Lack of dissemination of acquired resistance to β-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.

Lack of dissemination of acquired resistance to β-lactam in small wild mammals around an isolated village in the Amazonian forest

Nathalie Grall [1,2,3], Olivier Barraud [4,5,6], Ingrid Wieder [3], Anna Hua [3], Marion Perrier [3], Ana Babosan[7], Margaux Gaschet [4], Olivier Clermont [1,2], Erick Denamur [1,2], François Catzeflis [8], Dominique Decré[7,9,10], Marie-Cécile Ploy[4,5,6], Antoine Andremont[1,2,3]

[1] INSERM, IAME, UMR 1137, F-75018 Paris, France

[2] Univ Paris Diderot, IAME, UMR 1137, Sorbonne Paris Cité, F-75018 Paris, France

[3] AP-HP, Hôpital Bichat, Laboratoire de Microbiologie, F-75018 Paris, France

[4] CHU Limoges, Laboratoire de Bactériologie-Virologie-Hygiène, Limoges, France

[5] INSERM U1092, Limoges, France

[6] Univ Limoges, UMR-S1092, Limoges, France

[7] AP-HP, Hôpital Saint-Antoine, Laboratoire de Bactériologie-Hygiène, F-75012 Paris, France

[8] CNRS UMR-5554, Institut des Sciences de l'Evolution, Univ Montpellier-2, Montpellier, France

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

[10] 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
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 β-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 β-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). β-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; Catzeflis, 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 forestall 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 (Catzeflis 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; Catzeflis, 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.

Spatial distribution of β-lactam resistance in small wild mammal microbiota

All samples analyzed contained viable enterobacteria. Ticarcillin resistant enterobacteria were detected in 41/114 (36%) samples. The resistant species were *Klebsiella* spp. only in 30/114 (26.3%) samples, *E. coli* only in 5/114 (4.4%) samples and both in 6/114 (5.3%) samples. However, there were notable differences in the spatial distribution of the two species. Naturally resistant *Klebsiella* spp. strains were found in 23/73 (31.5%) samples from animals trapped in the village and in a very close ratio of 13/41 (31.7%) from those from animals trapped elsewhere along the transect (Tables 1, and S1). Moreover, the percentages did not vary significantly with increasing distance from the village, being 2/14 (14.3%), 4/12 (33.3%) and 7/15 (46.7%) on the 1st, the 2nd and the 3rd kilometers of the transect respectively (Fig. 2). By contrast, *E. coli* isolates with acquired resistance to ticarcillin were found in 10/73 (13.7%) samples from animals trapped in the village, but in only 1/41 (2.4%) of those from elsewhere along the transect, at 600 m from the village. As a reminder, the rate of this type of resistance in the dominant *E. coli* from the villagers was 20.4% (Lescat *et al.*, 2013).

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

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

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 $bla_{\text{TEM-1}}$.
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 $bla_{\text{TEM-1}}$, maybe trough Tn*3*-type
177 transpon (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 *fimH1* gene and 26/34 (76.5%) the *kfuABC* gene (Table 3). There was no phenotypically
189 hypermucoviscious 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*$_{\mathbf{TEM}}$**
206 We further investigated carriage by the animals of *bla* $_{\text{TEM}}$ 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* $_{\text{TEM}}$ 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*$_{\text{TEM}}$ 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 class1, 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

1 and S1). There was no detectable spatial distribution along it either, the prevalence being of 4/14 (28.6%), 2/12 (16.7%) 5/15 (33.3%) for the 1 st, 2 nd and 3 rd kilometers of the transect respectively (Fig. 4). Class 2 integrons, which were not further studied, were detected in 3 samples only, coming from one animal trapped in the village, one at the 300 m point and one at the 2400 m point. No class 3 integron was detected. The homogenous distribution of class 1 integrons between the village and the transect, independently of antibiotic selective pressure, is in appearance strikingly different from the heterogeneous distribution of acquired resistance described in previous studies (Cole *et al.*, 2005; Skurnik *et al.*, 2006), which found that integrons were only present when antibiotic selective pressure exceeds a certain threshold. The analysis of the gene cassettes in class 1 integrons showed little diversity with seven different gene cassette arrays (Table 4). The gene cassettes encoded resistance to trimethoprim (*drfA7*, *dfrA15*, *dfrA21*), spectinomycin and streptomycin (*aadA1*, *aadA2*, *aadA6*) or chloramphenicol (*cmlA4*). *aadA6* was the most frequently found gene cassette (6/22 (27.3%)). One sample (*Oecomys rutilus*) from the 2400 m trapping point had 2 different integrons, one with the *dfrA21* gene cassette and one with the *aadA1* gene cassette. However, we were not able to characterize the gene cassette arrays for 8/22 (36%) class 1 integron-positive samples because we failed to obtain amplification products with the endpoint PCR with 5'CS and 3'CS primers. We can hypothesize that these integrons do not contain the 3'-conserved segment in which the 3' CS primer hybridizes, as described especially for class 1 integrons detected in non-clinical strains (Gillings *et al.*, 2008). The genes cassette found in these integrons were coding for resistance towards antibiotics which were not used in the village. The presence of integrons carrying resistance gene cassettes in the intestinal microbiota of wild animals not exposed to antibiotic selective pressure has been observed before (Sunde, 2005; Goncalves *et al.*, 2013) but its significance has yet to be elucidated. All 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 Tn*21*-
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 ticarcillin 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 β-lactam. But resistance to β-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.

Acknowledgments/Funding

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

Potential conflict of interest

All authors: No reported conflicts.

This work has been presented at the 34th RICAI, 2014 Nov. 27-28, Paris.

References

Allen, S.E., Boerlin, P., Janecko, N., Lumsden, J.S., Barker, I.K., Pearl, D.L., *et al.* (2011). Antimicrobial Resistance in Generic *Escherichia coli* Isolates from Wild Small Mammals Living in Swine Farm, Residential, Landfill, and Natural Environments in Southern Ontario, Canada. Appl Environ Microbiol 77: 882-888.

Angebault, C., Djossou, F., Abelanet, S., Permal, E., Ben Soltana, M., Diancourt, L., *et al.* (2013). *Candida albicans* Is Not Always the Preferential Yeast Colonizing Humans: A Study in Wayampi Amerindians. J Infect Dis 208: 1705-1716.

Bachman, M.A., Oyler, J.E., Burns, S.H., Caza, M., Lepine, F., Dozois, C.M., and Weiser, J.N. (2011). *Klebsiella pneumoniae* Yersiniabactin Promotes Respiratory Tract Infection through Evasion of Lipocalin 2. Infect Immun 79: 3309-3316.

Bakour, R., Laroche, Y., and Cornelis, G. (1983). Study of the Incompatibility and Replication of the 70-Kb Virulence Plasmid of *Yersinia*. Plasmid 10: 279-289.

Barraud, O., Baclet, M.C., Denis, F., and Ploy, M.C. (2010). Quantitative Multiplex Real-Time PCR for Detecting Class 1, 2 and 3 Integrons. J Antimicrob Chemother 65: 1642-1645.

Bhullar, K., Waglechner, N., Pawlowski, A., Koteva, K., Banks, E.D., Johnston, M.D., *et al.* (2012). Antibiotic Resistance Is Prevalent in an Isolated Cave Microbiome. PLoS One 7: e34953.

Boucher, H.W., Talbot, G.H., Bradley, J.S., Edwards, J.E., Gilbert, D., Rice, L.B., *et al.* (2009). Bad Bugs, No Drugs: No Eskape! An Update from the Infectious Diseases Society of America. Clin Infect Dis 48: 1-12.

Brisse, S., Fevre, C., Passet, V., Issenhuth-Jeanjean, S., Tournebize, R., Diancourt, L., and Grimont, P. (2009). Virulent Clones of *Klebsiella pneumoniae*: Identification and Evolutionary Scenario Based on Genomic and Phenotypic Characterization. PLoS One 4: e4982.

Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K.L., and Threlfall, E.J. (2005). Identification of Plasmids by PCR-Based Replicon Typing. J Microbiol Methods 63: 219-228.

Carlet, J. (2012). The Gut Is the Epicentre of Antibiotic Resistance. Antimicrob Resist Infect Control 1: 39.

Catzeflis, F. (2012). A Survey of Small Non-Volant Mammals Inhabiting Wayampi Amerindian Houses in French Guiana. Mammalia 76: 327-330.

Chen, Y.T., Chang, H.Y., Lai, Y.C., Pan, C.C., Tsai, S.F., and Peng, H.L. (2004). Sequencing and Analysis of the Large Virulence Plasmid pLVPK of *Klebsiella pneumoniae* CG43. Gene 337: 189-198.

Chou, H.C., Lee, C.Z., Ma, L.C., Fang, C.T., Chang, S.C., and Wang, J.T. (2004). Isolation of a Chromosomal Region of *Klebsiella pneumoniae* Associated with Allantoin Metabolism and Liver Infection. Infect Immun 72: 3783-3792.

Clermont, O., Christenson, J.K., Denamur, E., and Gordon, D.M. (2013). The Clermont *Escherichia coli* Phylo-Typing Method Revisited: Improvement of Specificity and Detection of New Phylo-Groups. Environ Microbiol Rep 5: 58-65.

Cole, D., Drum, D.J., Stalknecht, D.E., White, D.G., Lee, M.D., Ayers, S., *et al.* (2005). Free-Living Canada Geese and Antimicrobial Resistance. Emerg Infect Dis 11: 935-938.

Deredjian, A., Colinon, C., Brothier, E., Favre-Bonte, S., Cournoyer, B., and Nazaret, S. (2011). Antibiotic and Metal Resistance among Hospital and Outdoor Strains of *Pseudomonas aeruginosa*. Res Microbiol 162: 689-700.

Fang, C.T., Chuang, Y.P., Shun, C.T., Chang, S.C., and Wang, J.T. (2004). A Novel Virulence Gene in *Klebsiella pneumoniae* Strains Causing Primary Liver Abscess and Septic Metastatic Complications. J Exp Med 199: 697-705.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Van den Bogaard, A.E., Jensen, L.B., and Stobberingh, E.E. (1997). Vancomycin-Resistant *Enterococci* in Turkeys and Farmers. N Engl J Med 337: 1558-1559.
Voss, R.S., and Jansa, S.A. (2009). Phylogenetic Relationships and Classification of Didelphid Marsupials, an Extant Radiation of New World Metatherian Mammals, Bull. American Museum Natural History 322: 1-177.
Voss, R.S., Lunde, D.P., and Simmons, B. (2001). The Mammals of Paracou, French Guiana: A Neotropical Lowland Rainforest Fauna. Part 2: Nonvolant Species., Bull. American Museum Natural History 263: 1-236.
Weksler, M., Percequillo, A.R., and Voss, R.S. (2006). Ten New Genera of Oryzomyine Rodents (Cricetidae: Sigmodontinae). American Museum Novitates 3537: 1-29.
Wilson, D.E., and Reeder, D.M. (2005). Mammal Species of the World. A Taxonomic and Geographic Reference. The Johns Hopkins University Press, Baltimore: 1-2142.
Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L.H.*, et al.* (2006). Sex and Virulence in *Escherichia coli*: An Evolutionary Perspective. Mol Microbiol 60: 1136-1151.
Woerther, P.L., Angebault, C., Jacquier, H., Clermont, O., El Mniai, A., Moreau, B.*, et al.* (2013a). Characterization of Fecal ESBL-Producing *Escherichia coli* in a Remote Community During a Long Term Period. Antimicrob Agents Chemother 57: 5060-5066.
Woerther, P.L., Angebault, C., Lescat, M., Ruppe, E., Skurnik, D., Mniai, A.E.*, et al.* (2010). Emergence and Dissemination of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* in the Community: Lessons from the Study of a Remote and Controlled Population. J Infect Dis 202: 515-523.
Woerther, P.L., Burdet, C., Chachaty, E., and Andremont, A. (2013b). Trends in Human Fecal Carriage of Extended-Spectrum Beta-Lactamases in the Community: Toward the Globalization of CTX-M. Clin Microbiol Rev 26: 744-758.
Yu, W.L., Ko, W.C., Cheng, K.C., Lee, C.C., Lai, C.C., and Chuang, Y.C. (2008). Comparison of Prevalence of Virulence Factors for *Klebsiella pneumoniae* Liver Abscesses between Isolates with Capsular K1/K2 and Non-K1/K2 Serotypes. Diagn Microbiol Infect Dis 62: 1-6.

Tables

Distance from the village (m)	Number of samples	Ticarcillin resistance[a]		Integrons[b]		
		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)

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

[a] Presence of ticarcillin resistant enterobacteria in the samples was screened by plating the global culture on Drigalski agar plates containing ticarcillin (32 mg/L).

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

482

Strain identity	Resistance Phenotype[a]	Virulence factors[b]													Virulence score	Phylogroup[c]	
		adhesins				toxins		iron capture systems					protectin				
		papC	*sfa/foc*	*ibeA*	*iha*	*hlyC*	*sat*	*iroN*	*ireA*	*fyuA*	*irp2*	*aer*	*neuC*	*usp*			
Ec-0-1	AMX, TIC, TMP, SSS	-	-	-	-	-	-	-	-	-	-	-	-	-	0	B1	
Ec-0-2	AMX, TIC, SSS, TET	-	-	-	+	-	-	-	-	+	+	+	-	-	4	A	
Ec-0-3	AMX, TIC	-	-	-	-	-	-	-	-	-	-	-	-	-	0	E	New ST (
Ec-0-4	AMX, TIC, TMP, SSS	-	-	-	-	-	-	-	-	-	-	-	-	-	0	A	New ST (6-
Ec-0-5	AMX, TIC	-	-	-	-	-	-	-	-	-	-	-	-	-		C	
Ec-0-6	AMX, TIC	-	-	-	+	-	-	-	-	+	+	-	-	-	3	A	
Ec-0-7	AMX, TIC, TMP, SSS	-	-	-	+	-	-	-	-	+	+	+	-	-	4	A	
Ec-0-8	AMX, TIC, TMP, SSS	-	-	-	-	-	-	-	-	-	-	-	-	+	1	E	
Ec-0-9	AMX, TIC, TMP, SSS	-	-	-	-	-	-	-	-	-	-	-	-	-	0	B1	
Ec-0-10	AMX, TIC, TMP, SSS	-	-	-	+	-	-	-	-	+	+	+	-	-	4	B1	New ST
Ec-0-11	AMX, TIC	-	-	-	-	-	-	-	-	-	-	-	-	-	0	C	New ST
Ec-0-12	AMX, TIC	-	-	-	-	-	-	-	-	-	-	-	-	-	0	C	
Ec-0-13	AMX, TIC, SSS	-	-	-	+	-	-	-	-	+	+	-	-	-	3	B1	New S
Ec-0-14	AMX, TIC, TMP, SSS, TET	-	-	-	-	-	-	-	-	-	-	-	-	-	0	D	New ST
Ec-0-15	AMX, TIC, KAN	-	-	-	-	-	-	-	-	-	-	-	-	-	0	B1	
Ec-600-1	AMX, TIC, TMP, SSS	-	-	-	-	-	-	-	-	+	+	-	-	-	2	D	

483

484 Table 2: Resistance phenotype, virulence factors and score, phylogroup and sequence type (ST) of *E. coli* stra

485 [a] The susceptibility of the following antibiotics were tested: amoxicillin (AMX), ticarcilline (TIC), amoxicilli

486 ceftazidime, cefepime, cefoxitin, ertapenem, gentamicin, amikacin, kanamycin (KAN), nalidixic acid, ofloxac

487 sulfonamide (SSS) and tetracycline (TET)

488 [b] *E. coli* virulence factors were detected by PCR as described elsewhere (Johnson *et al.*, 2006) and virulence s

489 2011).

490 [c] Phylogroups of *E. coli* strains were determined by quadruplex PCR as described elsewhere (Clermont *et al.*,

491 [d] Multilocus sequence typing (MLST) was performed using one of the MLST schemes developed for *E. coli*

492 (http://www.mlst.ucc.ie/mlst/dbs/Ecoli).

493 [e] 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* DH5α (Invitrogen) was attempted by el
495 DNA.
496 [f] Resistance genes bla_{TEM} were amplified with specific primers, as described elsewhere (Saladin *et al.*, 2002),
497 [g] NT, Not Typed. Plasmid replicons from transconjugants and transformants were typed by PCR, as described
498
499

Strain identity	Resistance phenotype[a]	Virulence factors[b]										ST[c]
		Adhesins						Iron capture systems				
		rmpA	*allS*	*ycfM*	*mrKD*	*kpn*	*fimH1*	*entB*	*iroN*	*ybtS*	*kfuABC*	
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)

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

[a] The susceptibility of the following antibiotics were tested: amoxicillin (AMX), ticarcilline (TIC), amoxicillin + clavulanate, cefotaxime, ceftazidime, cefepime, cefoxitin, ertapenem, gentamicin, amikacin, kanamycin, nalidixic acid, ofloxacin, trimethoprim, sulfonamide and tetracycline

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

[c] Multilocus sequence typing (MLST) was performed using the international MLST scheme of the Institut Pasteur, Paris, France (http://www.pasteur.fr/mlst)

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

Table 4: Gene cassettes arrays of class 1 integrons in microbiota of rodents and marsupials trapped in Trois-Sauts and at specific distances from the village.
ND: Not Determined
For the construction of gene cassette array libraries, primers 5'CS and 3'CS at 0.5 µM (Levesque *et al.*, 1995) were used to amplify and sequence the variable GC-containing region of class 1 integrons directly from the extracted DNA (5 µL) as described (Stalder *et al.*, 2013). At least 20 clones were used for sequencing.

Figure legends

Figure 1: Study site: Trois-Sauts (in the municipality of Camopi, French Guiana: 02°15′ N, 52°52′ W).
Red numbers represent the number of samples per trapping point (in the village and at specific distances from the village).
Rectal swabs of trapped animal were inoculated extemporaneously onto Drigalski agar slants in screw-cup tubes and sent to France at room temperature. There, the global Drigalski culture from each tube was suspended in 1.5 mL of brain-heart infusion (BHI) broth with 10% glycerol and stored at -80°C.

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

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

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

Supporting Information

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

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

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

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

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

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

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

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