



**HAL**  
open science

## **Lack of dissemination of acquired resistance to $\beta$ -lactams in small wild mammals around an isolated village in the Amazonian forest**

Nathalie Grall, Olivier Barraud, Ingrid Wieder, Anna Hua, Marion Perrier, Ana Babosan, Margaux Gaschet, Olivier Clermont, Erick Denamur, François M. Catzeflis, et al.

### ► To cite this version:

Nathalie Grall, Olivier Barraud, Ingrid Wieder, Anna Hua, Marion Perrier, et al.. Lack of dissemination of acquired resistance to  $\beta$ -lactams in small wild mammals around an isolated village in the Amazonian forest. *Environmental Microbiology Reports*, 2015, 7 (5), pp.698 - 708. 10.1111/1758-2229.12289 . hal-01836342

**HAL Id: hal-01836342**

**<https://hal.umontpellier.fr/hal-01836342v1>**

Submitted on 31 Jan 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# **Lack of dissemination of acquired resistance to $\beta$ -lactam in small wild mammals around an isolated village in the Amazonian forest**

Nathalie Grall <sup>1,2,3</sup>, Olivier Barraud <sup>4,5,6</sup>, Ingrid Wieder <sup>3</sup>, Anna Hua <sup>3</sup>, Marion Perrier <sup>3</sup>, Ana Babosan<sup>7</sup>, Margaux Gaschet <sup>4</sup>, Olivier Clermont <sup>1,2</sup>, Erick Denamur <sup>1,2</sup>, François Catzefflis <sup>8</sup>, Dominique Decré<sup>7,9,10</sup>, Marie-Cécile Ploy<sup>4,5,6</sup>, Antoine Andreumont<sup>1,2,3</sup>

<sup>1</sup> INSERM, IAME, UMR 1137, F-75018 Paris, France

<sup>2</sup> Univ Paris Diderot, IAME, UMR 1137, Sorbonne Paris Cité, F-75018 Paris, France

<sup>3</sup> AP-HP, Hôpital Bichat, Laboratoire de Microbiologie, F-75018 Paris, France

<sup>4</sup> CHU Limoges, Laboratoire de Bactériologie-Virologie-Hygiène, Limoges, France

<sup>5</sup> INSERM U1092, Limoges, France

<sup>6</sup> Univ Limoges, UMR-S1092, Limoges, France

<sup>7</sup> AP-HP, Hôpital Saint-Antoine, Laboratoire de Bactériologie-Hygiène, F-75012 Paris, France

<sup>8</sup> CNRS UMR-5554, Institut des Sciences de l'Evolution, Univ Montpellier-2, Montpellier, France

<sup>9</sup> Sorbonne Universités, UPMC Univ Paris 06, CR7, Centre d'Immunologie et des Maladies Infectieuses, CIMI, team E13 (Bacteriology), Paris, France

<sup>10</sup> INSERM, U1135, Centre d'Immunologie et des Maladies Infectieuses, CIMI, Team E13, Paris, France

**Keywords:** antibiotic resistance, wild animals, selective pressure, integron

**Running title:** Lack of dissemination of antibiotic resistance in the wild

**Corresponding author:** N. Grall

Hôpital Bichat – Claude Bernard

Laboratoire de Bactériologie

46, rue Henri Huchard

75018 Paris, France

[nathalie.grall@bch.aphp.fr](mailto:nathalie.grall@bch.aphp.fr)

Tel: + 33 (0)1 40 25 85 07

Fax: + 33 (0)1 40 25 85 81

## Summary

In this study we quantitatively evaluated the spread of resistance to  $\beta$ -lactams and of integrons in small rodents and marsupials living at various distances from a point of antibiotic's use. Rectal swabs from 114 animals were collected in Trois-Sauts, an isolated village in French Guiana, and along a 3 km transect heading through the nonanthropized primary forest. Prevalence of ticarcillin resistant enterobacteria was 36% (41/114). *Klebsiella* spp., naturally resistant to ticarcillin, were found in 31.1% (23/73) of animals from the village and in an equal ratio of 31.7% (13/41) of animals trapped along the transect. By contrast *E. coli* with acquired resistance to ticarcillin were found in 13.7% (10/73) of animals from the village and in only 2.4% (1/41) of those from the transect (600 m from the village). There was a huge diversity of *E. coli* and *K. pneumoniae* strains with very unique and infrequent sequence types. The overall prevalence of class 1 integrons carriage was 19.3% (22/114) homogenously distributed between animals from the village and the transect, which suggests a co-selection by a non-antibiotic environmental factor. Our results indicate that the anthropogenic acquired antibiotic resistance did not disseminate in the wild far from the point of selective pressure.

## Introduction

Antibiotic resistance is a threat to public health worldwide. Its rapid growth combined with the lack of antibiotic innovation leads to increase of infections due to totally resistant bacteria (Boucher *et al.*, 2009). We know a great deal about the genetics and biochemistry of bacterial resistance to antibiotics but much less on the paths of dissemination of resistance genes in nature. Ancestral resistance genes have been present in environmental bacteria for very long period of time where their actual role is not fully understood (Bhullar *et al.*, 2012). They disseminated to human bacteria only very recently. This is believe to be because resistance confers bacteria a decisive advantage to confront the antibiotic residues that are often present in the environmental and commensal ecosystems as a consequence of the human use of antibiotic, not only in medicine but also in the food-chain industry (Marshall and Levy, 2011; Lupo *et al.*, 2012). In these ecosystems bacteria of various origins can mix and exchange resistance genes. Concerning enterobacteria which are intestinal commensal but also major potential pathogens, resistance levels are extremely alarming worldwide (Woerther *et al.*, 2013b) and the intestinal microbiota is considered to be the “epicenter” where bacteria of various origins can exchange resistance genes and disseminate (Carlet, 2012). For instance, the transfer of bacterial resistance from animals, particularly food-chain ones, to humans through the intestinal microbiota is extensively studied to assess the risk associated with animal use of antibiotics for human health (Levy *et al.*, 1976; Van den Bogaard *et al.*, 1997). The reverse transfer of resistance from humans to wild animals is considered a marker of the impact of human usage of antibiotics on the environment (Skurnik *et al.*, 2006).

Wild animals are not normally exposed to antimicrobials, but they might come in contact with resistant bacteria through direct and indirect interactions with humans and domestic animals (Marshall *et al.*, 1990). Several studies showed higher bacterial resistance rates in wild

animals living in close proximity to humans or agriculture compared to those living in more isolated areas (Rolland *et al.*, 1985; Cole *et al.*, 2005; Skurnik *et al.*, 2006; Kozak *et al.*, 2009; Allen *et al.*, 2011). The presence of integrons has also been used as a marker of the level of selection pressure (Kang *et al.*, 2005; Skurnik *et al.*, 2006; Stalder *et al.*, 2012). The level of bacterial resistance in the intestinal microbiota of wild animals seems to depend on the intensity of their direct contacts with human populations and secondary transmission seems rare (Sjolund *et al.*, 2008). However, controlled studies are lacking and it is unknown how far resistance can spread in the wild through animal transmission due to the selective pressure that follows human use of antibiotics. Here we took advantage that we were studying bacterial resistance in a population of Wayampi Amerindians living isolated in the village of Trois-Sauts in the midst of the Amazonian forest. Resulting from the care they receive in the village health post, they are exposed to well characterized quantities of antibiotics (Woerther *et al.*, 2010; Woerther *et al.*, 2013a). In order to gather data on the consecutive dissemination of resistance genes we trapped small wild rodents and marsupials at various distances from the village and analyzed their feces. We explored as a model the dissemination of  $\beta$ -lactam resistance because it was the most commonly used class of antibiotics in the village of Trois-Sauts (58% of all treatments) followed by metronidazole (21%) and macrolides (11%). Others antibiotics (ofloxacin, doxycycline, cotrimoxazole and nitroxoline) were anecdotal (Woerther *et al.*, 2010).  $\beta$ -lactam resistance was analyzed in *E. coli* on one hand and in *Klebsiella* spp. on the other, taking advantage that it is an acquired trait in *E. coli* whereas it is a natural trait in *Klebsiella* spp.

## Results and discussion

The sampling was performed during three campaigns (October 2006, June 2008, and October 2010) that we carried out in the village of Trois-Sauts (in the municipality of Camopi, French Guiana: 02°15' N, 52°52' W) where we were studying bacterial resistance in the microbiota of the villagers (Skurnik *et al.*, 2008; Ruimy *et al.*, 2010; Woerther *et al.*, 2010; Catzefflis, 2012; Lebeaux *et al.*, 2012; Angebault *et al.*, 2013; Lescat *et al.*, 2013; Woerther *et al.*, 2013a). The setting is rather unique because it combines the presence of an isolated and stable human population still living in a traditional manner in a completely wild forest environment. However, this population is exposed to antibiotics due to the presence in the village of a health post where a resident paramedic treats villagers with antibiotics when necessary. In all, we collected 114 small wild mammals (rodents and marsupials) known to have a limited perimeter of life of few hectares (Catzefflis F., personal communication) by setting traps in the village (73 animals) and every 300 m from the village in a standardized manner (Mauffrey *et al.*, 2007; Catzefflis, 2012) along a 3000m transect that we draw in the forest in non anthropized zones (41 animals) (Fig. 1 and Table S1). There were 100 rodents and 14 marsupials, speciated as described (Voss *et al.*, 2001; Wilson and Reeder, 2005; Weksler *et al.*, 2006; Voss and Jansa, 2009), including 38 *Nectomys rattus*, 22 *Proechimys cuvieri*, 13 *Oecomys bicolor*, 13 *Hylaeamys megacephalus*, 4 *Oecomys rutilus*, 4 *Proechimys guyanensis*, 2 *Euryoryzomys macconnelli*, 2 *Makalata didelphoides*, 1 *Neacomys paracou*, 1 *Neusticomys oyapocki*, 8 *Marmosa murina*, 2 *Philander opossum*, 1 *Marmosops pinheiroi*, 1 *Didelphis imperfecta*, 1 *Didelphis marsupialis* and 1 *Marmosa demerarae*. A rectal swab was performed on each captured animal before it was released after being marked to avoid sampling twice the same animal.

121 **Spatial distribution of  $\beta$ -lactam resistance in small wild mammal microbiota**

122 All samples analyzed contained viable enterobacteria. Ticarcillin resistant enterobacteria were  
123 detected in 41/114 (36%) samples. The resistant species were *Klebsiella* spp. only in 30/114  
124 (26.3%) samples, *E. coli* only in 5/114 (4.4%) samples and both in 6/114 (5.3%) samples.  
125 However, there were notable differences in the spatial distribution of the two species.

126 Naturally resistant *Klebsiella* spp. strains were found in 23/73 (31.5%) samples from animals  
127 trapped in the village and in a very close ratio of 13/41 (31.7%) from those from animals  
128 trapped elsewhere along the transect (Tables 1, and S1). Moreover, the percentages did not  
129 vary significantly with increasing distance from the village, being 2/14 (14.3%), 4/12 (33.3%)  
130 and 7/15 (46.7%) on the 1<sup>st</sup>, the 2<sup>nd</sup> and the 3<sup>rd</sup> kilometers of the transect respectively (Fig. 2).  
131 By contrast, *E. coli* isolates with acquired resistance to ticarcillin were found in 10/73 (13.7%)  
132 samples from animals trapped in the village, but in only 1/41 (2.4%) of those from elsewhere  
133 along the transect, at 600 m from the village. As a reminder, the rate of this type of resistance  
134 in the dominant *E. coli* from the villagers was 20.4% (Lescat *et al.*, 2013).

135 Our current results indicate that acquired resistance to  $\beta$ -lactams did not spread far from the  
136 point of selective pressure in the wild. This probably results from cost fitness of acquired  
137 antibiotic resistance in the absence of selective pressure. Several studies have already shown  
138 in a qualitative way that the level of antibiotic resistance in the microbiota of wild animals is  
139 higher in those living in close proximity to human's activity than in those without contact  
140 with humans (Rolland *et al.*, 1985; Cole *et al.*, 2005; Skurnik *et al.*, 2006; Kozak *et al.*, 2009;  
141 Allen *et al.*, 2011). However, to our knowledge, our study is the first to assess quantitatively  
142 that acquired resistance to  $\beta$ -lactam does not disseminate far in the wild in absence of  
143 selective pressure.

144 Resistance to third generation cephalosporins was not detected in any sample.

145

146 **Characteristics of *E. coli* and *Klebsiella* spp. strains**

147 A total of 16 ticarcillin resistant *E. coli* strains were isolated from the 11 ticarcillin resistant *E.*  
148 *coli* positive samples. A TEM-1 penicillinase conferring resistance to amoxicillin and  
149 ticarcillin was present in all strains. Five (31.2%) were susceptible to all the others antibiotics  
150 tested (Table 2) while 7 (43.7%), including the one from the 600 m point (Ec-600-1), were co-  
151 resistant to trimethoprim and sulfonamides. The 4 remaining strains had each a unique pattern  
152 of co-resistance, combining sulfonamides and/or trimethoprim and/or tetracycline and/or  
153 kanamycin. Nine strains belonged to the two phylogroups most often shared by commensal *E.*  
154 *coli*, i.e. groups A (4 strains) and B1 (5 strains) while 3 strains were from group C, 2 from  
155 group D and 2 from group E (Table 2). Of note, no B2 strain was found. A high genetic  
156 diversity was observed. Indeed, except for 2 sequence type (ST) 2690 strains that were  
157 indistinguishable by rep-PCR (Ec-0-5 and Ec-0-12 from the village), each strain had a distinct  
158 rep-pattern (Fig. 3 (a)). Strains Ec-0-1 and Ec-0-15, both isolated from animals trapped in the  
159 village, were from ST155 but had different rep-patterns and antibiotic susceptibilities. The 12  
160 other strains had each a unique ST and half of them had never been described. Furthermore,  
161 the *gyrB* allele of Ec-0-4 had not been reported before. In all, 42.8% (6/14) of the ST found in  
162 these animal strains had never been described, in accordance with a previous work in the  
163 same region (Lescat *et al.*, 2013). The mean virulence score in these 16 strains was low at 1.3  
164 (range 0 to 4), with mainly virulence determinants involved in iron capture. The most frequent  
165 virulence genes were *fyuA* and *irp2*, which belong to the high pathogenicity island (HPI)  
166 found in enterobacteria. A great diversity was also observed among plasmid carrying *bla*<sub>TEM-1</sub>.  
167 The most commonly occurring TEM-1 plasmid replicon was IncHI1 (5 strains), followed by  
168 IncX (4 strains), IncFII (3 strains), IncFIA (1 strain) and IncB/O (1 strain). The replicons  
169 could not be determined for 5/16 (31.2%) strains. All incompatibility groups detected had  
170 been previously described in human and animal enterobacteria strains. However, the



171 distribution was unusual: no IncFIB and few IncFII were found in favor of IncHI1 (Johnson *et*  
172 *al.*, 2007; Marcade *et al.*, 2009). The diversity of plasmid replicons could also be explained by  
173 the diversity and connectivity of gamma-proteobacteria in the wild forest environment,  
174 resulting in highly promiscuous exchange of mobile genetic elements. The huge genetic  
175 diversity among the 16 ticarcillin resistant *E. coli* strains and their plasmids suggests that the  
176 dissemination of ticarcillin resistance was more likely due to *bla*<sub>TEM-1</sub>, maybe through Tn3-type  
177 transposon (Marcade *et al.*, 2009), than bacterial strains or plasmids spread.

178 A total of 36 *Klebsiella* spp. strains were isolated, including 34 *K. pneumoniae* and 2 *K.*  
179 *oxytoca*. A high genetic diversity without clonal spread was also observed among the 34 *K.*  
180 *pneumoniae* strains (Fig. 3(b) and Table 3) with only one pair sharing the same rep-patterns  
181 and the same ST (Kp-2400-2 and Kp-2400-3 from 2 samples from the 2400 m point). Two  
182 others pairs shared the same ST but a different rep-pattern (Kp-0-22 and Kp-600-1; Kp-0-9  
183 and Kp-2400-5). Each of the 28 remaining strains had a distinct ST. Among the 31 unique  
184 STs found, 24 (77.4%) were described for the first time, with new alleles for 7 of them. All  
185 strains had the usual natural resistance phenotype of *Klebsiella* spp., *i.e.* resistance to  
186 amoxicillin and ticarcillin, with no co-resistance. All carried the *entB* siderophore gene and  
187 the *ycfM* adhesin gene, 27/34 (79.4%) the *kpn* adhesin gene, 24/34 (70.6%) the fimbriae  
188 *fimHI* gene and 26/34 (76.5%) the *kfuABC* gene (Table 3). There was no phenotypically  
189 hypermucoviscous strain and *magA* (specific for K1 serotype) or *rmpA* genes which are  
190 associated with this phenotype were not detected in any strain. Interestingly, 3 of these  
191 commensal strains from wild rodents (3 *Proechimys cuvieri*, 2 from the 2400 m point and one  
192 from the village) had the *allS* gene which had been strongly associated with strains isolated  
193 from liver abscess (Chou *et al.*, 2004). Furthermore, one strain had the *ybtS* gene (*Proechimys*  
194 *cuvieri* from the 2100 m point), which comes from the Ybt operons of the HPI cited above  
195 (Geoffroy *et al.*, 2000). Capsular serotype was determined for two strains, Kp-0-8 (K20) and

196 Kp-0-17 (K5) from two animals trapped in the village. No strain from capsular serotype K1,  
197 K2, K54 or K57 was found. Altogether this showed that the *E. coli* and *K. pneumoniae* strains  
198 from these wild animals were particular and unfrequently described. It also suggested that the  
199 known ST-types are those prevalent in developed countries and that the number of different  
200 niches allowing enterobacteria proliferation is much larger in this complex forest  
201 environment, explaining enterobacteria local specialization and diversification. However, all  
202 the *K. pneumoniae* strains had the *entB* siderophore gene, and frequently the *kpn* and *ycfM*  
203 adhesin genes, fimbriae *fimH1* and *kfuABC* genes, just as strains of human origin.

204

#### 205 **Detection of intestinal carriage of *bla*<sub>TEM</sub>**

206 We further investigated carriage by the animals of *bla*<sub>TEM</sub> gene by PCR screening of the  
207 global Drigalski culture from each sample. We found only 5 positive samples, all from  
208 animals trapped in the village and none from animals living in any other place on the transect  
209 (data not shown). Ticarcillin resistant *E. coli* strains had indeed been isolated in these 5  
210 samples. By contrast the *bla*<sub>TEM</sub> PCR was negative in the 6 other samples from animals in  
211 whom ticarcillin resistant *E. coli* strains had also been isolated, including the one trapped at  
212 600 m from the village. This suggests that PCR on global Drigalski cultures was less sensitive  
213 than further screening of these cultures on selective media and performing PCR on the  
214 resulting strains for detection of *bla*<sub>TEM</sub> genes.

215

#### 216 **Prevalence of class 1, 2 and 3 integrons and gene cassettes characterization**

217 We also investigated the carriage by the animals of class 1, 2 and 3 integrons by multiplex  
218 qPCR screening of the global Drigalski culture from each sample. Class 1 integrons were  
219 detected in 22/114 (19.3%) of the samples, with no significant difference between those from  
220 animals trapped in the village or along the transect (11/73 (15.1%) vs 11/41 (26.8%)) (Tables

221 1 and S1). There was no detectable spatial distribution along it either, the prevalence being of  
222 4/14 (28.6%), 2/12 (16.7%) 5/15 (33.3%) for the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> kilometers of the transect  
223 respectively (Fig. 4). Class 2 integrons, which were not further studied, were detected in 3  
224 samples only, coming from one animal trapped in the village, one at the 300 m point and one  
225 at the 2400 m point. No class 3 integron was detected. The homogenous distribution of class 1  
226 integrons between the village and the transect, independently of antibiotic selective pressure,  
227 is in appearance strikingly different from the heterogeneous distribution of acquired resistance  
228 described in previous studies (Cole *et al.*, 2005; Skurnik *et al.*, 2006), which found that  
229 integrons were only present when antibiotic selective pressure exceeds a certain threshold.  
230 The analysis of the gene cassettes in class 1 integrons showed little diversity with seven  
231 different gene cassette arrays (Table 4). The gene cassettes encoded resistance to  
232 trimethoprim (*dhfrA7*, *dhfrA15*, *dhfrA21*), spectinomycin and streptomycin (*aadA1*, *aadA2*,  
233 *aadA6*) or chloramphenicol (*cmlA4*). *aadA6* was the most frequently found gene cassette  
234 (6/22 (27.3%)). One sample (*Oecomys rutilus*) from the 2400 m trapping point had 2 different  
235 integrons, one with the *dhfrA21* gene cassette and one with the *aadA1* gene cassette. However,  
236 we were not able to characterize the gene cassette arrays for 8/22 (36%) class 1 integron-  
237 positive samples because we failed to obtain amplification products with the endpoint PCR  
238 with 5'CS and 3'CS primers. We can hypothesize that these integrons do not contain the 3'-  
239 conserved segment in which the 3' CS primer hybridizes, as described especially for class 1  
240 integrons detected in non-clinical strains (Gillings *et al.*, 2008). The genes cassette found in  
241 these integrons were coding for resistance towards antibiotics which were not used in the  
242 village. The presence of integrons carrying resistance gene cassettes in the intestinal  
243 microbiota of wild animals not exposed to antibiotic selective pressure has been observed  
244 before (Sunde, 2005; Goncalves *et al.*, 2013) but its significance has yet to be elucidated. All  
245 genes cassette detected had been previously described in human or animal commensal strains

246 (Kang *et al.*, 2005; Peirano *et al.*, 2006; Kadlec and Schwarz, 2008; Michael *et al.*, 2008). It is  
247 of note that the method used didn't allowed to characterize the gene cassette arrays for 8/22  
248 (36%) class 1 integron positive samples, thus we cannot rule out that other gene cassettes  
249 could have been present there.

250

### 251 **Detection of *merA***

252 We hypothesized that the stable prevalence of class 1 integrons in the village and along the  
253 transect could be due to a co-selection by mercury. Indeed, the *mer* operon encoding for  
254 mercury resistance has often been described as associated to class 1 integron within the Tn21-  
255 like transposon (Skurnik *et al.*, 2006; Skurnik *et al.*, 2010) and this region of French Guiana  
256 has been heavily exposed to mercury in the past, because of gold mining prospection  
257 (Laperche *et al.*, 2014).

258 This was tested by screening the global Drigalski culture from each sample and the 16  
259 tetracycline resistant *E. coli* strains for *merA* gene by PCR amplification as described (Deredjian  
260 *et al.*, 2011). However, we found only 1 sample positive, (from a *Makalata didelphoides*,  
261 which is an arboreal echimyid Caviomorpha, trapped in the village) and none of the strains  
262 were positive (data not shown). Thus, class 1 integrons might have been selected by a non-  
263 antibiotic environmental factor which remains to be determined. It could also reflect other  
264 opportunities in such a complex environment for spontaneous horizontal genetic transfer and  
265 random drift-based enrichment of transconjugants.

266

267 Certainly our study was limited by the relatively small number of samples available from the  
268 transect. This was in spite of considerable efforts of trapping (Table S2) whose yield was low.  
269 The effort or trapping was indeed 7 times higher along the transect than in the village (7900 vs  
270 1047 trap-nights) and provided around twice less animals. The very small density of small

271 rodents and marsupials in the forest was probably due to reduced food resources (Catzeflis F.,  
272 personal communication). Another limitation of our work was that we studied the resistance  
273 to only one class of antibiotic, the  $\beta$ -lactam. But resistance to  $\beta$ -lactam was chosen as a model  
274 because it was by far the most frequent antibiotics used in the village. Despite these  
275 limitations, our results suggest that acquired resistance genes do not diffuse when antibiotic  
276 selective pressure stops which is a strong incentive to control the use of antibiotics and all  
277 kind of release of these molecules in the environment.

278 **Acknowledgments/Funding**

279 We thank the villagers for their help and their warm welcome and Gilles Peroz for excellent  
280 technical assistance. We are very grateful to Sylvie Nazaret and Catherine Branger for their  
281 help and technical support on mercury resistance and plasmid characterization respectively.  
282 The ERAES project was supported in part by the Agence Française de Sécurité Sanitaire de  
283 l'Environnement et du Travail (contracts ES-05-01 and EST-09-21), the Agence Nationale  
284 pour la Recherche (contract 05-9-114), the Institut National de la Santé et de la Recherche  
285 Médicale (INSERM; contracts C06-18 and C10-19), the Centre National de Référence  
286 “Résistance bactérienne dans les flores commensales”, and the French government’s  
287 investissement d’Avenir program, Laboratoire d’Excellence “Integrative Biology of Emerging  
288 Infectious Diseases” (grant ANR-10-LABX-62-IBEID). This work was also supported in part  
289 by EU-FP7 projects EVOTAR and R-Gnosis

290

291 **Potential conflict of interest**

292 All authors: No reported conflicts.

293

294 This work has been presented at the 34<sup>th</sup> RICAI, 2014 Nov. 27-28, Paris.

295 **References**

- 296 **Allen, S.E., Boerlin, P., Janecko, N., Lumsden, J.S., Barker, I.K., Pearl, D.L., et al.**  
 297 (2011). Antimicrobial Resistance in Generic *Escherichia coli* Isolates from Wild Small  
 298 Mammals Living in Swine Farm, Residential, Landfill, and Natural Environments in Southern  
 299 Ontario, Canada. *Appl Environ Microbiol* 77: 882-888.
- 300 **Angebault, C., Djossou, F., Abelanet, S., Permal, E., Ben Soltana, M., Diancourt, L., et**  
 301 **al.** (2013). *Candida albicans* Is Not Always the Preferential Yeast Colonizing Humans: A  
 302 Study in Wayampi Amerindians. *J Infect Dis* 208: 1705-1716.
- 303 **Bachman, M.A., Oyler, J.E., Burns, S.H., Caza, M., Lepine, F., Dozois, C.M., and**  
 304 **Weiser, J.N.** (2011). *Klebsiella pneumoniae* Yersiniabactin Promotes Respiratory Tract  
 305 Infection through Evasion of Lipocalin 2. *Infect Immun* 79: 3309-3316.
- 306 **Bakour, R., Laroche, Y., and Cornelis, G.** (1983). Study of the Incompatibility and  
 307 Replication of the 70-Kb Virulence Plasmid of *Yersinia*. *Plasmid* 10: 279-289.
- 308 **Barraud, O., Baclet, M.C., Denis, F., and Ploy, M.C.** (2010). Quantitative Multiplex Real-  
 309 Time PCR for Detecting Class 1, 2 and 3 Integrons. *J Antimicrob Chemother* 65: 1642-1645.
- 310 **Bhullar, K., Waglehner, N., Pawlowski, A., Koteva, K., Banks, E.D., Johnston, M.D., et**  
 311 **al.** (2012). Antibiotic Resistance Is Prevalent in an Isolated Cave Microbiome. *PLoS One* 7:  
 312 e34953.
- 313 **Boucher, H.W., Talbot, G.H., Bradley, J.S., Edwards, J.E., Gilbert, D., Rice, L.B., et al.**  
 314 (2009). Bad Bugs, No Drugs: No Escape! An Update from the Infectious Diseases Society of  
 315 America. *Clin Infect Dis* 48: 1-12.
- 316 **Brisse, S., Fevre, C., Passet, V., Issenhuth-Jeanjean, S., Tournebize, R., Diancourt, L.,**  
 317 **and Grimont, P.** (2009). Virulent Clones of *Klebsiella pneumoniae*: Identification and  
 318 Evolutionary Scenario Based on Genomic and Phenotypic Characterization. *PLoS One* 4:  
 319 e4982.
- 320 **Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K.L., and Threlfall, E.J.** (2005).  
 321 Identification of Plasmids by PCR-Based Replicon Typing. *J Microbiol Methods* 63: 219-228.
- 322 **Carlet, J.** (2012). The Gut Is the Epicentre of Antibiotic Resistance. *Antimicrob Resist Infect*  
 323 *Control* 1: 39.
- 324 **Catzeflis, F.** (2012). A Survey of Small Non-Volant Mammals Inhabiting Wayampi  
 325 Amerindian Houses in French Guiana. *Mammalia* 76: 327-330.
- 326 **Chen, Y.T., Chang, H.Y., Lai, Y.C., Pan, C.C., Tsai, S.F., and Peng, H.L.** (2004).  
 327 Sequencing and Analysis of the Large Virulence Plasmid pLVPK of *Klebsiella pneumoniae*  
 328 CG43. *Gene* 337: 189-198.
- 329 **Chou, H.C., Lee, C.Z., Ma, L.C., Fang, C.T., Chang, S.C., and Wang, J.T.** (2004).  
 330 Isolation of a Chromosomal Region of *Klebsiella pneumoniae* Associated with Allantoin  
 331 Metabolism and Liver Infection. *Infect Immun* 72: 3783-3792.
- 332 **Clermont, O., Christenson, J.K., Denamur, E., and Gordon, D.M.** (2013). The Clermont  
 333 *Escherichia coli* Phylo-Typing Method Revisited: Improvement of Specificity and Detection  
 334 of New Phylo-Groups. *Environ Microbiol Rep* 5: 58-65.
- 335 **Cole, D., Drum, D.J., Stalknecht, D.E., White, D.G., Lee, M.D., Ayers, S., et al.** (2005).  
 336 Free-Living Canada Geese and Antimicrobial Resistance. *Emerg Infect Dis* 11: 935-938.
- 337 **Deredjian, A., Colinon, C., Brothier, E., Favre-Bonte, S., Cournoyer, B., and Nazaret, S.**  
 338 (2011). Antibiotic and Metal Resistance among Hospital and Outdoor Strains of  
 339 *Pseudomonas aeruginosa*. *Res Microbiol* 162: 689-700.
- 340 **Fang, C.T., Chuang, Y.P., Shun, C.T., Chang, S.C., and Wang, J.T.** (2004). A Novel  
 341 Virulence Gene in *Klebsiella pneumoniae* Strains Causing Primary Liver Abscess and Septic  
 342 Metastatic Complications. *J Exp Med* 199: 697-705.

343 **Geoffroy, V.A., Fetherston, J.D., and Perry, R.D.** (2000). *Yersinia pestis* Ybtu and Ybtt  
344 Are Involved in Synthesis of the Siderophore Yersiniabactin but Have Different Effects on  
345 Regulation. *Infect Immun* 68: 4452-4461.

346 **Gillings, M., Boucher, Y., Labbate, M., Holmes, A., Krishnan, S., Holley, M., and Stokes,  
347 H.W.** (2008). The Evolution of Class 1 Integrons and the Rise of Antibiotic Resistance. *J*  
348 *Bacteriol* 190: 5095-5100.

349 **Goncalves, A., Igrejas, G., Radhouani, H., Santos, T., Monteiro, R., Pacheco, R., et al.**  
350 (2013). Detection of Antibiotic Resistant *Enterococci* and *Escherichia coli* in Free Range  
351 Iberian Lynx (*Lynx pardinus*). *Sci Total Environ* 456-457: 115-119.

352 **Johnson, J.R., Clermont, O., Menard, M., Kuskowski, M.A., Picard, B., and Denamur,  
353 E.** (2006). Experimental Mouse Lethality of *Escherichia coli* Isolates, in Relation to  
354 Accessory Traits, Phylogenetic Group, and Ecological Source. *J Infect Dis* 194: 1141-1150.

355 **Johnson, T.J., Wannemuehler, Y.M., Johnson, S.J., Logue, C.M., White, D.G., Doetkott,  
356 C., and Nolan, L.K.** (2007). Plasmid Replicon Typing of Commensal and Pathogenic  
357 *Escherichia coli* Isolates. *Appl Environ Microbiol* 73: 1976-1983.

358 **Kadlec, K., and Schwarz, S.** (2008). Analysis and Distribution of Class 1 and Class 2  
359 Integrons and Associated Gene Cassettes among *Escherichia coli* Isolates from Swine,  
360 Horses, Cats and Dogs Collected in the BfT-GermVet Monitoring Study. *J Antimicrob*  
361 *Chemother* 62: 469-473.

362 **Kang, H.Y., Jeong, Y.S., Oh, J.Y., Tae, S.H., Choi, C.H., Moon, D.C., et al.** (2005).  
363 Characterization of Antimicrobial Resistance and Class 1 Integrons Found in *Escherichia coli*  
364 Isolates from Humans and Animals in Korea. *J Antimicrob Chemother* 55: 639-644.

365 **Kozak, G.K., Boerlin, P., Janecko, N., Reid-Smith, R.J., and Jardine, C.** (2009).  
366 Antimicrobial Resistance in *Escherichia coli* Isolates from Swine and Wild Small Mammals  
367 in the Proximity of Swine Farms and in Natural Environments in Ontario, Canada. *Appl*  
368 *Environ Microbiol* 75: 559-566.

369 **Laperche, V., Hellal, J., Maury-Brachet, R., Joseph, B., Laporte, P., Breeze, D., and  
370 Blanchard, F.** (2014). Regional Distribution of Mercury in Sediments of the Main Rivers of  
371 French Guiana (Amazonian Basin). *Springerplus* 3: 322.

372 **Lebeaux, D., Barbier, F., Angebault, C., Benmahdi, L., Ruppe, E., Felix, B., et al.** (2012).  
373 Evolution of Nasal Carriage of Methicillin-Resistant Coagulase-Negative *Staphylococci* in a  
374 Remote Population. *Antimicrob Agents Chemother* 56: 315-323.

375 **Lefort, A., Panhard, X., Clermont, O., Woerther, P.L., Branger, C., Mentre, F., et al.**  
376 (2011). Host Factors and Portal of Entry Outweigh Bacterial Determinants to Predict the  
377 Severity of *Escherichia coli* Bacteremia. *J Clin Microbiol* 49: 777-783.

378 **Lescat, M., Clermont, O., Woerther, P.L., Glodt, J., Dion, S., Skurnik, D., et al.** (2013).  
379 Commensal *Escherichia coli* Strains in Guiana Reveal a High Genetic Diversity with Host-  
380 Dependant Population Structure. *Environ Microbiol Rep* 5: 49-57.

381 **Levesque, C., Piche, L., Larose, C., and Roy, P.H.** (1995). PCR Mapping of Integrons  
382 Reveals Several Novel Combinations of Resistance Genes. *Antimicrob Agents Chemother* 39:  
383 185-191.

384 **Levy, S.B., FitzGerald, G.B., and Maccone, A.B.** (1976). Changes in Intestinal Flora of Farm  
385 Personnel after Introduction of a Tetracycline-Supplemented Feed on a Farm. *N Engl J Med*  
386 295: 583-588.

387 **Lupo, A., Coyne, S., and Berendonk, T.U.** (2012). Origin and Evolution of Antibiotic  
388 Resistance: The Common Mechanisms of Emergence and Spread in Water Bodies. *Front*  
389 *Microbiol* 3: 18.



390 **Marcade, G., Deschamps, C., Boyd, A., Gautier, V., Picard, B., Branger, C., et al.** (2009).  
391 Replicon Typing of Plasmids in *Escherichia coli* Producing Extended-Spectrum Beta-  
392 Lactamases. *J Antimicrob Chemother* 63: 67-71.

393 **Marshall, B., Petrowski, D., and Levy, S.B.** (1990). Inter- and Intraspecies Spread of  
394 *Escherichia coli* in a Farm Environment in the Absence of Antibiotic Usage. *Proc Natl Acad*  
395 *Sci U S A* 87: 6609-6613.

396 **Marshall, B.M., and Levy, S.B.** (2011). Food Animals and Antimicrobials: Impacts on  
397 Human Health. *Clin Microbiol Rev* 24: 718-733.

398 **Mauffrey, J.F., Steiner, C., and Catzefflis, F.** (2007). Small-Mammal Diversity and  
399 Abundance in a French Guianan Rain Forest: Test of Sampling Procedures Using Species  
400 Rarefaction Curves. *Journal of Tropical Ecology* 23: 419-425.

401 **Michael, G.B., Cardoso, M., and Schwarz, S.** (2008). Molecular Analysis of Multiresistant  
402 Porcine *Salmonella enterica* subsp. *enterica* Serovar Bredeney Isolates from Southern Brazil:  
403 Identification of Resistance Genes, Integrons and a Group II Intron. *Int J Antimicrob Agents*  
404 32: 120-129.

405 **Peirano, G., Agero, Y., Aarestrup, F.M., dos Reis, E.M., and dos Prazeres Rodrigues,**  
406 **D.** (2006). Occurrence of Integrons and Antimicrobial Resistance Genes among *Salmonella*  
407 *enterica* from Brazil. *J Antimicrob Chemother* 58: 305-309.

408 **Rolland, R.M., Hausfater, G., Marshall, B., and Levy, S.B.** (1985). Antibiotic-Resistant  
409 Bacteria in Wild Primates: Increased Prevalence in Baboons Feeding on Human Refuse. *Appl*  
410 *Environ Microbiol* 49: 791-794.

411 **Ruimy, R., Angebault, C., Djossou, F., Dupont, C., Epelboin, L., Jarraud, S., et al.**  
412 (2010). Are Host Genetics the Predominant Determinant of Persistent Nasal *Staphylococcus*  
413 *aureus* Carriage in Humans? *J Infect Dis* 202: 924-934.

414 **Saladin, M., Cao, V.T., Lambert, T., Donay, J.L., Herrmann, J.L., Ould-Hocine, Z., et**  
415 **al.** (2002). Diversity of CTX-M Beta-Lactamases and Their Promoter Regions from  
416 *Enterobacteriaceae* Isolated in Three Parisian Hospitals. *FEMS Microbiol Lett* 209: 161-168.

417 **Sjolund, M., Bonnedahl, J., Hernandez, J., Bengtsson, S., Cederbrant, G., Pinhassi, J., et**  
418 **al.** (2008). Dissemination of Multidrug-Resistant Bacteria into the Arctic. *Emerg Infect Dis*  
419 14: 70-72.

420 **Skurnik, D., Bonnet, D., Bernede-Bauduin, C., Michel, R., Guette, C., Becker, J.M., et al.**  
421 (2008). Characteristics of Human Intestinal *Escherichia coli* with Changing Environments.  
422 *Environ Microbiol* 10: 2132-2137.

423 **Skurnik, D., Ruimy, R., Andremont, A., Amorin, C., Rouquet, P., Picard, B., and**  
424 **Denamur, E.** (2006). Effect of Human Vicinity on Antimicrobial Resistance and Integrons in  
425 Animal Faecal *Escherichia coli*. *J Antimicrob Chemother* 57: 1215-1219.

426 **Skurnik, D., Ruimy, R., Ready, D., Ruppe, E., Bernede-Bauduin, C., Djossou, F., et al.**  
427 (2010). Is Exposure to Mercury a Driving Force for the Carriage of Antibiotic Resistance  
428 Genes? *J Med Microbiol* 59: 804-807.

429 **Stalder, T., Barraud, O., Casellas, M., Dagot, C., and Ploy, M.C.** (2012). Integron  
430 Involvement in Environmental Spread of Antibiotic Resistance. *Front Microbiol* 3: 119.

431 **Stalder, T., Barraud, O., Jove, T., Casellas, M., Gaschet, M., Dagot, C., and Ploy, M.C.**  
432 (2013). Quantitative and Qualitative Impact of Hospital Effluent on Dissemination of the  
433 Integron Pool. *ISME J* 8: 768-777.

434 **Sunde, M.** (2005). Class I Integron with a Group II Intron Detected in an *Escherichia coli*  
435 Strain from a Free-Range Reindeer. *Antimicrob Agents Chemother* 49: 2512-2514.

436 **Turton, J.F., Baklan, H., Siu, L.K., Kaufmann, M.E., and Pitt, T.L.** (2008). Evaluation of  
437 a Multiplex PCR for Detection of Serotypes K1, K2 and K5 in *Klebsiella* sp. And  
438 Comparison of Isolates within These Serotypes. *FEMS Microbiol Lett* 284: 247-252.

439 **Van den Bogaard, A.E., Jensen, L.B., and Stobberingh, E.E.** (1997). Vancomycin-  
440 Resistant *Enterococci* in Turkeys and Farmers. *N Engl J Med* 337: 1558-1559.

441 **Voss, R.S., and Jansa, S.A.** (2009). Phylogenetic Relationships and Classification of  
442 Didelphid Marsupials, an Extant Radiation of New World Metatherian Mammals, *Bull.*  
443 *American Museum Natural History* 322: 1-177.

444 **Voss, R.S., Lunde, D.P., and Simmons, B.** (2001). The Mammals of Paracou, French  
445 Guiana: A Neotropical Lowland Rainforest Fauna. Part 2: Nonvolant Species., *Bull.*  
446 *American Museum Natural History* 263: 1-236.

447 **Weksler, M., Percequillo, A.R., and Voss, R.S.** (2006). Ten New Genera of Oryzomyine  
448 Rodents (Cricetidae: Sigmodontinae). *American Museum Novitates* 3537: 1-29.

449 **Wilson, D.E., and Reeder, D.M.** (2005). *Mammal Species of the World. A Taxonomic and*  
450 *Geographic Reference.* The Johns Hopkins University Press, Baltimore: 1-2142.

451 **Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L.H., et al.** (2006). Sex and  
452 Virulence in *Escherichia coli*: An Evolutionary Perspective. *Mol Microbiol* 60: 1136-1151.

453 **Woerther, P.L., Angebault, C., Jacquier, H., Clermont, O., El Mniai, A., Moreau, B., et**  
454 **al.** (2013a). Characterization of Fecal ESBL-Producing *Escherichia coli* in a Remote  
455 Community During a Long Term Period. *Antimicrob Agents Chemother* 57: 5060-5066.

456 **Woerther, P.L., Angebault, C., Lescat, M., Ruppe, E., Skurnik, D., Mniai, A.E., et al.**  
457 (2010). Emergence and Dissemination of Extended-Spectrum Beta-Lactamase-Producing  
458 *Escherichia coli* in the Community: Lessons from the Study of a Remote and Controlled  
459 Population. *J Infect Dis* 202: 515-523.

460 **Woerther, P.L., Burdet, C., Chachaty, E., and Andremont, A.** (2013b). Trends in Human  
461 Fecal Carriage of Extended-Spectrum Beta-Lactamases in the Community: Toward the  
462 Globalization of CTX-M. *Clin Microbiol Rev* 26: 744-758.

463 **Yu, W.L., Ko, W.C., Cheng, K.C., Lee, C.C., Lai, C.C., and Chuang, Y.C.** (2008).  
464 Comparison of Prevalence of Virulence Factors for *Klebsiella pneumoniae* Liver Abscesses  
465 between Isolates with Capsular K1/K2 and Non-K1/K2 Serotypes. *Diagn Microbiol Infect Dis*  
466 62: 1-6.

467

468

469 **Tables**

470

471

Distance from the village (m)	Number of samples	Ticarcillin resistance <sup>a</sup>		Integrans <sup>b</sup>		
		Natural n (%)	Acquired n (%)	Class 1 n (%)	Class 2 n (%)	Class 3 n (%)
0	73	23 (31.5)	10 (13.7)	11 (15.1)	1 (1.4)	0 (0)
300	4	0 (0)	0 (0)	2 (50)	1 (25)	0 (0)
600	9	2 (22.2)	1 (11.1)	2 (22.2)	0 (0)	0 (0)
900	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1200	11	3 (27.3)	0 (0)	2 (18.2)	0 (0)	0 (0)
1500	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
2100	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
2400	11	6 (54.5)	0 (0)	5 (45.5)	1 (9.1)	0 (0)
2700	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
3000	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

472

473 **Table 1:** Frequencies of natural and acquired ticarcillin resistance and of class 1, 2 and 3  
 474 integrans in microbiota of rodents and marsupials trapped in the village of Trois-Sauts and at  
 475 specific distances from the village, i.e. the source of antibiotic selection pressure.

476 <sup>a</sup> Presence of ticarcillin resistant enterobacteria in the samples was screened by plating the  
 477 global culture on Drigalski agar plates containing ticarcillin (32 mg/L).

478 <sup>b</sup> The carriage of class 1, 2, and 3 integrans by animals was detected on total DNA extracted  
 479 from the global Drigalski culture by a multiplex Taqman-based qPCR with amplification of  
 480 *intI1*, *intI2*, and *intI3* genes, as described (Barraud *et al.*, 2010).

481



493 <sup>e</sup> T, transfer by electroporation; C, transfer by mating. The transferability of TEM-1 genes was assessed by ma  
494 (Bakour *et al.*, 1983). When mating failed, transformation into *E. coli* DH5 $\alpha$  (Invitrogen) was attempted by el  
495 DNA.

496 <sup>f</sup> Resistance genes *bla*<sub>TEM</sub> were amplified with specific primers, as described elsewhere (Saladin *et al.*, 2002),

497 <sup>g</sup> NT, Not Typed. Plasmid replicons from transconjugants and transformants were typed by PCR, as described

498

499

Strain identity	Resistance phenotype <sup>a</sup>	Virulence factors <sup>b</sup>										ST <sup>c</sup>
		Adhesins					Iron capture systems					
		<i>rmpA</i>	<i>allS</i>	<i>ycfM</i>	<i>mrKD</i>	<i>kpn</i>	<i>fimH1</i>	<i>entB</i>	<i>iroN</i>	<i>ybtS</i>	<i>kfuABC</i>	
Kp-0-1	AMX, TIC	-	-	+	+	+	+	+	-	-	-	New ST (2-3-1-1-7-1-1)
Kp-0-2	AMX, TIC	-	-	+	-	-	-	+	-	-	+	New ST (16-24-36-27-47-17-67)
Kp-0-3	AMX, TIC	-	-	+	-	-	+	+	-	-	+	New ST (18-23-25-96-79-20-51)
Kp-0-4	AMX, TIC	-	-	+	-	-	+	+	-	-	+	New ST (18-23-56-New allele-11-43-New allele)
Kp-0-5	AMX, TIC	-	-	+	-	+	+	+	-	-	+	New ST (16-24-21-27-54-22-105)
Kp-0-6	AMX, TIC	-	-	+	-	+	-	+	-	-	+	New ST (28-24-21-53-137-22-67)
Kp-0-7	AMX, TIC	-	-	+	-	+	-	+	-	-	-	682
Kp-0-8	AMX, TIC	-	-	+	-	+	-	+	-	-	-	1294
Kp-0-9	AMX, TIC	-	-	+	+	+	-	+	-	-	+	New ST (2-1-2-1-27-1-39)
Kp-0-10	AMX, TIC	-	-	+	-	+	-	+	-	-	+	New ST (16-24-43-27-47-17-New allele)
Kp-0-11	AMX, TIC	-	-	+	-	+	+	+	-	-	+	New ST (16-24-21-53-47-17-215)
Kp-0-12	AMX, TIC	-	-	+	-	-	-	+	-	-	+	New ST (16-24-21-106-68-59-188)
Kp-0-13	AMX, TIC	-	-	+	-	-	+	+	-	-	+	New ST (16-24-21-33-104-New allele-New allele)
Kp-0-14	AMX, TIC	-	-	+	-	+	+	+	-	-	+	New ST (18-22-18-22-New allele-13-50)
Kp-0-15	AMX, TIC	-	-	+	-	+	-	+	-	-	-	999
Kp-0-16	AMX, TIC	-	-	+	+	+	+	+	-	-	-	New ST (4-4-1-1-6-2-12)
Kp-0-17	AMX, TIC	-	-	+	+	+	-	+	-	-	+	New ST (2-1-5-3-4-4-8)
Kp-0-18	AMX, TIC	-	+	+	-	+	+	+	-	-	+	New ST (16-24-New allele-33-76-33-67)
Kp-0-19	AMX, TIC	-	-	+	-	+	+	+	-	-	+	1208
Kp-0-20	AMX, TIC	-	-	+	-	+	+	+	-	-	+	New ST (28-24-21-33-68-New allele-225)
Kp-0-21	AMX, TIC	-	-	+	+	+	+	+	-	-	-	New ST (7-1-1-1-12-1-123)
Kp-0-22	AMX, TIC	-	-	+	-	+	-	+	-	-	-	442
Kp-600-1	AMX, TIC	-	-	+	-	+	-	+	-	-	-	442
Kp-600-2	AMX, TIC	-	-	+	-	+	-	+	-	-	-	197
Kp-1200-1	AMX, TIC	-	-	+	-	+	+	+	-	-	+	New ST (16-18-43-27-47-93-67)
Kp-1200-2	AMX, TIC	-	-	+	-	+	+	+	-	-	+	New ST (16-18-21-33-50-22-67)
Kp-1200-3	AMX, TIC	-	-	+	-	+	+	+	-	-	+	New ST (18-22-18-16-25-13-165)
Kp-2100-1	AMX, TIC	-	-	+	-	+	+	+	-	+	+	New ST (16-24-36-27-47-22-67)
Kp-2400-1	AMX, TIC	-	-	+	-	+	-	+	-	-	-	347
Kp-2400-2	AMX, TIC	-	+	+	-	-	+	+	-	-	+	New ST (18-22-26-63-115-13-New allele)
Kp-2400-3	AMX, TIC	-	+	+	-	+	+	+	-	-	+	New ST (18-22-26-63-115-13-New allele)
Kp-2400-4	AMX, TIC	-	-	+	-	+	-	+	-	-	+	New ST (16-18-43-27-47-93-75)
Kp-2400-5	AMX, TIC	-	-	+	+	+	+	+	-	-	+	New ST (2-1-2-1-27-1-39)
Kp-2400-6	AMX, TIC	-	-	+	+	+	-	+	-	-	-	New ST (2-20-1-1-4-4-4)

500

501 **Table 3:** Resistance phenotype, virulence factors and sequence type (ST) of *K. pneumoniae*  
502 strains.

503 <sup>a</sup> The susceptibility of the following antibiotics were tested: amoxicillin (AMX), ticarcilline  
504 (TIC), amoxicillin + clavulanate, cefotaxime, ceftazidime, cefepime, ceftoxitin, ertapenem,  
505 gentamicin, amikacin, kanamycin, nalidixic acid, ofloxacin, trimethoprim, sulfonamide and  
506 tetracycline

507 <sup>b</sup> *K. pneumoniae* capsular serotypes K1 (*magA*), K2, K5, K20, K54 and K57 were determined  
508 using multiplex PCR, as described (Turton *et al.*, 2008). Virulence genes were searched by  
509 PCR. Primers used are reported in Table S3. Two reference strains of capsular serotypes K1  
510 (NTUH K2044) and K2 (CG43) were used as controls (Chen *et al.*, 2004; Fang *et al.*, 2004).

511 <sup>c</sup> Multilocus sequence typing (MLST) was performed using the international MLST scheme  
512 of the Institut Pasteur, Paris, France (<http://www.pasteur.fr/mlst>)

513

514  
515

Cassette array	Number of samples					Total
	0 (Village)	300 m	600 m	1200 m	2400 m	
<i>dfrA15-cmlA4-aadA2</i>	2 - - - -					2
<i>dfrA21-aadA2</i>	1 - - - -					1
<i>aadA1</i>	- - - -	1				1
<i>aadA2</i>	2 - - - -					2
<i>aadA6</i>	1 -	1	1	3	6	6
<i>dfrA7</i>	1 - - - -					1
<i>dfrA21</i>	1 - - -		1	2		2
ND	3	2	1	1	1	8

516

517 Table 4: Gene cassettes arrays of class 1 integrons in microbiota of rodents and marsupials  
518 trapped in Trois-Sauts and at specific distances from the village.

519 ND: Not Determined

520 For the construction of gene cassette array libraries, primers 5'CS and 3'CS at 0.5 µM  
521 (Levesque *et al.*, 1995) were used to amplify and sequence the variable GC-containing region  
522 of class 1 integrons directly from the extracted DNA (5 µL) as described (Stalder *et al.*,  
523 2013). At least 20 clones were used for sequencing.

524

525

526

527 **Figure legends**

528

529

530 Figure 1: Study site: Trois-Sauts (in the municipality of Camopi, French Guiana: 02°15' N,  
531 52°52' W).

532 Red numbers represent the number of samples per trapping point (in the village and at specific  
533 distances from the village).

534 Rectal swabs of trapped animal were inoculated extemporaneously onto Drigalski agar slants  
535 in screw-cup tubes and sent to France at room temperature. There, the global Drigalski culture  
536 from each tube was suspended in 1.5 mL of brain-heart infusion (BHI) broth with 10%  
537 glycerol and stored at -80°C.

538

539

540 Figure 2: Percentage of samples bearing natural (*Klebsiella* spp.) or acquired (*E. coli*)  
541 ticarcillin resistance according to the distance from the village, i.e. the source of antibiotic  
542 selection pressure.

543

544

545 Figure 3: Dendrogram and rep-PCR fingerprints of *E. coli* (a) and *K. pneumoniae* (b) strains.  
546 Strains were named by their initials (Ec for *E. coli* and Kp for *K. pneumoniae*), followed by  
547 the distance from the village of the isolation site, followed by the order number. The genetic  
548 relatedness was analyzed by rep-PCR DNA fingerprinting with the DiversiLab system  
549 (bioMérieux, Marcy l'Etoile, France) as in Woerther *et al.*, 2013a.

550

551

552 Figure 4: Percentage of samples bearing class 1 or class 2 integrons according to the distance  
553 from the village, i.e. the source of antibiotic selection pressure.

554

555

556



557 **Supporting Information**

558

559

Sample	Distance from the village (m)	Animal	Rodent / Marsupial	Ticarcillin resistant <i>E. coli</i>	<i>Klebsiella</i> spp.	Class 1 integron	Class 2 integron
1	0	<i>Nectomys rattus</i>	Rodent	Ec-0-1 No		--	
2	0	<i>Nectomys rattus</i>	Rodent	No Kp-0-1		--	
3	0	<i>Nectomys rattus</i>	Rodent	No Kp-0-2		--	
4	0	<i>Nectomys rattus</i>	Rodent	No No		--	
5	0	<i>Philander opossum</i>	Marsupial	No No		--	
6	0	<i>Makalata didelphoides</i>	Rodent	No Kp-0-3		--	
7	0	<i>Nectomys rattus</i>	Rodent	No No		--	
8	0	<i>Makalata didelphoides</i>	Rodent	No Kp-0-4		--	
9	0	<i>Marmosa murina</i>	Marsupial	No Kp-0-5		--	
10	0	<i>Nectomys rattus</i>	Rodent	No No		--	
11	0	<i>Nectomys rattus</i>	Rodent	No No		--	
12	0	<i>Nectomys rattus</i>	Rodent	No No		--	
13	0	<i>Marmosa murina</i>	Marsupial	No No		--	
14	0	<i>Oecomys bicolor</i>	Rodent	No Kp-0-6		+-	
15	0	<i>Hylaeamys megacephalus</i>	Rodent	No No		--	
16	0	<i>Nectomys rattus</i>	Rodent	Ec-0-2 Kp-0-7		--	
17	0	<i>Marmosa murina</i>	Marsupial	No No		+-	
18	0	<i>Nectomys rattus</i>	Rodent No No			--	
19	0	<i>Proechimys cuvieri</i>	Rodent No No			--	
20	0	<i>Oecomys bicolor</i>	Rodent No No			--	
21	0	<i>Nectomys rattus</i>	Rodent No No			--	
22	0	<i>Nectomys rattus</i>	Rodent No No			--	
23	0	<i>Nectomys rattus</i>	Rodent Ec-0-3 No			--	
24	0	<i>Nectomys rattus</i>	Rodent No No			--	
25	0	<i>Oecomys bicolor</i>	Rodent No No			--	
26	0	<i>Oecomys bicolor</i>	Rodent No No			--	
27	0	<i>Oecomys bicolor</i>	Rodent No No			--	
28	0	<i>Hylaeamys megacephalus</i>	Rodent No No			-+	
29	0	<i>Nectomys rattus</i>	Rodent No No			--	
30	0	<i>Nectomys rattus</i>	Rodent Ec-0-4, Ec-0-5 Kp-0-8			+-	
31	0	<i>Oecomys bicolor</i>	Rodent No <i>K. oxytoca</i>			+-	
32	0	<i>Oecomys bicolor</i>	Rodent Ec-0-6, Ec-0-7 Kp-0-9			+-	
33	0	<i>Oecomys bicolor</i>	Rodent No No			--	
34	0	<i>Nectomys rattus</i>	Rodent No No			--	
35	0	<i>Neusticomys oyapocki</i>	Rodent No Kp-0-10			--	
36	0	<i>Nectomys rattus</i>	Rodent No No			--	
37	0	<i>Nectomys rattus</i>	Rodent No Kp-0-11			--	
38	0	<i>Proechimys cuvieri</i>	Rodent No No			--	
39	0	<i>Nectomys rattus</i>	Rodent Ec-0-8 No			--	
40	0	<i>Nectomys rattus</i>	Rodent No No			+-	
41	0	<i>Nectomys rattus</i>	Rodent No No			--	
42	0	<i>Nectomys rattus</i>	Rodent No Kp-0-12			--	
43	0	<i>Nectomys rattus</i>	Rodent No Kp-0-13			--	
44	0	<i>Nectomys rattus</i>	Rodent No Kp-0-14			--	
45	0	<i>Nectomys rattus</i>	Rodent No No			--	
46	0	<i>Nectomys rattus</i>	Rodent No No			--	
47	0	<i>Nectomys rattus</i>	Rodent No No			--	
48	0	<i>Marmosa murina</i>	Marsupial No Kp-0-15			+-	

49 0		<i>Nectomys rattus</i>	Rodent	No	No	--
50 0		<i>Marmosa murina</i>	Marsupial	No	Kp-0-16	+-
51 0		<i>Hylaeamys megacephalus</i>	Rodent	No	Kp-0-17	--
52 0		<i>Nectomys rattus</i>	Rodent	Ec-0-9	No	--
53 0		<i>Hylaeamys megacephalus</i>	Rodent	No	No	--
54 0		<i>Nectomys rattus</i>	Rodent	No	No	--
55 0		<i>Proechimys cuvieri</i>	Rodent	No	Kp-0-18	--
56 0		<i>Nectomys rattus</i>	Rodent	No	No	--
57 0		<i>Oecomys bicolor</i>	Rodent	No	No	--
58 0		<i>Philander opossum</i>	Marsupial	Ec-0-10	Kp-0-19	--
59 0		<i>Hylaeamys megacephalus</i>	Rodent	No	No	--
60 0		<i>Nectomys rattus</i>	Rodent	No	No	--
61 0		<i>Marmosa murina</i>	Marsupial	No	No	--
62 0		<i>Oecomys bicolor</i>	Rodent	No	No	+-
63 0		<i>Oecomys bicolor</i>	Rodent	No	No	--
64 0		<i>Oecomys bicolor</i>	Rodent	No	No	--
65 0		<i>Nectomys rattus</i>	Rodent	No	No	--
66 0		<i>Hylaeamys megacephalus</i>	Rodent	No	No	--
67 0		<i>Nectomys rattus</i>	Rodent	No	No	--
68 0		<i>Oecomys bicolor</i>	Rodent	No	No	--
69 0		<i>Oecomys bicolor</i>	Rodent	No	No	--
70 0		<i>Oecomys bicolor</i>	Rodent	No	Kp-0-20	--
71 0		<i>Nectomys rattus</i>	Rodent	No	No	--
72 0		<i>Nectomys rattus</i>	Rodent	Ec-0-11, Ec-0-12	Kp-0-21	+-
73 0		<i>Marmosa murina</i>	Marsupial	Ec-0-13, Ec-0-14, Ec-0-15	Kp-0-22	+-
74	300	<i>Marmosa murina</i>	Marsupial	No	No	--
75	300	<i>Nectomys rattus</i>	Rodent	No	No	--
76	300	<i>Proechimys cuvieri</i>	Rodent	No	No	+-
77	300	<i>Oecomys rutilus</i>	Rodent	No	No	++
78	600	<i>Didelphis marsupialis</i>	Marsupial	Ec-600-1	No	+-
79	600	<i>Hylaeamys megacephalus</i>	Rodent	No	Kp-600-1	--
80	600	<i>Neacomys paracou</i>	Rodent	No	No	--
81	600	<i>Hylaeamys megacephalus</i>	Rodent	No	No	+-
82	600	<i>Proechimys cuvieri</i>	Rodent	No	No	--
83	600	<i>Proechimys cuvieri</i>	Rodent	No	No	--
84	600	<i>Hylaeamys megacephalus</i>	Rodent	No	No	--
85	600	<i>Proechimys cuvieri</i>	Rodent	No	Kp-600-2	--
86	600	<i>Hylaeamys megacephalus</i>	Rodent	No	No	--
87	900	<i>Proechimys cuvieri</i>	Rodent	No	No	--
88	1200	<i>Proechimys cuvieri</i>	Rodent	No	Kp-1200-1	--
89	1200	<i>Proechimys cuvieri</i>	Rodent	No	No	--
90	1200	<i>Hylaeamys megacephalus</i>	Rodent	No	No	+-
91	1200	<i>Proechimys cuvieri</i>	Rodent	No	No	--
92	1200	<i>Proechimys cuvieri</i>	Rodent	No	No	--
93	1200	<i>Proechimys guyannensis</i>	Rodent	No	Kp-1200-2	--
94	1200	<i>Proechimys cuvieri</i>	Rodent	No	No	--
95	1200	<i>Euryoryzomys macconnelli</i>	Rodent	No	No	--
96	1200	<i>Hylaeamys megacephalus</i>	Rodent	No	Kp-1200-3	+-
97	1200	<i>Didelphis imperfecta</i>	Marsupial	No	No	--
98	1200	<i>Proechimys cuvieri</i>	Rodent	No	No	--
99	1500	<i>Proechimys cuvieri</i>	Rodent	No	<i>K. oxytoca</i>	--
100	2100	<i>Proechimys cuvieri</i>	Rodent	No	Kp-2100-1	--

101	2400	<i>Marmosa demerarae</i>	Marsupial	No	No	+ -
102	2400	<i>Oecomys rutilus</i>	Rodent	No	No	+ +
103	2400	<i>Proechimys cuvieri</i>	Rodent	No	No	- -
104	2400	<i>Proechimys cuvieri</i>	Rodent	No	Kp-2400-1	- -
105	2400	<i>Proechimys cuvieri</i>	Rodent	No	Kp-2400-2	+ -
106	2400	<i>Proechimys cuvieri</i>	Rodent	No	Kp-2400-3	+ -
107	2400	<i>Proechimys cuvieri</i>	Rodent	No	Kp-2400-4	+ -
108	2400	<i>Proechimys guyannensis</i>	Rodent	No	No	- -
109	2400	<i>Proechimys cuvieri</i>	Rodent	No	Kp-2400-5	- -
110	2400	<i>Marmosops pinheiroi</i>	Marsupial	No	Kp-2400-6	- -
111	2400	<i>Hylaeamys megacephalus</i>	Rodent	No	No	- -
112	2700	<i>Proechimys guyannensis</i>	Rodent		No No	- -
113	3000	<i>Proechimys cuvieri</i>	Rodent	No	No	- -
114	3000	<i>Euryoryzomys macconnelli</i>	Rodent	No	No	- -

560

561 Table S1: Animals trapped in the village and along the transect, with the distance of the trap  
562 from the village and the carriage of tetracycline resistant *E. coli* or *Klebsiella* spp. strains and  
563 class 1 and 2 integrons in the rectal swab samples.

564

565

Distance from the village (m)	Trap-nights (n)
0	1047
300	786
600	1341
900	241
1200	1341
1500	241
1800	786
2100	241
2400	1341
2700	241
3000	1341

566 Table S2: Sampling effort (trap-nights) over the three campaigns (October 2006, June 2008,  
567 and October 2010) according to the distance from the village.

568  
569

Name DNA sequence	Target site	Amplicon size (pb)	Reference
ycfM-F 5'-CGATTGAGCATCAGGATCAG-3' <i>ycfM</i> ycfM-R 5'-GTTGGTGCGGTTGTTAC-3'		161	This study
mrkD-1 5'-TAT(T/C)G(G/T)CTTAATGGCGCTGG-3' <i>mrkD</i> mrkD-2 5'-TAATCGTACGTCAGGTAAAGA(C/T)C-3'		920	Brisse <i>et al.</i> (Brisse <i>et al.</i> , 2009)
entB-F 5'-CGCCCAGCCGAAAGAGCAGA-3' entB-R 5'-CATCGGCACCGAATCCAGAC-3'	<i>entB</i>	508	This study
ybtS-F 5'-CAAAAATGGGCGGTGGATTC-3' <i>ybtS</i> ybtS-R 5'-CCTGACGGAACATAAACGAGCG-3'			Bachman <i>et al.</i> (Bachman <i>et al.</i> , 2011)
kfu-F 5'-ATAGTAGGCGAGCACCGAGA-3' <i>kfu</i> kfu-R 5'-AGAACCTTCCTCGCTGAACA-3'		520	Yu <i>et al.</i> (Yu <i>et al.</i> , 2008)
iroN-F 5'-GCATAGGCGGATACGAACAT-3' <i>iroN</i> iroN-R 5'-CACAGGGCAATTGCTTACCT-3'		556	Yu <i>et al.</i> (Yu <i>et al.</i> , 2008)
magA-F 5'-GGTGCTCTTTACATCATTGC-3' <i>magA</i> magA-R 5'-GCAATGGCCATTTGCGTTAG-3'		1280	Fang <i>et al.</i> (Fang <i>et al.</i> , 2004)
1416R 5'-CCGTTAGGCAATCCAGAC-3' <i>allS</i> 336F2 5'TCTGATTTA(A/T)CCCACATT-3'		1090	Chou <i>et al.</i> (Chou <i>et al.</i> , 2004)
rmpA-F 5'-ACTGGGCTACCTCTGCTTCA-3' <i>rmpA</i> rmpA-R 5'-CTTGCATGAGCCATCTTTCA-3'		535	Brisse <i>et al.</i> (Brisse <i>et al.</i> , 2009)

570  
571  
572  
573  
574  
575

Table S3: Primers used for characterization of *K. pneumoniae* virulence factors