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SGI0, a relative of *Salmonella* genomic islands SGI1 and SGI2, lacking a class 1 integron, found in *Proteus mirabilis*

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1 **Abstract**

2 Several groups of integrative mobilizable elements (IMEs) that harbour a class 1
3 integron carrying antibiotic resistance genes have been found at the 3'-end of the
4 chromosomal *trmE* gene. Here, a new IME, designated SGI0, was found in *trmE* in
5 the sequenced and assembled genome of a French clinical, multiply antibiotic
6 resistant *Proteus mirabilis* strain, Pm1LENAR. SGI0 shares the same gene content
7 as the backbones of SGI1 and SGI2 (overall 97.6% and 97.7% nucleotide identity,
8 respectively) but it lacks a class 1 integron. However, SGI0 is a mosaic made up
9 of segments with >98.5% identity to SGI1 and SGI2 interspersed with segments
10 sharing 74-95% identity indicating that further diverged backbone types exist
11 and that recombination between them is occurring. The structure of SGI1-V, here
12 re-named SGI-V, which lacks two SGI1 (SGI2 and SGI0) backbone genes and
13 includes a group of additional genes in the backbone, was re-examined. In
14 regions shared with SGI1, the backbones shared 97.3% overall identity with the
15 differences distributed in patches with various levels of identity. The class 1
16 integron is also in a slightly different position with the target site duplication
17 AAATT instead of ACTTG for SGI1 and variants, indicating that it was acquired
18 independently. The Pm1LENAR resistance genes are in the chromosome, in Tn7
19 and an ISEcp1-mobilised segment.

20

21 **Keywords**

22 *Salmonella* genomic island; Integrative mobilizable element; IncC plasmid

23

24 **Abbreviations**

25 AGI1: *Acinetobacter* Genomic Island 1; IME: Integrative Mobilizable Element; Orf:
26 open reading frame; PGI1: *Proteus* Genomic Island 1; SNP: Single nucleotide
27 polymorphism; SGI: *Salmonella* Genomic Island
28

29

30 1. Introduction

31 *Salmonella* genomic island 1 (SGI1) which confers ampicillin,
32 chloramphenicol/florfenicol, streptomycin/spectinomycin, sulphonamides and
33 tetracycline resistances, was first reported in the epidemic multidrug resistant
34 *Salmonella enterica* subsp. *enterica* serovar Typhimurium DT104 clone (Boyd et al.,
35 2000; Threlfall, 2000). SGI1 is an integrative mobilizable element (IME), integrated
36 at the 3'-end of the *trmE* gene (formerly called *thdF*) (Douard et al., 2010; Doublet et
37 al., 2005). SGI1 is made up of a 27.4 kb backbone containing 28 open reading frames
38 (orfs) and a 15 kb complex class 1 integron, designated In104 that includes five
39 resistance genes (Boyd et al., 2001; Levings et al., 2005). In104 is inserted upstream
40 of the resolvase gene (*resG*, formerly S027) and flanked by a 5 bp duplication.

41 Since the first report of SGI1, many variants (SGI1-A to SGI1-Z, etc) have
42 been reported (Hall, 2010; Lei et al., 2015; Qin et al., 2015). They have identical or
43 nearly identical (>99.78% nucleotide identity) backbones and the variation usually
44 results from changes in the class 1 integron that affect the resistance gene content (Bie
45 et al., 2018; de Curraize et al., 2018; Harmer et al., 2016; Lei et al., 2015, 2014;
46 Siebor and Neuwirth, 2013; Xiao et al., 2019). Most SGI1 variants have been found in
47 *S. enterica* serovars or in *Proteus mirabilis* but recently SGI1 variants were also
48 reported in *Morganella morganii* (SGI1-L) (Schultz et al., 2017), in *Providencia*
49 *stuartii* (SGI1-W) (Soliman et al., 2019) and in *Escherichia coli* (SGI1-B) (Cummins
50 et al., 2019).

51 However, not all IMEs found in *trmE* are directly derived from SGI1. SGI2,
52 reported in *S. Emek* is related to SGI1 and was initially called SGI1-J (Levings et al.,
53 2005). However, it is an independently derived genomic island (Levings et al., 2008).

54 There is 0.53% nucleotide difference between the SGI1 and SGI2 backbones and the
55 integron in SGI2 is located in S023 instead of being upstream of *resG* (Hamidian et
56 al., 2015a; Levings et al., 2008; Wilson and Hall, 2010). SGI1 and SGI2 are
57 specifically mobilized by IncA and IncC (A/C) plasmids (Douard et al., 2010;
58 Doublet et al., 2005; Harmer et al., 2016; Siebor et al., 2016). The transcriptional
59 activator complex AcaCD of A/C plasmids promotes the excision of SGI1 and
60 mobilization of SGI1 thanks to the presence of AcaCD binding sites on SGI1 (Carraro
61 et al., 2014; Kiss et al., 2015).

62 Further IMEs found in *trmE* that include regions related to parts of the SGI1
63 backbone and carry a class 1 integron, have been reported. The backbone of the
64 element called SGI1-V has some differences in the gene content of its backbone in
65 comparison to SGI1, though it was reported as a variant of SGI1 (Siebor and
66 Neuwirth, 2011). More distantly related IME are *Proteus* genomic island 1 (PGI1)
67 (Siebor and Neuwirth, 2014), PGI2 (Lei et al., 2018), and *Acinetobacter* genomic
68 island 1 (AGI1)(Hamidian et al., 2015b). These IMEs are also not confined to the
69 species they were found in. PGI1 has been found in *P. mirabilis* and *S. enterica*
70 (Siebor and Neuwirth, 2014) and IME related to AGI1, first found in *Acinetobacter*
71 *baumannii*, have also been detected in *Enterobacter cloacae*, *S. enterica* and *Vibrio*
72 *cholerae* (Siebor et al., 2019).

73 All of the IMEs described above that target *trmE* harbour a class 1 integron
74 that contains genes conferring resistance to various antibiotics including critical
75 antibiotics: *bla*_{NDM-1} (carbapenem resistance) (Girlich et al., 2015), *bla*_{VEB-6} (Siebor
76 and Neuwirth, 2011) and *bla*_{CTX-M-15} (de Curraize et al., 2018) (resistance to broad
77 spectrum cephalosporins) or *rmtC* (resistance to aminoglycosides) (Wilson and Hall,
78 2010). Though the location of the integron is the same in all genuine SGI1 variants, it

79 is not the same in the other types indicating that an integron has been acquired
80 independently by each of them.

81 Here, we report an IME found in *trmE* in a *P. mirabilis* isolate that consists of
82 a backbone closely related to the SGI1 and SGI2 backbones but lacks a class 1
83 integron. The original classification of SGI1-V as an SGI1 variant is also re-assessed.

84

85 **2. Materials and methods**

86 *2.1 Strain*

87 A multiply antibiotic resistant *P. mirabilis*, Pm1LENAR was recovered in the
88 course of continuous surveillance of *P. mirabilis* isolates for SGI1 or PGI1 from
89 clinical samples in our French hospital (Siebor and Neuwirth, 2013). This isolate was
90 identified using the ApiE 20E system (bioMérieux, Marcy l'Étoile, France) and the
91 antibiotic susceptibility tests were performed in accordance with the guidelines of the
92 French Committee on Antimicrobial Susceptibility Testing (Comité de
93 l'Antibiogramme de la Société Française de Microbiologie CASFM 2013)
94 <https://www.sfm-microbiologie.org/>.

95 *2.2 Screen for IME related to SGI1 and PGI1*

96 DNA from *P. mirabilis* isolates was extracted by heat lysis of cells and
97 screened by PCR for the presence of the antitoxin gene, *sgiA* (S026) (Huguet et al.,
98 2016), which is present in both SGI1 and PGI1, using primers (Table S1) and
99 conditions previously reported (Siebor and Neuwirth, 2011).

100 *2.3 Genome Sequencing and assembly*

101 Bacterial DNA was extracted with the Gentra[®] Puregene[®] purification kit
102 (Qiagen, Courtaboeuf, France) and was quantified using the Qubit[®] system (Life
103 Technologies, Paysey, United Kingdom). The library was prepared with the

104 Nextera[®]XT kit (Illumina, Eindhoven, Netherlands) following the manufacturer's
105 instructions. The sample was multiplexed using Illumina-supplied barcodes and
106 paired-end sequencing was performed on the Illumina MiSeq with the 300 cycle
107 (MiSeq[®] Reagent Kit v2) (Illumina, Eindhoven, Netherlands). The reads (length 300
108 bp) of the genome of *Pm1LENAR* were assembled using SPAdes (Bankevich et al.,
109 2012) via the PATRIC web-based platform <https://www.patricbrc.org>. Contigs were
110 analyzed with the Basic Local Alignment Search Tool (BLAST)
111 (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). PCR and Sanger sequencing were
112 performed to check the single nucleotide polymorphisms (SNPs) not present in SGI1
113 (KU499918) (Siebor et al., 2016). This sequence was chosen for comparison because
114 the original sequence (AF261825) (Boyd et al., 2001) differs slightly from most
115 recent SGI1 sequences.

116 The short reads from Leekitcharoenphon's study (Leekitcharoenphon et al.,
117 2016) were retrieved and assembled using SPAdes (Bankevich et al., 2012) from the
118 PATRIC web-based platform <https://www.patricbrc.org>.

119 *2.4 Sequence analysis*

120 BLAST, Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) and
121 Artemis Comparison Tool (Carver et al., 2005) were used to compare nucleic acid
122 (na) and amino acid (aa) sequences. SGI2 (AY963803) (Hamidian et al., 2015a) and
123 SGI1-V (HQ888851) (Siebor and Neuwirth, 2011) sequences were used for alignment
124 analysis.

125 *2.5 Plasmid transfer*

126 The IncC plasmid, pPV835TEM24 (GenBank accession number MN148427),
127 originally from a *Proteus vulgaris* strain, Pv835, was used to induce IME excision. It
128 harbours a *floR* gene encoding for chloramphenicol and florfenicol resistance, an

129 *aacA4* gene encoding kanamycin, tobramycin, netilmicin and amikacin resistances, a
130 *dfrA1* gene encoding trimethoprim resistance, an *aadA1* encoding streptomycin,
131 spectinomycin resistances and a *sulI* encoding sulphonamides resistance. The
132 *Enterobacter aerogenes* BOL (EABOL) strain harbouring the IncC plasmid
133 pPV835TEM24 (donor) was mated with the *Proteus mirabilis* Pm1LENAR which
134 had an IME (recipient) in a 4:1 ratio at the end of the exponential growth phase in
135 Luria Bertani broth at 37°C as previously described (Siebor et al., 2016). After
136 overnight incubation at 37°C without shaking of the mating broth, 10-fold serial
137 dilutions of the bacterial cells were plated on Drigalski agar media (ThermoFisher
138 Scientific®) (which inhibits the swarming of *P. mirabilis*), supplemented with 15
139 mg/L of colistin to select for *P. mirabilis* and 50 mg/L of chloramphenicol to select
140 for pPV835TEM24 and were incubated 48h at 37°C. The transconjugants that had
141 acquired the pPV835TEM24 plasmid were recovered on this media.

142 2.6 Loss of the IME in cells

143 IMEs and IncC plasmids are unstable together (Harmer et al., 2016; Huguet et
144 al., 2016). Therefore, DNA was extracted from transconjugants grown on media
145 selecting for the retention of the IncC plasmid (media with 15 mg/L of colistin and 50
146 mg/L of chloramphenicol), and PCR assays were performed to check whether they
147 have lost the IME. Primers used are listed in Table S1. The presence or absence of the
148 IME was screened by PCR targeting the antitoxin gene *sgiA* (S026), the left and right
149 junctions between the IME and the *trmE* gene in the chromosome. To confirm the
150 absence of the IME in the *trmE* gene, a PCR of the intact *trmE* (*attB*) was also
151 performed. When loss of the IME from the chromosome was detected, colonies were
152 subcultured on the same selective media. DNA was extracted and screened by a two-
153 step DNA amplification of the *attP* site to ensure no extra-chromosomal circular form

154 of the IME remained in cells. A negative and a positive control were run for all PCR
155 assays. The negative and positive controls were DNA extracts of *P. mirabilis* with or
156 without an SGI1.

157 2.7 Nucleotide sequence accession numbers

158 The nucleotide sequences of the complete SGI0 and the pPV835TEM24
159 plasmid were assigned the GenBank accession numbers MH734355 and MN148427,
160 respectively. The Whole Genome Shotgun project for the *Pm1LENAR* strain has been
161 deposited at DDBJ/ENA/GenBank under the accession VIYO00000000. The version
162 described in this paper is version VIYO01000000.

163

164 3. Results

165 3.1 Characteristics of the *P. mirabilis* isolate, *Pm1LENAR*

166 The multiply antibiotic resistant *P. mirabilis*, *Pm1LENAR*, was isolated in 2013 in
167 France, from stool of a patient hospitalized in the haematology intensive care unit.
168 This isolate had an unusual antibiotic resistance profile; it was resistant to amoxicillin
169 (32 mg/L), amoxicillin + clavulanic acid (32 mg/L), cefotaxime (cephalosporinase
170 producer) (32 mg/L), kanamycin (256 mg/L), streptomycin (256 mg/L),
171 spectinomycin (256 mg/L) and trimethoprim (32 mg/L). *P. mirabilis* is intrinsically
172 resistant to tetracyclines (256 mg/L) and colistin (>2 mg/L) (Magalhães et al., 1998;
173 Sud and Feingold, 1970). As the SGI1/PGII antitoxin gene, *sgiA* (S026) was detected
174 by PCR; the genome of *Pm1LENAR* was sequenced and assembled. An IME located,
175 at the 3'-end of the chromosomal *trmE* gene and related to SGI1, was found in one
176 contig (coverage 51X) (MH734355). However, an integron carrying resistance genes
177 was not contained in this structure and for this reason; we have called it, SGI0.

178 The resistance genes were found in three different contigs in the *Pm1LENAR*
179 draft genome. The first 50.42 kb contig (number 00025) harboured a structure
180 containing an *ISEcpI* with a *bla_{CMY-2}* gene encoding a cephalosporinase. An 11.63 kb
181 segment flanked by 5 bp duplication and therefore inserted by an *ISEcpI*- mediated
182 transposition event was found in a 37.6 kb integrative element. An identical segment
183 harbouring *ISEcpI* with a *bla_{CMY-2}* (99.85% identity) was previously reported in a *S.*
184 Newport plasmid (DQ164214) (Kang et al., 2006). A 44.47 kb contig (number 00028)
185 contained a Tn7 transposon (at 7421 bp to 21488 bp) with *dfrAI* and *aadAI* genes
186 conferring resistance to trimethoprim, and streptomycin and spectinomycin,
187 respectively. This Tn7 is inserted downstream the *glmS* gene as is usual (Mitra et al.,
188 2010). The third 1.07 kb contig (number 00075) harboured an *aphA1b* gene encoding
189 resistance to kanamycin and neomycin.

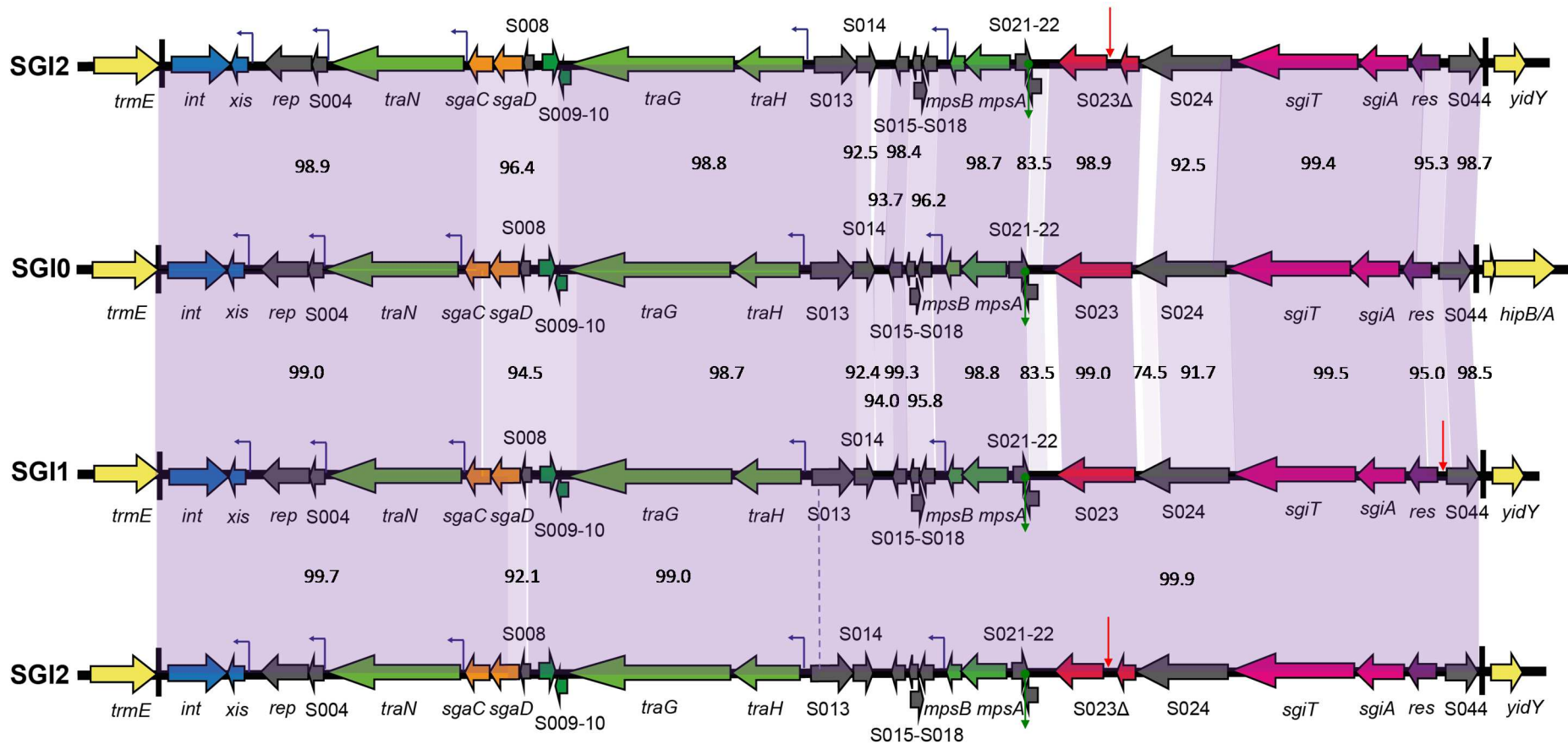
190 ResFinder also recorded the intrinsic chromosomal gene *tet(J)* conferring
191 tetracycline resistance as expected (Magalhães et al., 1998) and a *cat* gene encoding a
192 chloramphenicol acetyltransferase. However, *Pm1LENAR* was susceptible to
193 chloramphenicol. It has been reported that this *cat* gene is often present in *P. mirabilis*
194 and can result in an intermediate level of chloramphenicol resistance (Charles et al.,
195 1985; Okamoto et al., 1967).

196

197 3.2 SGI0, a new IME in *trmE*,

198 SGI0 was 27.3 kb and contained 28 orfs closely related to those in the
199 backbones of SGI1 and SGI2. However, it did not harbour a class 1 integron and it
200 was not interrupted by other elements. SGI0 shared 97.59% (26123/26767) nucleotide
201 identity with the SGI1 backbone and 97.72% (26158/26767) nucleotide identity with
202 the SGI2 backbone. Most of the backbone shared >98.5% identity with SGI1 and

203 SGI2 but the nucleotide identity in different segments, ranged from 74.5% to 99.5%
204 for SGI1 and from 83.5% to 99.4% for SGI2 clearly indicating a mosaic structure
205 (Fig. 1).
206



207
208
209
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211
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Figure 1: Comparison of SGI0 backbone with SGI1 and SGI2 backbones

The percentages of nucleotide identity are indicated between the backbones. The following colours were used for characterizing the orfs: chromosomal genes (yellow), DNA recombination (blue), conjugal transfer (green), regulatory genes (orange), toxin/antitoxin system (pink), helicase (red), resolvase (purple), unknown function (grey). The blue hooked arrows indicate the AcaCD binding sites, the vertical green arrows indicate the *oriT* site, the vertical red arrows indicates the position of the class 1 integron. The thick black vertical bars indicate the attachment sites, *attL* and *attR* of SGI.

213 SgaC, MpsA and SgiT of SGI0 shared more aa identities with SGI1 than with SGI2,
214 whereas SgaD, S008, TraG, S016, S017 and S044 shared more identity with SGI2.
215 SNPs were distributed throughout the backbone but there were also some spots with
216 more SNPs such as in a region between *sgaD* gene (S007) and S009 and also in S024
217 (Table 1) (Fig. 1). Some of SNPs result in variation in the length of the predicted
218 polypeptide sequence. For instance, S014 and S021 of SGI0 were 21 aa and 42 aa
219 longer respectively than for SGI1 and SGI2. S017 and S023 were 72 aa and 2 aa
220 shorter respectively due to the presence of a point-nonsense mutation. S024 shared
221 only 85.65% and 86.12% aa identity with S024 from SGI1 and SGI2, respectively.
222 All of these orfs have **unknown** function. All of the essential known proteins were
223 conserved. Hence, the SGI0 integrase shared 99.75% aa identity 99.10% nucleotide
224 identity with SGI1 and SGI2 ones. The toxin/antitoxin system, SgiAT (Huguet et al.,
225 2016), was also very conserved > 99.5% aa identical to SGI1 and SGI2. Moreover,
226 the recently described mobilization proteins, MpsB/MpsA (Kiss et al., 2019), were
227 also very conserved > 99% aa identical to SGI1 and SGI2. Interestingly, the DNA
228 segment immediately after the *oriT* in SGI0 shared only a 83.5% percentage of
229 nucleotide identity with SGI1 and SGI2. **The *oriT* region seems to be a hot spot for**
230 **recombination events as previously reported for plasmids (Llosa et al., 1994).**

231

Table 1: Comparison of the 28 orfs of SGI0 with those of SGI1, SGI2 and SGI-V

^a orf	gene name	^b SGI0 (aa length)	^c SGI1 identity (%)	^b SGI1 (aa length)	^b number of aa changed	^c SGI2 identity (%)	^b SGI2 (aa length)	^b number of aa changed	^c SGI-V identity (%)	^b SGI-V (aa length)	^b number of aa changed
S001	<i>integrase</i>	405	99.75	405	1	99.75	405	1	99.51	405	2
S002	<i>excisionase</i>	122	99.18	122	1	99.18	122	1	99.18	122	1
S003	<i>replicase</i>	317	99.37	317	2	99.37	317	2	99.05	317	3
S004		96	98.96	96	1	98.96	96	1	98.96	96	1
S005	<i>traN</i>	919	99.56	919	4	99.56	919	4	99.35	919	6
S006	<i>sgaC</i>	177	100.00	177	0	99.44	177	1	99.44	177	1
S007	<i>sgaD</i>	203	91.63	203	17	93.60	203	13	92.12	203	16
S008		73	87.67	73	9	97.26	73	2	86.30	73	10
S009		96	96.88	96	3	95.83	96	4	95.83	96	4
S010		84	98.81	84	1	98.81	84	1	97.62	84	2
S011	<i>traG</i>	1134	98.68	1134	15	99.21	1134	9	99.03	1134	11
S012	<i>traH</i>	474	98.95	474	5	98.52	474	7	98.10	474	9
S013		285	98.60	285	4	98.60	285	4	96.14	285	11
S014		152	90.84	131	12	90.84	131	12	insertion in	104	-
S015		89	98.88	89	1	98.88	89	1	98.88	89	1
S016		61	95.08	61	3	96.72	61	2	96.72	61	2
S017		41	92.68	84	6	95.12	84	4	95.12	84	4
S018		83	92.77	105	8	92.77	105	8	92.77	105	8
S019	<i>mpsB</i>	98	100.00	98	0	100.00	98	0	98.98	98	1
S020	<i>mpsA</i>	321	99.69	321	1	99.38	321	2	97.51	321	8
S021		153	99.10	111	1	99.10	111	1	98.69	153	2
S022		91	72.53	91	25	72.53	91	25	100.00	91	0
S023		544	96.14	546	21	insertion in	-	-	absent	-	-
S024		642	85.65	641	91	86.12	641	89	absent	-	-
S025	<i>sgiT</i>	847	99.76	847	2	99.65	847	3	90.20	847	83
S026	<i>sgiA</i>	329	100.00	329	0	100.00	329	0	99.39	194	1
S027	<i>resG</i>	194	98.97	194	2	98.97	194	2	100.00	209	0
S044		209	98.56	209	3	99.04	209	2	truncated	-	-

232

233

^a orf: open reading frame; ^b aa: amino acid; ^c Percentages represent nucleotide identity

234 3.3 Loss of SGI0

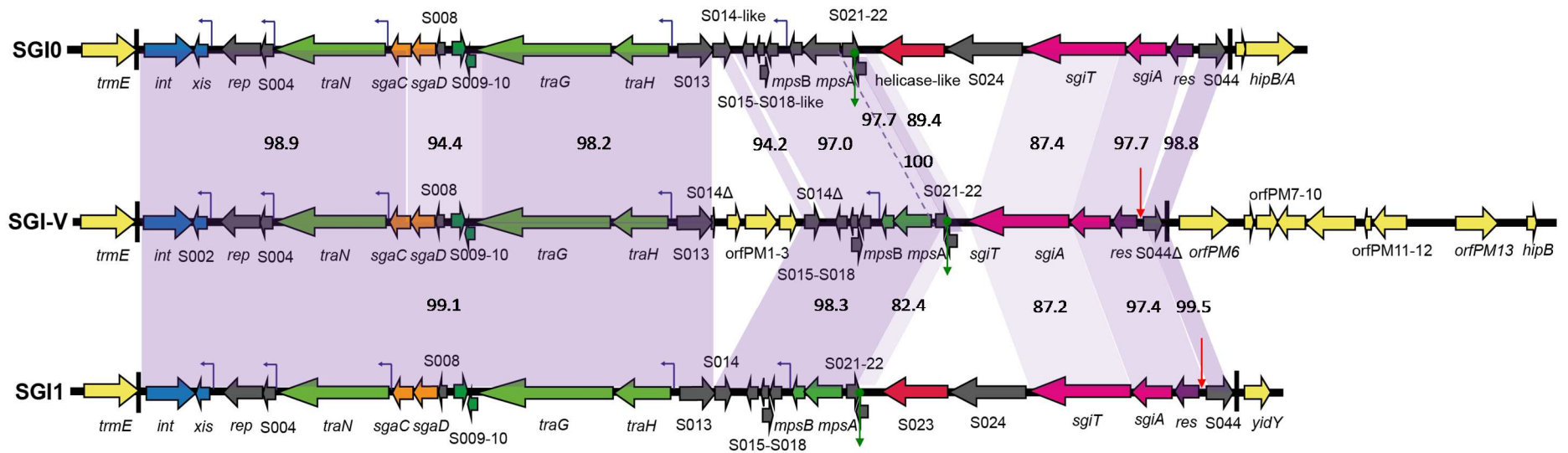
235 As previously reported for SGI1, SGI0 also harboured the five AcaCD binding sites
236 (Fig. 1). The sequences were same as in SGI1, except the one upstream of the *xis* gene, which
237 had one mutation. The presence of these AcaCD binding sites and the high percentage of
238 identity of the SGI0 integrase with SGI1, led us to test whether SGI0 could be excised when
239 an IncC plasmid was present. Hence, the IncC plasmid pPV835TEM24 was introduced into
240 *Pm1LENAR* harbouring SGI0. Ten colonies were detected on the selective media for the
241 retention of the plasmid. All of them kept the IncC plasmid. PCR screening revealed that a
242 few colonies kept both the IncC plasmid and SGI0 but most of them had lost SGI0 as
243 the *sgiA* gene, as well as the left and right junctions of SGI1 in *trmE* were not detected. In
244 addition, the PCR targeting the intact *trmE* gene (*attB* site) yielded a product of the correct
245 size confirming that SGI0 was not present in the chromosome. The complete loss of SGI0
246 from these cells was confirmed by the fact that the extra-chromosomal form of SGI0 was not
247 detected by PCR. As SGI0 did not harbour a resistance gene, transfer of SGI0 into another
248 bacterium was not looked for.

249

250 3.4 SGI1-V is also an independently derived SGI

251 The backbone of SGI1-V reported previously (Siebor and Neuwirth, 2011) has some
252 significant differences from the SGI1 backbone (Fig. 2). Therefore, SGI1-V was re-
253 examined. In addition to the deletion and the insertion in SGI1-V relative to SGI1 (Fig. 2), in
254 the backbone regions shared with the SGI1, the SGI1-V backbone was on average 97.25%
255 (22671/23312 nucleotides) identical to that of SGI1. There are also polymorphisms in the
256 SGI1-V backbone relative to SGI0 and SGI2. It shared 96.48% (22499/23320) and 97.03%
257 (22620/23312) nucleotide identity, respectively. As for SGI2, SgaD, TraG, S016, S017,
258 S022, ResG shared more aa identities with SGI0 than with SGI1 (Table 1).

259 In addition, the 5 bp duplication flanking the SGI1-V integron (AAATT) is different
260 to that flanking SGI1 (ACTTG), indicating that the class 1 integron was inserted in a
261 different position to In104 in SGI1 and hence was acquired in an independent event. The
262 backbone differences and the different position of the class 1 integron indicate that, like SGI2
263 (Wilson and Hall, 2010), SGI1-V is an independently derived IME. Therefore, we have re-
264 named it SGI-V.
265



266

267

Figure 2: Comparison of SGI-V backbone with SGI0 and SGI1 backbones

268

The percentages of nucleotide identity are indicated between the backbones. The following colours were used for characterizing the orfs:

269

chromosomal genes (yellow), DNA recombination (blue), conjugal transfer (green), regulatory genes (orange), toxin/antitoxin system (pink), helicase

270

(red), resolvase (purple), unknown function (grey). The blue hooked arrows indicate the AcaCD binding sites, the vertical green arrows indicate the

271

oriT site, the vertical red arrows indicates the position of the class 1 integron. The thick black vertical bars indicate the attachment sites, *attL* and *attR*

272

of SGI.

273

274 4. Discussion

275 This study describes an IME, SGI0, in a *P. mirabilis* isolate that is related to the
276 backbones of SGI1 and SGI2. SGI0 shared varying levels of identity with parts of the SGI1
277 or SGI2 backbones. It also shared regions with the SGI-V backbone. Hence, SGI0 is a mosaic
278 that includes segments shared with the backbones of three SGIs. All of the SGI types that
279 include a class 1 integron, namely SGI1, SGI2 and SGI-V, acquired the integron
280 independently. SGI0 likely represents a group of integron-free IMEs that share a common
281 ancestor and can be the forerunners of an SGI type that carries an integron.

282 SGI-like structures without any resistance gene have been reported in previous
283 studies. The first was reported in 2008 in a susceptible *S. Typhimurium* DT104 that came
284 from Health Protection Agency, in United Kingdom. In this isolate, the presence of a SGI-
285 like structure was found using an SGI1-fingerprinting assay but the class 1 integron was not
286 detected (Amar et al., 2008). However, no sequence is available. A later study detected the
287 presence of an SGI-like element without a class 1 integron in a *P. mirabilis* isolate, which
288 was collected from a dog in 2013, in France (Schultz et al., 2015). Only 3.9 kb of this
289 structure was sequenced (some parts of S007 (*sgaD*), S008 and S011) (GenBank accession
290 number KR296941). Interestingly, these parts are identical to the corresponding parts of
291 SGI0, reported here and it might be identical to SGI0. The isolate harbouring this SGI, came
292 from a different area (Pyrénées orientales) than ours (Bourgogne). However, like
293 *Pm1LENAR*, this isolate was also resistant to amoxicillin, amoxicillin + clavulanic acid,
294 kanamycin, spectinomycin, trimethoprim. It would be interesting to completely sequence it to
295 determine whether it belongs to the same clone as *Pm1LENAR*.

296 A third study that analyzed the genomes of *S. Typhimurium* DT104 from worldwide
297 isolates collected from 1969 to 2012, reported a few isolates harbouring SGI1 but without

298 any resistance genes (Leekitcharoenphon et al., 2016). However, we retrieved, assembled,
299 and analyzed the short reads and found that all genomes harbouring an SGI1 derivative had at
300 least part of a class 1 integron. Hence, they do not resemble SGI0.

301 Finally, the presence of AcaCD binding sites and the **loss of SGI0** in presence of an
302 IncC plasmid suggest that SGI0 might be mobilized by A/C plasmids as the other IMEs in
303 *trmE* (Doublet et al., 2005; Harmer et al., 2016; Siebor et al., 2016). SGI0 and further **related**
304 IMEs in *trmE* without class 1 integron might be also prevalent.

305

306 **5. Conclusion**

307 SGI0 is a new IME in *trmE* closely related to the backbones of SGI1, SGI2 and **parts**
308 **of SGI-V** but characterized by the lack of class 1 integron. All of these SGIs backbones are
309 likely to be derived from an original common ancestor, which has diverged overtime and the
310 lineages have repeatedly recombined. Further **IME like SGI0** without class 1 integron might
311 **be circulating** but have not yet been detected.

312

313 **Conflict of interest**

314 The authors declare no conflict of interest.

315

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318

319 **Author contributions**

320 CC conceptualized, performed loss assay, analysed data and wrote the manuscript. ES
321 conceptualized and performed the sequencing and assembly of the genomes and reviewed the
322 preparation of manuscript. CN conceptualized, supervised, revised and reviewed the

323 preparation of manuscript. RMH analysed data and supervised, revised and reviewed the
324 preparation of manuscript.

325

326 **References**

- 327 Amar, C.F.L., Arnold, C., Bankier, A., Dear, P.H., Guerra, B., Hopkins, K.L., Liebana, E.,
328 Mevius, D.J., Threlfall, E.J., 2008. Real-time PCRs and fingerprinting assays for the
329 detection and characterization of *Salmonella* Genomic Island-1 encoding multidrug
330 resistance: application to 445 European isolates of *Salmonella*, *Escherichia coli*,
331 *Shigella*, and *Proteus*. *Microb. Drug Resist.* 14, 79–92.
332 <https://doi.org/10.1089/mdr.2008.0812>
- 333 Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin,
334 V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V.,
335 Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new
336 genome assembly algorithm and its applications to single-cell sequencing. *J. Comput.*
337 *Biol.* 19, 455–477. <https://doi.org/10.1089/cmb.2012.0021>
- 338 Bie, L., Fang, M., Li, Z., Wang, M., Xu, H., 2018. Identification and characterization of new
339 resistance-conferring SGIs (*Salmonella* Genomic Island 1) in *Proteus mirabilis*.
340 *Front Microbiol* 9, 3172. <https://doi.org/10.3389/fmicb.2018.03172>
- 341 Boyd, D., Peters, G.A., Cloeckert, A., Boumedine, K.S., Chaslus-Dancla, E., Imberechts, H.,
342 Mulvey, M.R., 2001. Complete nucleotide sequence of a 43-kilobase genomic island
343 associated with the multidrug resistance region of *Salmonella enterica* serovar
344 Typhimurium DT104 and its identification in phage type DT120 and serovar Agona.
345 *J. Bacteriol.* 183, 5725–5732. <https://doi.org/10.1128/JB.183.19.5725-5732.2001>
- 346 Boyd, D.A., Peters, G.A., Ng, L., Mulvey, M.R., 2000. Partial characterization of a genomic
347 island associated with the multidrug resistance region of *Salmonella enterica*
348 Typhimurium DT104. *FEMS Microbiol. Lett.* 189, 285–291.
- 349 Carraro, N., Matteau, D., Luo, P., Rodrigue, S., Burrus, V., 2014. The master activator of
350 IncA/C conjugative plasmids stimulates genomic islands and multidrug resistance
351 dissemination. *PLoS Genet.* 10, e1004714.
352 <https://doi.org/10.1371/journal.pgen.1004714>
- 353 Carver, T.J., Rutherford, K.M., Berriman, M., Rajandream, M.-A., Barrell, B.G., Parkhill, J.,
354 2005. ACT: the Artemis Comparison Tool. *Bioinformatics* 21, 3422–3423.
355 <https://doi.org/10.1093/bioinformatics/bti553>
- 356 Charles, I.G., Harford, S., Brookfield, J.F., Shaw, W.V., 1985. Resistance to chloramphenicol
357 in *Proteus mirabilis* by expression of a chromosomal gene for chloramphenicol
358 acetyltransferase. *J. Bacteriol.* 164, 114–122.
- 359 Cummins, M.L., Chowdhury, P.R., Marena, M.S., Browning, G.F., Djordjevic, S.P., 2019.
360 *Salmonella* Genomic Island 1B variant found in a Sequence Type 117 avian
361 pathogenic *Escherichia coli* Isolate. *mSphere* 4, e00169-19.
362 <https://doi.org/10.1128/mSphere.00169-19>
- 363 de Curraize, C., Neuwirth, C., Bador, J., Chapuis, A., Amoureux, L., Siebor, E., 2018. Two
364 new *Salmonella* genomic islands 1 from *Proteus mirabilis* and description of *bla*_{CTX-}
365 *M-15* on a variant (SGI1-K7). *J. Antimicrob. Chemother.* 73, 1804–1807.
366 <https://doi.org/10.1093/jac/dky108>

367 Douard, G., Praud, K., Cloeckaert, A., Doublet, B., 2010. The *Salmonella* genomic island 1 is
368 specifically mobilized in *trans* by the IncA/C multidrug resistance plasmid family.
369 PLoS ONE 5, e15302. <https://doi.org/10.1371/journal.pone.0015302>

370 Doublet, B., Boyd, D., Mulvey, M.R., Cloeckaert, A., 2005. The *Salmonella* genomic island
371 1 is an integrative mobilizable element. *Molecular Microbiology* 55, 1911–1924.
372 <https://doi.org/10.1111/j.1365-2958.2005.04520.x>

373 Girlich, D., Dortet, L., Poirel, L., Nordmann, P., 2015. Integration of the *bla*_{NDM-1}
374 carbapenemase gene into *Proteus* genomic island 1 (PGI1-*PmPEL*) in a *Proteus*
375 *mirabilis* clinical isolate. *J Antimicrob Chemother* 70, 98–102.
376 <https://doi.org/10.1093/jac/dku371>

377 Hall, R.M., 2010. *Salmonella* genomic islands and antibiotic resistance in *Salmonella*
378 *enterica*. *Future Microbiol* 5, 1525–1538. <https://doi.org/10.2217/fmb.10.122>

379 Hamidian, M., Holt, K.E., Hall, R.M., 2015a. The complete sequence of *Salmonella* genomic
380 island SGI2. *J. Antimicrob. Chemother.* 70, 617–619.
381 <https://doi.org/10.1093/jac/dku407>

382 Hamidian, M., Holt, K.E., Hall, R.M., 2015b. Genomic resistance island AGI1 carrying a
383 complex class 1 integron in a multiply antibiotic-resistant ST25 *Acinetobacter*
384 *baumannii* isolate. *J Antimicrob Chemother* 70, 2519–2523.
385 <https://doi.org/10.1093/jac/dkv137>

386 Harmer, C.J., Hamidian, M., Ambrose, S.J., Hall, R.M., 2016. Destabilization of IncA and
387 IncC plasmids by SGI1 and SGI2 type *Salmonella* genomic islands. *Plasmid* 87–88,
388 51–57. <https://doi.org/10.1016/j.plasmid.2016.09.003>

389 Huguet, K.T., Gonnet, M., Doublet, B., Cloeckaert, A., 2016. A toxin antitoxin system
390 promotes the maintenance of the IncA/C-mobilizable *Salmonella* Genomic Island 1.
391 *Scientific Reports* 6. <https://doi.org/10.1038/srep32285>

392 Kang, M.-S., Besser, T.E., Call, D.R., 2006. Variability in the region downstream of the
393 *bla*_{CMY-2} beta-lactamase gene in *Escherichia coli* and *Salmonella enterica* plasmids.
394 *Antimicrob. Agents Chemother.* 50, 1590–1593.
395 <https://doi.org/10.1128/AAC.50.4.1590-1593.2006>

396 Kiss, J., Papp, P.P., Szabó, M., Farkas, T., Murányi, G., Szakállas, E., Olasz, F., 2015. The
397 master regulator of IncA/C plasmids is recognized by the *Salmonella* Genomic island
398 SGI1 as a signal for excision and conjugal transfer. *Nucl. Acids Res.* 43, 8735–8745.
399 <https://doi.org/10.1093/nar/gkv758>

400 Kiss, J., Szabó, M., Hegyi, A., Douard, G., Praud, K., Nagy, I., Olasz, F., Cloeckaert, A.,
401 Doublet, B., 2019. Identification and characterization of *oriT* and two mobilization
402 genes required for conjugative transfer of *Salmonella* Genomic Island 1. *Front*
403 *Microbiol* 10, 457. <https://doi.org/10.3389/fmicb.2019.00457>

404 Leekitcharoenphon, P., Hendriksen, R.S., Hello, S.L., Weill, F.-X., Baggesen, D.L., Jun, S.-
405 R., Ussery, D.W., Lund, O., Crook, D.W., Wilson, D.J., Aarestrup, F.M., 2016.
406 Global genomic epidemiology of *Salmonella enterica* Serovar Typhimurium DT104.
407 *Appl. Environ. Microbiol.* 82, 2516–2526. <https://doi.org/10.1128/AEM.03821-15>

408 Lei, C.-W., Chen, Y.-P., Kong, L.-H., Zeng, J.-X., Wang, Y.-X., Zhang, A.-Y., Wang, H.-N.,
409 2018. PGI2 is a novel SGI1-related multidrug-resistant genomic island characterized
410 in *Proteus mirabilis*. *Antimicrob Agents Chemother* 62.
411 <https://doi.org/10.1128/AAC.00019-18>

412 Lei, C.-W., Zhang, A.-Y., Liu, B.-H., Wang, H.-N., Guan, Z.-B., Xu, C.-W., Xia, Q.-Q.,
413 Cheng, H., Zhang, D.-D., 2014. Molecular characteristics of *Salmonella* Genomic
414 Island 1 in *Proteus mirabilis* isolates from poultry farms in China. *Antimicrob.*
415 *Agents Chemother.* 58, 7570–7572. <https://doi.org/10.1128/AAC.03992-14>

- 416 Lei, C.-W., Zhang, A.-Y., Liu, B.-H., Wang, H.-N., Yang, L.-Q., Guan, Z.-B., Xu, C.-W.,
417 Zhang, D.-D., Yang, Y.-Q., 2015. Two novel *Salmonella* Genomic Island 1 variants
418 in *Proteus mirabilis* isolates from swine farms in China. *Antimicrob Agents*
419 *Chemother* 59, 4336–4338. <https://doi.org/10.1128/AAC.00120-15>
- 420 Levings, R.S., Djordjevic, S.P., Hall, R.M., 2008. SGI2, a relative of *Salmonella* Genomic
421 Island SGI1 with an independent origin. *Antimicrob. Agents Chemother.* 52, 2529–
422 2537. <https://doi.org/10.1128/AAC.00189-08>
- 423 Levings, R.S., Lightfoot, D., Partridge, S.R., Hall, R.M., Djordjevic, S.P., 2005. The
424 Genomic Island SGI1, containing the multiple antibiotic resistance Region of
425 *Salmonella enterica* Serovar Typhimurium DT104 or variants of it, is widely
426 distributed in other *S. enterica* serovars. *J Bacteriol* 187, 4401–4409.
427 <https://doi.org/10.1128/JB.187.13.4401-4409.2005>
- 428 Llosa, M., Bolland, S., Grandoso, G., Cruz, F. de la, 1994. Conjugation-independent, site-
429 specific recombination at the *oriT* of the IncW plasmid R388 mediated by TrwC.
430 *Journal of Bacteriology* 176, 3210–3217. [https://doi.org/10.1128/jb.176.11.3210-
431 3217.1994](https://doi.org/10.1128/jb.176.11.3210-3217.1994)
- 432 Magalhães, V.D., Schuman, W., Castilho, B.A., 1998. A new tetracycline resistance
433 determinant cloned from *Proteus mirabilis*. *Biochimica et Biophysica Acta (BBA) -*
434 *Gene Structure and Expression* 1443, 262–266. [https://doi.org/10.1016/S0167-
435 4781\(98\)00210-3](https://doi.org/10.1016/S0167-4781(98)00210-3)
- 436 Mitra, R., McKenzie, G.J., Yi, L., Lee, C.A., Craig, N.L., 2010. Characterization of the
437 TnsD-attTn7 complex that promotes site-specific insertion of Tn7. *Mob DNA* 1, 18.
438 <https://doi.org/10.1186/1759-8753-1-18>
- 439 Okamoto, S., Suzuki, Y., Mise, K., Nakaya, R., 1967. Occurrence of chloramphenicol-
440 acetylating enzymes in various Gram-negative bacilli. *Journal of Bacteriology* 94,
441 1616–1622.
- 442 Qin, S., Qi, H., Zhang, Q., Zhao, D., Liu, Z.-Z., Tian, H., Xu, L., Xu, H., Zhou, M., Feng, X.,
443 Liu, H.-M., 2015. Emergence of extensively drug-resistant *Proteus mirabilis*
444 harboring a conjugative NDM-1 plasmid and a novel *Salmonella* Genomic Island 1
445 variant, SGI1-Z. *Antimicrobial Agents and Chemotherapy* 59, 6601.
446 <https://doi.org/10.1128/AAC.00292-15>
- 447 Schultz, E., Barraud, O., Madec, J.-Y., Haenni, M., Cloeckert, A., Ploy, M.-C., Doublet, B.,
448 2017. Multidrug resistance *Salmonella* Genomic Island 1 in a *Morganella morganii*
449 subsp. *morganii* human clinical isolate from France. *mSphere* 2, e00118-17.
450 <https://doi.org/10.1128/mSphere.00118-17>
- 451 Schultz, E., Haenni, M., Mereghetti, L., Siebor, E., Neuwirth, C., Madec, J.-Y., Cloeckert,
452 A., Doublet, B., 2015. Survey of multidrug resistance integrative mobilizable
453 elements SGI1 and PGI1 in *Proteus mirabilis* in humans and dogs in France, 2010–
454 13. *J. Antimicrob. Chemother.* 70, 2543–2546. <https://doi.org/10.1093/jac/dkv154>
- 455 Siebor, E., Curraize, C. de, Amoureux, L., Neuwirth, C., 2016. Mobilization of the
456 *Salmonella* genomic island SGI1 and the *Proteus* genomic island PGI1 by the A/C2
457 plasmid carrying *bla*_{TEM-24} harboured by various clinical species of
458 Enterobacteriaceae. *J. Antimicrob. Chemother.* 71, 2167–2170.
459 <https://doi.org/10.1093/jac/dkw151>
- 460 Siebor, E., de Curraize, C., Neuwirth, C., 2019. Identification of AGI1-A, a variant of
461 *Acinetobacter* genomic island 1 (AGI1), in a French clinical isolate belonging to the
462 *Enterobacter cloacae* complex. *J. Antimicrob. Chemother.* 74, 311–314.
463 <https://doi.org/10.1093/jac/dky442>

464 Siebor, E., Neuwirth, C., 2014. *Proteus* genomic island 1 (PGI1), a new resistance genomic
465 island from two *Proteus mirabilis* French clinical isolates. J. Antimicrob. Chemother.
466 69, 3216–3220. <https://doi.org/10.1093/jac/dku314>
467 Siebor, E., Neuwirth, C., 2013. Emergence of *Salmonella* genomic island 1 (SGI1) among
468 *Proteus mirabilis* clinical isolates in Dijon, France. J. Antimicrob. Chemother. 68,
469 1750–1756. <https://doi.org/10.1093/jac/dkt100>
470 Siebor, E., Neuwirth, C., 2011. The new variant of *Salmonella* genomic island 1 (SGI1-V)
471 from a *Proteus mirabilis* French clinical isolate harbours *bla*_{VEB-6} and *qnrA1* in the
472 multiple antibiotic resistance region. J. Antimicrob. Chemother. 66, 2513–2520.
473 <https://doi.org/10.1093/jac/dkr335>
474 Soliman, A.M., Shimamoto, Toshi, Nariya, H., Shimamoto, Tadashi, 2019. Emergence of
475 *Salmonella* Genomic Island 1 variant SGI1-W in a clinical isolate of *Providencia*
476 *stuartii* from Egypt. Antimicrobial Agents and Chemotherapy 63, e01793-18.
477 <https://doi.org/10.1128/AAC.01793-18>
478 Sud, I.J., Feingold, D.S., 1970. Mechanism of polymyxin B resistance in *Proteus mirabilis*.
479 Journal of Bacteriology 104, 289–294.
480 Threlfall, E.J., 2000. Epidemic *Salmonella* Typhimurium DT 104—a truly international
481 multiresistant clone. J. Antimicrob. Chemother. 46, 7–10.
482 <https://doi.org/10.1093/jac/46.1.7>
483 Wilson, N.L., Hall, R.M., 2010. Unusual class 1 integron configuration found in *Salmonella*
484 Genomic Island 2 from *Salmonella enterica* serovar Emek. Antimicrob. Agents
485 Chemother. 54, 513–516. <https://doi.org/10.1128/AAC.00895-09>
486 Xiao, T., Dai, H., Lu, B., Li, Z., Cai, H., Huang, Z., Kan, B., Wang, D., 2019. Distribution
487 and characteristics of SGI1/PGI2 genomic island from *Proteus* strains in China.
488 Infect. Genet. Evol. 70, 123–130. <https://doi.org/10.1016/j.meegid.2019.02.027>
489