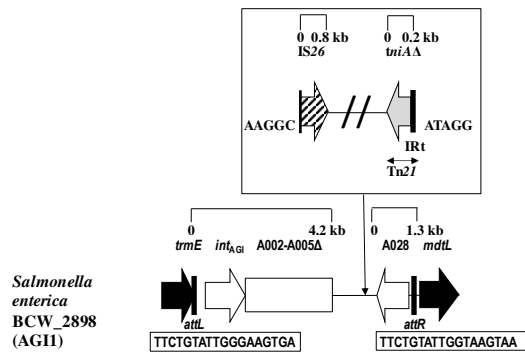
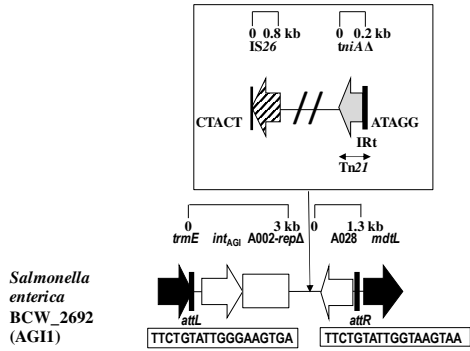
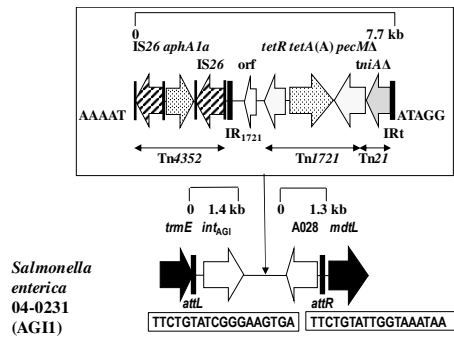


Table 1. Antimicrobial profiles of the donor, recipient strains, and transconjugants (according to the Antibiogram Committee of the French Society for Microbiology (EUCAST guidelines).

	<i>E. hormaechei</i> EclCSP2185 AGI1-A	<i>E. coli</i> K12 C600 pEA409TEM24 IncC	<i>E. coli</i> K12 C600 AGI1-A
ticarcillin	S*	R	S
cefalotin	R*	R	S
ceftazidime	S (1 mg/L)	R (128 mg/L)	S (<0.06 mg/L)
streptomycin	R (64 mg/L)	I (8 mg/L)	R (64 mg/L)
spectinomycin	R	I	R
kanamycin	S	R	S
tobramycin	S	R	S
sulfamethoxazole	R	R	R
trimethoprim	R	R	R
nalidixic acid	R (>64 mg/L)	S (2 mg/L)	S (2 mg/L)
rifampicin	I* (16 mg/L)	R (256 mg/L)	R (256 mg/L)

* S: susceptible, R: resistant, I: intermediate

Resistances in boldface letters are due to AGI1-A. Minimum inhibitory concentrations are indicated for antibiotics used in conjugation experiments.

Table 2. Characteristics of the backbones of AGI1-related elements and insertion sites of the MDR region for the 132 strains of this study.

Species (total number of strains)	Number of contigs ^a	Number of strains	Backbone types ^b	Backbone Number assigned ^c	Number of strains	Insertion site ^d ATAGG (AGI1) ^e	Insertion site ^d CATAG (AGI3) ^e	Insertion site TAGGT (AGI4) ^e	Insertion site TGCAC (AGI5) ^e	Insertion site ^d CCATA (AGI2) ^e	Absence of MDR region
<i>Acinetobacter baumannii</i> (2)	1	1	like AGI1: KP054476	1	1	1	-	-	-	-	-
	>1	1	like AGI1	1	1	1	-	-	-	-	-
<i>Vibrio cholerae</i> (33)	1	15	AGI1 with A025del (834 nt)	1del	14	14 (RJ)	-	-	-	-	-
	>1	18	AGI1 with A025del (702 nt)	1del	1	-	-	-	-	1 (RJ)	-
			AGI1 with A025del (834 nt)	1del	16	16 (RJ)	-	-	-	-	-
			Shew (918 nt)	2A	1	-	-	-	-	-	1
		Shew (918 nt) + Vcamp-P013-P017	2C	1	-	1	-	-	-	-	
<i>Enterobacter hormaechei</i> (12)	>1	12	like AGI1, A025del (702 nt)	1del	12	-	-	-	-	12 (RJ)	-
<i>Salmonella enterica</i> (83)	1	40	Shew (918 nt)	2A	36	-	36	-	-	-	-
	>1	43	Shew del (252 nt)	2Adel	1	1 (RJ)	-	-	-	-	-
			Large deletion	ind	2	2 (RJ)	-	-	-	-	-
			Shew (918 nt) + P012-P014	2B	1	-	-	-	1	-	-
			Shew (918 nt)	2A	34	-	32	2	-	-	-
			Shew del (252 nt)	2Adel	2	2 (RJ)	-	-	-	-	-
		Large deletion	ind	3	2 (RJ)	1 (RJ)	-	-	-	-	
		Shew (918 nt) + P012-P014	2B	4	-	-	-	4	-	-	
<i>Escherichia coli</i> (1)	>1	1	Shew del (252 nt)	2Adel	1	1 (RJ)	-	-	-	-	-
<i>Klebsiella pneumoniae</i> (1)	>1	1	Shew (918 nt)	2A	1	-	-	1	-	-	-

^a: number of contigs for the entire backbone.

^b: A025del: remaining nt of A025 in parenthesis and deletion of A026-*resG*; Shew: *Shewanella* (in parenthesis, nt similar (98.7%) to locus Sputw3181_4076 from *Shewanella* sp. W3-18-1 (CP000503)) between A022 and A025; Vcamp-P013-P017: segment of 597 bp from *Vibrio campbellii* (CP020076) including locus Vc3S01_0342 (93.0% identity) followed by P013-P017 (96.0% nt identity) between A014 and A020; Shew del: remaining nt of *Shewanella* in parenthesis; Large deletion: including at least A015-*resG* not allowing to determine the presence of *Shewanella* segment after A022; P012-P014: about 1077 nt similar (96.0%) to PGI1 between A014 and A016.

^c: del: part of the backbone deleted, particularly *resG* missing; ind: indeterminate

^d : RJ: insertion site determined at the right junction of the MDR region.

^e : AGI type