Antibiotics produced by natural organisms play a role in their interactions shaping the lifestyle and homeostasis of bacterial populations and communities (Yakimov, 1991; Davies, 2006; Fujardo and Martínez, 2008; Aminov, 2009). Such interactions might be of antagonistic nature as the production of antibiotics serves to inhibit other bacterial populations. Inhibition does not necessarily intend to kill competitive bacterial organisms, but rather prevent undesirable local overgrowth of partners in shared ecosystems. The diffusion of antibiotics in the environment assures an “exclusive zone” at a certain distance from the producer population. At the borders of such a zone, the potentially competing organisms are confronted with very low antibiotic concentrations, probably sufficient to decrease their growth rate, but not to kill the competing neighbor. In this sense, it is highly possible that the production of antagonistic (allelopathic) substances by microorganisms has more a defensive than offensive nature (Chao and Levin, 1981). In addition, mutual inhibition is frequently desirable for the maintenance of healthy species diversity in a particular ecosystem (Czażan et al., 2002; Becker et al., 2012; Cordero et al., 2012). It is of note that natural antibiotic production, decreasing the growth rate of the competing population, not only restricts its local predominance, but also assures that this population is preserved, as antibiotic-promoted cessation of growth is a highly effective system to avoid antibiotic killing.

However, the production of natural antibiotics might only have functions unrelated with inter-bacterial antagonisms. Antagonism might arise in particular contexts as a side-effect of cell-to-cell signaling effects resulting in self-regulation of growth, virulence, sporulation, motility, mutagenesis, SOS stress response, phage induction, transformation, lateral gene transfer, intra-chromosomal recombination, or biofilm formation (Goh et al., 2002; Ubeda et al., 2005; Linares et al., 2006; Yim et al., 2007; Martínez, 2008; Couce and Blázquez, 2009; Kohanski et al., 2010; Allen et al., 2011; Baharoglu and Mazel, 2011; Pedró et al., 2011; Loof et al., 2012; Looft and Allen, 2012; Looft et al., 2012).

Natural antibiotic resistance modulates the effect of the natural production of antibiotics, so antibiotic production and antibiotic resistance act as two complementary sides of the same process.
mechanisms for self-protection, " which have been considered
"antibiotic environment" is extremely low (Halling-Sørensen et al.,
Aminov and Mackie, 2007; Mindlin et al., 2008; Allen et al., 2009;
derives from the huge escalation of the amount of antibi-
dominate in the wild environments.
other kinds of "resistance" genes, those of the "intrinsic resistome, 
plex evolutionary processes pre-existing the industrial release of
that "modern" resistance genes might have evolved along com-
mitted to duplications and frequent horizontal gene transfer, so
organisms. Nevertheless, they might have been historically sub-
not be explained by recent horizontal gene transfer from these
studies suggest that current clinical resistance genes are not found
as the origin of modern functions involved in clinical antibiotic
resistance genes in bacterial populations (Biel and Hartl, 1983;
Martínez et al., 2008; Alvarez-Ortega et al., 2011). That does not mean that
these genes were not submitted to horizontal gene transfer before the
crisis provoked by the industrial antibiotics pollution, illus-
trating that besides direct selection by clinical antibiotics other
factors contribute to dissemination and maintenance of antibiotic
resistance genes in bacterial populations (Biel and Hartl, 1983; 
Aminov and Mackie, 2007; Mindlin et al., 2008; Allen et al., 2009;
D’Costa et al., 2011). In any case, it has been recently suggested that the
close identity of resistance genes (and resistance platforms)
from clinical strains and environmental strains might indicate recent exchange events (Forsberg et al., 2012). From this perspec-
tive, natural antibiotics and antibiotic "resistance" mechanisms
have a natural regulatory role in shaping both population biology
and evolutionary biology of bacterial organisms. However, the
amount of active antibiotic determining this physiological natural
"antibiotic environment" is extremely low (Halling-Sørensen et al.,
1998). Of course antibiotic-producer organisms are endowed with "mechanisms for self-protection," which have been considered
as the origin of modern functions involved in clinical antibiotic
resistance (Biem and Davies, 1973). However, phylogenetic
studies suggest that current clinical resistance genes are not found
in antibiotic producers, and its emergence in clinical strains can
not be explained by recent horizontal gene transfer from these
organisms. Nevertheless, they might have been historically sub-
mittet to duplications and frequent horizontal gene transfer, so
that "modern" resistance genes might have evolved along com-
plicated evolutionary processes pre-existing the industrial release of
antibiotics. In fact many identical "resistance genes" are found in
environmental and clinical organisms (Cantón et al., 1999; Fens-
berg et al., 2012). In any case, what we denominate "antibiotic resistance" for clinical microorganisms is extremely rare in nature;
other kinds of "resistance" genes, those of the "intrinsic resistome, 
able to protect cells from tiny concentrations of natural antibiotics,
dominate in the wild environments.
The main problem that we are examining in this manuscript derives from the huge escalation of the amount of antibi-
tics released into the microbial environments as an effect of
assuring the homeostasis of microbial populations and commu-
nities. In fact, communities with a cohesive habitat association
might act as units in terms of antibiotic production and resistance.
In these clusters, antibiotics are frequently produced by few bac-
terial organisms, whereas other members of the club are resistant
(Cordero et al., 2012).
As in the case of natural antibiotic production, natural antibi-
" fmicb-04-00015 " — 2013/3/5 — 11:06 — page 2 — #2

oresis might not only focus on "defense against antibiotics" or "self-protection" in antibiotic producers. In fact, this "defense" is
frequently a side-effect of other functions of the "natural resistance mechanism," including nutrition, metabolism, detoxification of
noxious substances, and catabolic processes (Dantas et al., 2008; 
Martínez, 2008; Alvarez-Ortega et al., 2011; Martínez and Rojo,
2011; Qu and Spain, 2011).
The so-called "intrinsic resistome," the ensemble of non-
acquired genes and functions normally present in bacterial cells
which influence the susceptibility to antibiotics, might account for
3% of the bacterial genome (Fajardo et al., 2008). Obviously, such a huge number of "defensive" genes reflects the unspecific nature of
their functions in which antibiotic resistance is concerned (Fajardo
et al., 2008, Alvarez-Ortega et al., 2011). That does not mean that these genes were not submitted to horizontal gene transfer before the
presence of every possible antibiotic concentration in contact
with bacteria. The consequences of such an extensive release of
inhibitory and regulatory molecules have an important impact on
the population biology and evolutionary biology of bacteria.

POPULATION BIOLOGY OF THE UNITS OF SELECTION
The units of selection define the evolutionary individuals (Lewon-
tin, 1970; Brandon, 1987; Mayr, 1997; Okasha, 2004; Dupré and
O’Malley, 2007; Lloyd, 2008; Doolittle and Zhaxybayeva, 2010;
Baquero, 2011; Rodrigues-Valera and Ussery, 2012). But what
is selected in the case of antibiotic resistance? Possible units of
selection in antibiotic resistance are discrete genetic sequences,
genomes, plasmids, plasmids part of cells, cells part of clones, clones part
of species, and so on. Each unit is a "vessel" for the other(s),
affecting not only its potential dissemination but also its rate of
intrusive descent and evolution (Doolittle and Zhaxybayeva,
2010; Baquero, 2011; Baptiste et al., 2012; Cordero et al., 2012).
The investigation of such a trans-hierarchical landscape clearly
requires a multi-level population genetic approach (Baquero and
Coque, 2011; Day et al., 2011; Cordero et al., 2012).
What we propose in this work is essentially a mental heuristic
exercise. Let us imagine that we are aware of a kind of replicators
called genes but we still ignore the existence of cells. We could
observe changes in the frequency and variety of genes, and we
might consider populations of genes, submitted to evolutionary
dynamics and natural selection. If we were only conscious of the
existence of transposons, we would establish population biology of
transposons. If we considered plasmids, we would refer to the
pan-plasmidome, the plasmid population harbored by a particular
microbiome or a particular bacterial group (Fondi and Fani, 2010;
Mizrahi, 2012). This would apply for every unit of selection. We
would of course be able to observe changes in the abundance and
variety of each unit involving a resistance trait as a consequence of
the presence of antibiotics in the environment. Each unit of selec-
tion is a self-interested entity (Rankin et al., 2011b) exploiting the
higher hierarchical unit for its own benefit (resistance plasmids
exploit successful bacterial clones), but the higher unit might
acquire critical traits for its spread because of the exploitation
of the lower hierarchy unit (bacterial clones, bacterial communi-
ties, or microbiomes) might be successful because of resistance
plasmids, plasmids, or at the cellular and supra-cellular
levels, genomes and cells (organisms), clones, clonal complexes,
species, communities, and ecosystems. Note that all these possi-
bile units belong to different hierarchical levels, ranging from the
relatively simple to the complex, as resistance genes are part of
integrons, integrons part of transposons, transposons part of
plasmids, plasmids part of cells, cells part of clones, clones part
of species, and so on. Each unit is a "vessel" for the other(s),
affecting not only its potential dissemination but also its rate of
intrusive descent and evolution (Doolittle and Zhaxybayeva,
2010; Baquero, 2011; Baptiste et al., 2012; Cordero et al., 2012).
The so-called "intrinsic resistome, " the ensemble of non-
acquired genes and functions normally present in bacterial cells
which influence the susceptibility to antibiotics, might account for
3% of the bacterial genome (Fajardo et al., 2008). Obviously, such a huge number of "defensive" genes reflects the unspecific nature of
their functions in which antibiotic resistance is concerned (Fajardo
et al., 2008, Alvarez-Ortega et al., 2011). That does not mean that these genes were not submitted to horizontal gene transfer before the
presence of every possible antibiotic concentration in contact
with bacteria. The consequences of such an extensive release of
inhibitory and regulatory molecules have an important impact on
the population biology and evolutionary biology of bacteria.
natural selection processes, from the selection of a resistant cell to multi-level selections. Such processes should increase the absolute density (number) of all pieces involved in antibiotic resistance, and consequently might favor their interactions and emergence of novel combinatorial patterns (Baquero, 2004). Prediction of which pieces and patterns will evolve is a crucial issue for the management of multiantibiotic resistance, and might be possible if we have the right data (Martínez et al., 2007; Baquero, 2011; Partridge, 2011).

ANTIBIOTICS AND POPULATIONS OF RESISTANCE GENES

Have antibiotics increased the abundance of highly effective resistance genes in the bacterial world? Confronted with antibiotics, bacterial populations might adapt by selecting “more effective” mutants of wild genes endowed with other functions, but providing low level of resistance. Such process is favored by gene duplication, so that wild genes having a “small effect” on resistance could increase in number to increase protection. It is of note that this process might be much more frequent than mutation (Naßvall et al., 2012). A high number of gene-copies might in fact transiently accumulate during selection, producing a full resistance phenotype. The question is if such gene duplications might contribute to the emergence of novel resistance genes. Once the permanence of a functional copy of a given gene is guaranteed, the second (or n+1) copy has the evolutionary freedom (liberation from purifying selection) to be modified, eventually leading to a variant or novel gene (Kondrashov et al., 2002; Naßvall et al., 2012). It is of note that not all genes have equal chances of duplication, and certainly there are adaptive genes with a higher potential variability, containing highly variable regions interspersed among well-conserved, “segmentally variable genes,” as ABC transporters involved in multidrug resistance (Zheng et al., 2004). Mutational changes in genes, leading to novel resistance genes, are facilitated under circumstances of enhanced mutagenesis in the host strain. Hyper-mutable bacteria (“mutators”) are enriched in allelic variants of resistance genes, eventually providing wider resistance spectrum, as in the case of beta-lactamases (Baquero et al., 2005). Indeed organisms with enhanced mutation rates (frequently involving failures in the mismatch repair system) see their possibility of survival increased, and inside these strains, other genes could be modified to provide antibiotic resistance. Note that hyper-mutation and gene variation at large, might result from the effects of the antibiotic themselves (Blázquez et al., 2012).

Indeed, intra- and inter-bacterial gene movement and recombinational events between genetic platforms contribute to the total amount of resistance genes. In fact, the “biological success” of a resistance gene is dependent on its wider genetic context (Walsh, 2006; Wozniak and Waldor, 2010; Bertels and Rainey, 2011). Moreover, hybrid resistance genes resulting from recombinational events are not infrequent in nature (Goffin and Ghuysen, 1998; Maiden, 1999; Novais et al., 2012b). Under antibiotic exposure, bacterial pathogens in humans and animals (and commensals) might fix and further refine acquired resistance genes originated in areas less exposed to antimicrobials, as in the soil (including the rhizosphere), or water bodies (including sewage) (Aminov, 2009; Lupo et al., 2012).

If antibiotics have polluted the entire globe, including wild environments, specialized antibiotic resistance genes, identical to those found in hospitals, can be found everywhere else, including the most remote and wild regions (Gilliver et al., 1999; Osterblad et al., 2001; Sjölund et al., 2008; Allen et al., 2009; Quinteira et al., 2011; Stalder et al., 2012). However, the variety of natural bacterial genes that can provide antibiotic resistance in a heterologous host is much larger than that actually found in human pathogens (Dansa et al., 2008). Why only a very small fraction of “resistance genes” present in the “global resistome” have entered in human pathogens is a poorly addressed question. Of course genes from phylogenetically remote organisms should have functional connectivity and concert with the host systems, and that certainly constitutes an important bottleneck for their acceptance (Halary et al., 2010; Martínez, 2011). However, relatively “independent” functionally connected gene clusters (Zheng et al., 2005) determining resistance might be better tolerated and eventually fixed (Pepa et al., 2011). In any case, as stated recently (Skippington and Ragan, 2011), the network and evolutionary dynamics that allow the stoichiometric participation of horizontally transferred genes in cellular networks remains poorly addressed, even though new bioinformatic advances have recently been made available (Cohen et al., 2012).

Considering potential sets of “acceptable” resistance genes able to evolve in bacterial populations, eventually only the “fittest genes” resulting from competition among genes might finally reach high densities. Competition is expected to occur mostly among orthologs or paralogs (for instance resulting from recent duplications) occupying the same functional niches (Kondrashov et al., 2002; Francino, 2012). Antibiotics could have enriched the more efficient adaptive genes among competing genes (for instance the more detoxifying ones; Novais et al., 2012b). However, the “fittest genes” are not necessarily those with the best intrinsic activity in terms of providing antibiotic resistance. Different resistance genes impose different biological costs for the host strain. As it was stated above, successful novel resistance genes should be fit in a particular genetic context, that is, the epigenetic compatibility of a new gene with the host genome is critical in the acquisition of resistance (Sánchez and Martínez, 2012). Such fitness bottleneck will select, in combination with the detoxifying efficiency, the novel successful genes.

Alternatively, the quantitative success of a particular gene (as beta-lactams in Escherichia coli, or blz in Staphylococcus aureus) might result only from a “founder effect” (Livermore, 2000; Martínez et al., 2009), that is, the first gene that by chance conferred a selective advantage in particular conditions was fixed and that resulted in a successful wide spread. This founder effect in human and animal pathogens might have occurred because of multiple selective events in environmental organisms exposed to dynamic landscapes. In fact, founder effects are expected to occur in a continuous and cumulative way (Aguilée et al., 2009). In turn, such emergent events might have been facilitated by changes in environmental conditions (as animal crowding in farms) resulting in local fluctuations in the size of particular bacterial populations, thus favoring acquisition by lateral genetic transfer of adaptive traits from environmental bacteria (Baloue, 2010). Similarly, the changes in the environment, the landscape dynamics, influence the probability of founders fixation, as well as the possibilities...
for extinction and re-emergence (Aguilée et al., 2011). It is not impossible that many resistance genes, even those rarely found or never found in the clinical environment, could have also been enriched by environmental antibiotic exposure (Martínez et al., 2009; Sommier et al., 2009).

How can we explain that the same resistance gene of plausible environmental origin (as βlactamase, or βlactamase) appears to have been captured in separate events by different gene-capturing elements as IS650, ISCR1, or IS260 (Barlow and Hall, 2002; Tolman et al., 2006; Valverde et al., 2009; Partridge, 2011). There is a contemporary enrichment in organisms as Enterobacteriaceae of captured gene(s) with apparently the same function, and a bloom of a diversity of new genes coding for resistance to beta-lactams (Cantón and Coque, 2006; Coque et al., 2008; Poirot, 2012). This might be due to a dense interactive field resulting from an in the number of environmental species (donors) where it originated from, as well as an environmental increase in population density of a variety of “good recipients” as E. coli or Klebsiella pneumoniae. Recipients increase could result from both the augmentation of the total number of Enterobacteriaceae in the gut microbiota of mammals, probably due to antibiotic exposure, and the massive release of human and animal sewage to the environment (see later).

Other less-successful resistance or pre-resistance genes might not be relevant in the clinical setting, but constitute an increasing reservoir of unpredictable consequences, and undoubtedly might influence the population ecology of bacteria. On the other hand, the selection of variant genes might occur at very low antibiotic concentrations (Henderson-Begg et al., 2006; Gulberg et al., 2011) particularly among natural concentration gradients (Baquero and Nørgaard, 1999; Nørgaard et al., 2000; Hermson et al., 2012). It can be suggested that the overall increase in the amount of resistance genes on Earth also has a positive effect in maintaining the desirable homeostasis of bacterial populations in a heavily antibiotic-polluted environment.

An interesting point in gene population biology is the question of why a number of resistance genes maintain their full sequence integrity through myriads of replications in spite of an apparently insufficient level of antibiotic selection. Even considering that they are co-selected with genes under active selection (for instance being part of the collection of gene cassettes of an integron), their functionality seems to be better preserved than could be expected. This might suggest that the current function of a number of classic “resistance genes” is something other than antibiotic resistance (exaptation, Alonso and Gready, 2006; Petrosa et al., 2011; Sánchez and Martínez, 2012).

Resistance genes tend to be collected in particular clades and clustered in common genetic platforms (Partridge and Hall, 2004; Kiar et al., 2006; Partridge, 2011; Petron et al., 2013), probably following the “genetic capitalism principle,” that is, the more resistant clones and the most fit resistance-providing platforms are selected, and then their ability to acquire novel adaptive traits is favored (Lawrence, 1997; Baquero, 2004). As we will see along this review, the extensive antibiotic-promoted selection of resistant bacterial organisms is a selection of the “vehicles” where antibiotic resistance genes are located, and the selection of “vehicles” assures the selection of the genes that they contain. Consequently, the total amount of resistance genes should increase under selection. Interestingly, some “vehicles” (as MGEs are more frequently associated with resistance genes than others. As complementary explanations, we can recall here the founder effect (advantages for the first gene-capturing MGEs), the influence of genes and platforms on the overall fitness of the recipient cells, or the higher prevalence of particular MGEs in the organisms more exposed to antibiotics, heavy metals, biocides, or other ecological stressors (Stokes and Gillings, 2011).

**ANTIBIOTS AND POPULATIONS OF RESISTANCE INTEGRONS**

Have antibiotics increased the abundance, in the microbial world, of integrons able to capture resistance genes? Integrons possess a site-specific recombination system able to integrate, rearrange, and express adaptive genes, including antibiotic resistance genes (Mazel, 2006; Partridge, 2011; Stokes and Gillings, 2011; Moura et al., 2012). These genetic platforms are ancient structures (several hundred million years old) that were already involved in the initial outbreaks of antibiotic resistance in the 1950s (Liebert et al., 1999; Mazel, 2006; Revilla et al., 2008). The same type of integrons, now carrying a diversity of antibiotic resistance genes, are preserved in the current bacterial world, and have installed themselves in natural environments along extended periods of time (Petrosa et al., 2011; Stokes and Gillings, 2011; Stalder et al., 2012). Integrons evolution often results in the local array of resistance genes, and other genes of adaptive nature (Lábate et al., 2009; Moura et al., 2012; Stalder et al., 2012), which increases the possibilities of their selective multiplication.

In other words, exposure to antibiotics, biocides, or heavy metals and a high multiplicity of other different environmental factors results in an increase of cells containing integrons (Gaze et al., 2005, 2011; Wright et al., 2008; Stalder et al., 2012). Moreover, exposure to different antibiotics (aminoglycosides, beta-lactams, fluoroquinolones, trimethoprim, metronidazole) facilitates extensive gene cassette recombination; occasionally involving the SOS-triggered IntI1 integrase over-expression (Guarrin et al., 2009; Hocquet et al., 2012). Other recombinational events (often mediated by ISs) influence shuffling of resistance genes contained in different integrons, giving rise to multi-resistance regions (Partridge, 2011) and further facilitating the evolution and selection of the upper-level host vehicles (Domingues et al., 2012). In fact, integrons are not mobile, but are frequently associated with transposons and/or plasmids and therefore should increase in abundance as a result of conjugation or transposition events mediated by Tn21-like and IS26-like elements. For instance class 1 integrons located in Tn402, which are often part of Tn501-like transposons on conjugative plasmids, have greatly contributed to the spread of integrons among γ and β-proteobacteria (Tato et al., 2010; Partridge, 2011).

The frequent association of integrons with a variety of specialized DNA recombination systems enhances both transferability and genetic diversification. For instance, insertion sequences (IS) of the IS110/IS482 family as IS432 and IS8075 (members of the IS1111 subgroup) target the terminal inverted repeats of Tn21 family transposons (Partridge and Hall, 2003; Novais et al., 2010b). The outcome is the initiation of a non-standard transposition
resulting in only a single copy appearing in the transposed product (Cain et al., 2010; Martinez et al., 2012). Such mobilization of integrons by specialized transposition is a powerful mechanism for integrons spread in both environmental competent and non-competent bacteria (Domínguez et al., 2012; Stokes et al., 2012). Also, IS-mediated mobilization of relevant antibiotic resistance genes contained in the integron contributes to enhance gene expression and mosaic genetic diversity, which should be reflected in higher possibilities of dissemination. That is the case for insertion sequences targeting the pseudo-palindromes of integron attC sites, as the IS1111attC group elements of the IS1210/IS492 family (Tetu and Holmes, 2008), or IS4-like elements as IS1999 (Aubert et al., 2006; Post and Hall, 2009; Poirel et al., 2010). Note that the antibiotic-mediated selection of strains, plasmids, or transposons containing integrons necessarily implies selection of IS sequences, and therefore the capture of resistance genes and the combinatorial evolution of resistance platforms. In fact, ISs mobilizing adjacent sequences by rolling-circle (RC) transposition, as IS5epI and the insertion sequence common regions (ISC)5b, favor the capture and mobilization of a full series of antibiotic resistance genes leading to complex multi-resistance class 1 integrons (del Pilar Garcíllan-Barcia et al., 2001; Garciillan-Barcia and de la Cruz, 2002; Aubert et al., 2006; Tolemen et al., 2006; Poirel et al., 2010).

ANTIBIOTICS AND POPULATIONS OF RESISTANCE TRANSPOSABLE ELEMENTS

Have antibiotics increased the abundance of resistance transposable elements in the microbiosphere? Transposable elements encode an enzyme, transposase, which is required for excising and inserting the mobile element. Transposases (revised in Hickman et al., 2010 and references herein) seem to be the most abundant genes in known sequenced genomes and environmental metagenomes (Aziz et al., 2010).

Among transposases, class II dsDNA transposases constitute the most common group, followed by serine and tyrosine recombinases and RC transposases which are linked to different MGEs (IS, composite transposons, class II transposons, bacteriophages, and genetic islands). The wide spread and maintenance of different classes of transposable elements in bacterial populations has been obviously favored by antibiotic selection because of their association with antibiotic resistance genes. However, most of the contemporary antibiotic resistance transposable elements belong to a few families that have been detected in ancient isolates, often linked to alternative functional roles, thus suggesting antibiotic resistance might have overshadowed previous selection forces.

For instance, some transposable elements, as IS or composite transposons, as Tn5 or Tn10, confer growth rate advantages under different conditions of nutrient availability, enabling populations to rapidly adapt to different physiological conditions (Biel and Hartl, 1983; Hartl et al., 1983; Bilò et al., 1994). Also, the highly specialized targeting system of Tn7 able to selectively direct transposition into both mobile and stationary DNA pools (see later), avoids the occurrence of deleterious insertions and allows the host population or community to recruit genes through a variety of mobile DNAs, thus favoring the adaptation of diverse groups of bacteria to survive or adapt to different conditions (Parks and Peters, 2009; Parks et al., 2009). Selection by different ecological conditions and stressors (including antibiotics) multiplies the chances for expansion, recombination, and diversification (Partridge and Iredell, 2012; Segatini et al., 2012).

The main group within dsDNA transposases corresponds to DDE transposases (designation given due to the presence of a highly conserved catalytic triad of two aspartate (D) and one glutamate (E) residue, originally identified in the retrovirus integrase and having a role during the transfer of the DNA strand. Most IS families use this catalytic reaction for transposition with the exception of IS1111 and the RCR IS91-like elements. IS-DDE transposases have been detected in more than 70 bacterial genera, more than one third being iso-elements (>95% of identity at protein level, >90% at DNA level). They are frequently located on plasmids associated with composite transposons containing antibiotic resistance genes (Merlin et al., 2000).

Tn7 poses an unique fine-tuned regulated transposition array (TnABCIDE) involved in the regulation of transposition (the core machinery coding for TnsA, TnsB, TnsC) and the mobilization of the element (TnsE, TnsD; Parks and Peters, 2009, Parks et al., 2009). Such regulation allows Tn7 to use two target-site selection pathways and move to different hosts. Tn7 belongs to a family of MGE that encodes a transposase and an ATP-utilizing protein (TnsC) that controls the activity of such transposase and often its target site selection. Members of the ATP-subunit superfamily comprise widespread AbR transposons that differ in the transposase (also a DDE enzyme) and the number of proteins in the transposition module (for revision see Craig, 2002). Some examples are Tn1625/Tn1626, Tn552/Ts21, and Tn402/Tm5013 (Craig, 2002). All are widely distributed and they are related with trimethoprim and heavy metals resistance (Kholodii et al., 2003; Partridge, 2011). Please note that in this case trimethoprim resistance is the currently recognizable phenotype, but the genes might have been selected before trimethoprim exposure for other reasons (Alonso and Greary, 2006).

Members of the Tn3 family are mainly derivatives of transposon subfamilies Tn3 (Tn3, Tn3393, Tn5403) or Tn501 (Tn22, Tn301, TnJ271) and all of them were already detectable in ancient bacteria from permafrost (Tolmasky, 1990; Graidy, 2002; Kholodii et al., 2003; Mindlin et al., 2008). Members of the Tn501 subfamily of Tn3 transposons could have been enriched by mercury exposure, as they carry mercury-detoxifying genes. These genes probably originated in hydrothermal environments, where geochemically derived mercury is at high concentrations (Boyd and Barkay, 2012). Mercury-transposons provide target sites for Tn301-type transposons, and include a diversity of Tn21, Tn6986, and Tn301 related elements carrying class 1 integrons. Enrichment of Tn3-type transposons by environmental pollutants, as heavy metals, might have contributed to increase the connectivity with organisms and genetic platforms harboring resistance genes, eventually included in integrons (most frequently of class 1), and have converted this family in the “flagship” of floating resistance genes (Lübert et al., 1999; Partridge and Hall, 2004; Partridge and Iredell, 2012).

Besides classic antibiotic resistance gene cassettes, emerging beta-lactamase genes as βLACT-βLACTARE, βLACTARE, or βLACX are located in integrons on different Tn301 derivatives (Partridge, 2011). Other widespread “new” beta-lactamase genes have been
directly recruited by host-specific Tn3-like transposons as Tn1440 carrying blaTEM-1, blaSHV-2, or Tn3 (carrying blaSHV-14; Novais et al., 2010a; Bailey et al., 2011), probably because of the reduced numbers and connectivities (influencing success) of the vehicles in which they were located (plasmids, clones; Cain et al., 2010; Novais et al., 2010a; Partridge, 2011).

Transposable elements also increase in number by inserting extra copies in the host genomes. Of course this might cause an “intergenomic conflict,” as such insertions might affect chromosomal balance and produce mutations, being so, transposons might be a “bitter–sweet” pill for host bacteria (Toleman and Walsh, 2011). This is why genomes have evolved suppressors limiting transposon spread (Pomiankowski, 1999). Eventually, equilibrium is reached by diminishing the transposition frequency. Successful transpositions as Tn3 have this kind of “transposition immunity” to ensure a maximum of two copies per replication.

On the other hand, transposition might compete with the host genome replication. Some transposases as that of Tn5 (and possibly Tn917) can bind to “processivity factors” involved DNA replication; competition for this interaction could limit their proliferation. However, a benefit for the transposons appear to be derived from the fact that the interaction of transposases with processivity factors favors “target site selection,” so that activation of transposition with Tn7 (transposon excision and insertion) does not occur until an appropriate target has been identified, most frequently mobile plasmids, providing Tn7 with a means of spreading to a new host (Parks et al., 2009).

As in the case of integrons, transposable elements are very ancient on Earth, but the very same molecular structures are found in modern resistance-bearing transposons (Bugarcic and Ochman, 1993; Mindlin et al., 2005; Vishnivetskaya and Kathariou, 2005; Petrova et al., 2009; Aziz et al., 2010). The acquisition of resistance genes seems to have occurred preferentially by particular transposable elements that were afterward amplified by antibiotics. Interestingly, a number of transposons carrying resistance genes have been recovered from ancient permafrost and seem to have been selected before the antibiotic era (Mindlin et al., 2008).

Widespread transposons in our days, as those belonging to Tn7 or Tn3 superfamilies, certainly have a very ancient origin. Most probably they were selected by pre-antibiotic forces, increasing their absolute amount, followed by their spread and diversification in different plasmids and organisms. Exposure to early chemotherapeutic agents has reinforced these evolutionary events.

**ANTIBIOTICS AND POPULATIONS OF RESISTANCE MOBILE GENETIC ELEMENTS (PLASMIDS, ICEs)**

Have antibiotics increased the abundance of plasmids and ICEs carrying resistance genes in bacterial populations and communities? Conjugative plasmids and ICEs are quite similar genomic objects, in fact they appear to be short-term variants of identical backbone elements (Guglielmini et al., 2013); the main difference is that the replication of ICEs occurs only by integration in the host’s chromosome (Wozniak and Waldor, 2010). For instance a close relationship resulting from a common phylogeny can be found between IncA/C plasmids and SXT/R391 ICEs (Wozniak and Waldor, 2010; Toleman and Walsh, 2011). Note that the traditional association of highly transmissible elements with plasmids is not necessarily true.

Plasmids are abundant in nature and consistently isolated from microbial communities of different habitats with and without anthropogenic exposure (Coombs, 2009; Soheky and Hazen, 2009). In fact, contemporary resistance plasmids are based on plasmid backgrounds existing in the pre-antibiotic era (Datta and Hughes, 1983; Hughes and Datta, 1983). Maintenance of plasmids and ICEs in bacterial populations results from both the selfish features that promote acquisition and persistence within bacterial populations (“the parasitic hypothesis”) and the beneficial effects they confer to individual bacterial hosts and communities (“the evolutionarily hypothesis”; Verren, 2011). Plasmids are increasingly being considered within this multi-hierarchical model, as clonal-, species-, or community-specific mobile elements (Carattoli, 2009; Garcillán-Barcia et al., 2009; Lim et al., 2010; Sheaer et al., 2011; Heuer et al., 2012; Williams et al., 2012; Clewell et al., 2013; Guglielmini et al., 2013). Between and also within these hierarchical levels, plasmids may eventually evolve toward mutualism (Rankin et al., 2011b). Plasmids might assure their permanent linkage with a particular host, bacterial lineage, or multi-specific community by post-segregation killer strategies that cause the death of non-carrying bacterial offspring. This is caused by toxin-antitoxin (TA), restriction-modification (R-M), or clustered regularly interspersed short palindromic repeats (CRISPR) systems, and also probably by “plasmid domestication,” which is produced by changes either in the plasmid and host genome that lead to a more stable coexistence (Bouma and Lenski, 1988; Jones, 2010; Marraffini and Sontheimer, 2010; Garcia-Quiñanilla and Casadesús, 2011).

The diversity of bacterial communities, the relative population densities of their components, the spatial separation, and nutrient availability greatly influence plasmid host-range, content, and transferability (Coombs, 2009). It is interesting to suggest that the selective processes exerted by antibiotics will modify bacterial diversity and population densities, forcing the coexistence of plasmids and particular hosts, favoring recombination and other processes that lead to plasmid domestication (Boyd et al., 1996). The robustness of interactions between particular plasmids and particular clones is shaped by epistatic processes (Silva et al., 2011; San Millán et al., 2012) mediated by nucleoid-associated transcriptional regulators (Doyle et al., 2007; Yun et al., 2010; Fernández-Alarcón et al., 2011; Humphrey et al., 2012) and the clonal interferences that might result from these interactions (Hughes et al., 2012). A robust interaction is reflected in a non-cost or even negative-cost coexistence, and will tie the fate of plasmid density to the abundance of their specific bacterial hosts, resulting in a necessary “in-host” plasmid evolution linked to “with plasmid” host evolution (Dionisio et al., 2005; Halary et al., 2010).
A major topic in plasmid population biology is the consider-
eration of advantages and inconveniences of plasmid or ICE
broad-host-range. It is of note that host-range does not nec-
essarily mean transfer ability to a particular host or long-term
maintenance in bacterial populations, but stable replication in a
new host (De Gelder et al., 2007; Suzuki et al., 2010). Certainly,
broad-host-range conjugative plasmids favor the penetration of
adaptive traits as “new” antibiotic resistance genes (Fernández-
Alarcón et al., 2011; Sen et al., 2011; Hamprecht et al., 2012; Heuer
and Smalla, 2012) and, in turn, antibiotics can favor the abundance
of these plasmids promoting transfer and by selection of plasmid
containing bacteria (Lang et al., 2012). The frequent observation of
different systems of replication (recognizing host primases) in the
same plasmid suggests adaptive ways of increasing host-range (Del
Solar et al., 1996; Clewell et al., 2013). Globally spread plasmids
identified in hospitals, soils, agriculture, and marine habitats have
a complex mosaic structure that reflects inter-genomic historical
adaptations to phylogenetically distant bacterial hosts (Schütter
et al., 2007; Norberg et al., 2011; Heuer et al., 2012; Partridge and
Iredell, 2012). In addition, broad-host-range plasmids lacking
transfer systems can be transferred to phylogenetically close or
distantly related bacteria by helper conjugation systems located in
narrow-host-range plasmids containing a conjugation system
(Simorawinska et al., 2012).

Long-term maintenance and dispersion of broad-host-range
plasmids in bacterial populations and communities seems to be
related to the local availability of hosts (De Gelder et al., 2007),
but also with the “social interactions between plasmids”
eventually leading to unbearable costs for their hosts (Smith,
2012). Eventually, exclusion mechanisms between plasmids (one
plasmid excludes the incoming one) might be softened by inter-
plasmid recombination that might result in hybrids able to
evade exclusion. CRISPR is a genetic interference system by
which bacteria with CRISPR regions carrying DNA copies of
previously encountered plasmids can abort the replication of
plasmids with these sequences. Hypothetically that might favor
plasmid dispersal among different strains, providing a weak
selective advantage for the host cell (Levin, 2010), although an
increased benefit could be predicted for host coalitions, as genetic
exchange communities (GECs; see later). This system also controls
phages, but the possible populational interactions (competition–
cooperation) between phages and plasmids have scarcely been
investigated.

Antibiotic exposure might have increased the absolute number
of plasmids with resistance determinants in bacterial populations
due to the selection of clones harboring them. The possibility that
broad-host conjugative plasmids have been submitted to a more
effective enrichment than narrow-host plasmids (because of selec-
tion in multiple hosts and environmental compartments) poses an
interesting question. Eventually, the biological cost of resistance
plasmids for the host could be compensated by higher transmis-
sion (Garcillán-Burcia et al., 2011). As Stokes and Gillings pointed
out, an increase in the general tempo of resistance genes dissemi-
nation is highly probable, due to selection of cells with inherently
higher rates of lateral transfer (Stokes and Gillings, 2014).

Finally, we can consider bacteriophages as mobile mediators
of inter-bacterial transfer of resistance genes. Also in this case
antibiotics might modulate phage–bacteria population dynamics
by processes as “phage-antibiotic synergy,” a non-SOS mechanism
of virulent phage induction caused by exposure to sub-inhibitory
concentrations of beta-lactams (Comas et al., 2007; Allen et al.,
2011; Looft et al., 2012). Antibiotics promote the number of
phages and pro-phages linked to antibiotic resistance platforms,
favoring dissemination of these platforms, and consequently
amplifying the dissemination of resistance and virulence genes
(Allen et al., 2011).

ANTIBIOTICS AND POPULATIONS OF BACTERIAL CLONES
AND SPECIES
Bacterial clones are constituted by isolates that have a close com-
mon phylogenetical origin. High-risk clones are defined as clones
with an enhanced ability to colonize, spread, and persist in a variety
of niches, which acquire adaptive traits that increase pathogenicity
or antibiotic resistance (Baquero and Coque, 2011). They consti-
tute the main vehicles dispersing antibiotic resistance at a global
scale (Willems et al., 2011; Woodford et al., 2011). Examples of
these high-risk clones are E. coli ST131 (phylogroup B2), ST135
and ST393 (phylogroup B1), or ST69, ST405, and ST648 (phy-
logroup D); K. pneumoniae ST258, ST14 or ST37; Enterococcus
faecium, ST18, ST17, ST78; Enterococcus faecalis ST6, S. aureus,
ST45, ST5, ST8, ST30, or ST22. A number of these clones were
ancient lineages, well-adapted to colonization and transmission
between particular hosts, that acquired antibiotic resistance and
consequently enhanced capabilities of dispersal (McBride et al.,
2007; Brisse et al., 2009; Chambers and Deleo, 2009; Willems
et al., 2012). Multiplication and spread of highly successful clones
implies multiplication and spread of all the antibiotic resistance
genes and platforms they contain, increasing their absolute num-
bers. In fact, it is not unusual that a single successful clone might
contribute to the spread of different plasmids, genetic platforms,
and resistance genes, both in Gram-positives (Chambers and
Deleo, 2009) and in Gram-negatives (Carattoli, 2009; Andrade
et al., 2011; Woodford et al., 2011; Novais et al., 2012a; Partridge
and Iredell, 2012). Such multi-lateral collaboration probably con-
tributes to the local ecological success of variants arising in these
clones, leading to a clonal diversification (clonalization) which
assures a long-term permanence in complex adaptive landscapes,
following the “never put all the eggs in the same basket” principle
(Wiedenbeck and Cohani, 2011; de Regt et al., 2012; Freitas et al.,
2013). Focusing only on mutational evolution, it has been sug-
gested that there is an acceleration of emergence of bacterial antibi-
otic resistance in connected microenvironments (Zhang et al.,
2011; Hallatschek, 2012) but this might also occur in the case
of gene flow.

Local clonalization might result in a restricted gene flow
among resulting subpopulations (Willems et al., 2012). However,
recombinational events might spare those regions required for
adaptation to local microenvironments, ensuring divergence, and be
maintained for other regions (ecological speciation with-gene-flow;Via,
2012). The increased recovery of isolates belonging to high-risk
clonal complexes of important human pathogens as E. coli, S.
aureus, E. faecalis, or E. faecium, that cause both human infec-
tions and mucosal colonization, and even the expansion of these
clones to novel hosts is most probably related with the acquisition

www.frontiernb.org
March 2013 | Volume 4 | Article 15 | 7
of antibiotic resistance genes (Hidron et al., 2008; Baquero, 2012; Novais et al., 2012a, 2013)

An interesting question is if the selection of particular clones because of antibiotic resistance might decrease the overall clonal diversity as might be inferred from recent studies (Ghosh et al., 2011). Actually, that might be compensated by clonal diversification, in a “ex pluribus unamexit” evolutionary dynamics (Baquero, 2011). It is easy to conclude that any outbreak produced by antibiotic resistant bacteria will locally enrich the involved evolutionary units, facilitating further events of antibiotic resistance development and possibly transmission (Chambers and Deleo, 2009; Freitas et al., 2013).

**ANTIBIOTICS AND POPULATIONS OF BACTERIAL COMMUNITIES**

Imbedded into the high complexity of the microbiomes, of humans and animals, in the soil or in the water sediments, it is possible to recognize “clubs” of bacterial clones and species where genes and genetic platforms circulate via lateral transfer, the GECs. In a very restrictive manner, Skippington and Ragan (2011) have recently defined a GEC as a group of organisms (entities) in which each entity has over time both donated genetic material to, and received genetic material from every other entity in that GEC, via a path of lateral gene transfer. What do the members of such clubs have in common? Network modeling and co-occurrence statistical approaches indicate that lifestyle and shared environments, functional complementarities, and most probably, continued physical clustering (granularity) determine the size and connectivity of GECs (Freilich et al., 2010; Smilie et al., 2011; Faust and Raes, 2012; Faust et al., 2012). In many cases, this also means a closed shared phylogeny (Skippington and Ragan, 2012). In fact, GECs members are linked not only by genetic exchanges, but also by metabolic and functional cooperation, providing a certain ecological compartmentalization inside particular microbial megasystems (as intestinal microbiota; Faust and Raes, 2012; Faust et al., 2012). We can consider here some kind of cooperative “niche construction” (Laland et al., 1999; Kylin and Loreau, 2011). Genetic transfer, particularly considering mixed-granular “surface-associated populations” with a kind of “lattice reciprocity” (Zhong et al., 2010, 2012) might assure a “collective” adaptation of such functional GEC units, increasing relatedness among members and fixing common evolutionary boundaries (Nogueira et al., 2009; Rankin et al., 2011a,b). The development of more studies on the “physics” of genetic transfer, is certainly advisable, for instance, to investigate to what extent genetic transfer can be influenced by mixing movements or the viscosity or fluidity of the surrounding medium, as in the intestinal content (particularly during enteric diseases) or in water bodies, influencing cell-to-cell contacts and therefore GECs integrity (Zhong et al., 2010; Jeffery et al., 2012).

An important topic is the possibility of “multifocal GECs” as a form of organization of the lifestyle of a particular species or closely phylogenetically related coalitions in changing environments (Skippington and Ragan, 2012), where different sub-specific ecotypes exploiting neighbor nano-niches (Wiedenbeck and Cohan, 2011) and taking advantage of a common “public good” are frequently encoded by conjugative elements (Norman et al., 2009; Rankin et al., 2011b). The distribution among GECs members of such plasmid-mediated “public goods” is favored by common characteristics in the consortium, as nearly identical immune phenotypes mediated by CRISPR, or common DNA uptake mechanisms or quorum-sensing responses. Plasmid-mediated common benefits will probably lead to GEC-plasmid coevolution (Skippington and Ragan, 2012). Along the same line, addiction-type TA complexes can spread on plasmids, favoring coexistence and/or competition in spatially structured environments (Rankin et al., 2012) highlighting the role of kin effects in GECs selection (taking “kin” in a wider sense than just intra-specific ties). It is of note, however, that possibly most organisms and environments might act as conduits for resistance gene flow (Stokes and Gillings, 2011), acting as “sources” from where resistance genes are directed to GECs, acting as “sink,” according to the Perring et al. (2007) metaphor. The possibility of “go-between” organisms traveling from GECs of different microbiotic systems (humans, animals, rhizosphere, and water sediments) should be taken into consideration, as they might contribute to the inter-environmental globalization of antibiotic resistance genes. The existence of “ubiquitous” organisms or species able to transit in different environments has been suggested (Fondi and Fani, 2010; Freilich et al., 2010; Tamames et al., 2010). Candidates for efficient “go-between” organisms are groups of the same bacterial species but specialized in particular environments, as the case of human or animal-adapted versus environmental E. coli clades, where probably only ecological barriers prevent gene flow (Freilich et al., 2010; Luo et al., 2011). Mixing of human or animal derived water effluents into the environment, a practice that is surprisingly perpetuated even in modern societies will facilitate conduits for resistance gene flow (Baquero et al., 2008; Czekalski et al., 2012; Lupe et al., 2012). Such flow occurs because of the confluence of human microbiota from different human hosts, different animals and the indigenous environmental microbiota. Among these GECs, the most relevant for the transmission of antibiotic resistance are those including species from Gammaproteobacteria (as *E. coli*) and Firmicutes (as *Enterococcus*, *Antonopoulos et al., 2009; Freilich et al., 2010; Skippington and Ragan, 2011; Faust et al., 2012). These groups of organisms are enriched in the microbiota during antibiotic exposure (Antonopoulos et al., 2009; Sommer et al., 2009). Antibiotic-mediated reduction in number or loss of some species, favors bacterial species able to explore and temporarily exploit empty niches due to short generation times (Allen et al., 2011; Loof and Allen, 2012). Antibiotic exposure will increase the absolute number (overgrowth) of GECs-associated organisms, for instance, by antibiotic exposure of the infant gut (Fouhy et al., 2012; Loof and Allen, 2012). Probably the same might occur in environmental GECs submitted either to antibiotic pollution or sanitation procedures (Baquero et al., 2008). For instance, metagenomic analysis indicates that drinking water chlorination could concentrate populations containing insertion sequences, integrons, and antibiotic resistance plasmids (Shi et al., 2013). These effects will contribute to the dissemination of resistance genes and the genetic platforms in which they are located.
The identification of GECs among members of the “microbial guilds” in the three identified major microbiome “enterotypes” (Arumugam et al., 2011) needs to be investigated. These major enterotypes (clusters of species) should probably be tightly maintained into host populations, and therefore the local spread of antibiotic resistance genes could serve to maintain their integrity in an antibiotic-polluted environment. At the same time, low-abundance enterotype-species, but providing critical functions, could have low possibilities of getting resistance genes in the absence of GECs. Consequently, a deep and permanent contamination by antibiotic resistance genes of the normal microbiota of humans and animals is a reasonable possibility. Complex microbiota of humans and animals are reproducible systems, not only along time in the same individual, but also across individuals. These systems are frequently based in a microbiotic “core” composed by organisms belonging to a relatively small number of phyla/phylgroups, and probably metabolically linked with the host (Dethlefsen et al., 2006; Ley et al., 2006; Marchesi, 2011). Newborns have a sterile gut, but the human microbiota is “reproduced” with a relatively low number of variations on each of them (Baquero and Nombela, 2012; Valles et al., 2012). Possibly there is also an “epidemiology of bacterial consortia” even in hospitals, which remains to be investigated.

Exposure to antimicrobial agents might affect the frequency and absolute number of GECs; in a number of cases, antibiotic resistance might contribute to the temporal stability and resilience of microbiomes in an antibiotic-polluted environment (Allison and Martiny, 2008; Antonopoulos et al., 2009). Of course that occurs at the expenses of maintenance and spread of the full range of antibiotic resistance evolutionary units.

Finally, we cannot discard individual variations in the microbiotic communities caused by diet, host genetics, particular illnesses, inflammation, or infectious agents including viruses and parasites which might lead to microbial communities more prone to capture and propagate antibiotic resistance (Marchesi, 2010; Claesson et al., 2012; Looff and Allen, 2012). For instance, a high-fat diet determines the composition of the murine gut microbiome independently of obesity, with an increase of Proteobacteria and Firmicutes, heavily involved in resistance gene mobilization (Hildebrandt et al., 2009; Taglialove and Elii, 2012). In several microbiota communities studied in the elderly, the proportion of phylum Proteobacteria, very active in the mobilization of antibiotic resistance genes and vehicles, was ten times higher than average (20 versus 2%); Claesson et al., 2011). E. coli numbers are higher in the microbiota of women with excessive weight gain than in women with normal weight gain during pregnancy (Santacruz et al., 2010). A number of surgical interventions (as surgery for morbid obesity) increases Proteobacteria even in a higher proportion (50 times increase; Li et al., 2011; Graeser et al., 2012). Unfortunately, these populational microbiotic shifts favoring the active populations and communities contributing to the emergence, dispersal and maintenance of antibiotic resistance might also occur as a consequence of undernutrition (10 times increase in Proteobacteria, 46 versus 5% in healthy children in Bangladesh; Monira et al., 2011). Possibly the deleterious effect of antibiotics in promoting antibiotic resistance will be significantly increased. Finally, it could be considered, under certain circumstances, as during the colonization of the neonatal intestinal tract, that rapidly growing populations might be more prone to the dissemination of antibiotic resistance. Also the unexpected possibility of resistance gene exchange between Actinobacteria (Bifidobacterium belongs to this group!) and Gammaproteobacteria has been recently shown under the same conditions (Tamminen et al., 2012).

ANTIBIOTICS IN THE ANTHROPOCENE: EFFECTS ON GLOBAL ECOLOGY AND EVOLUTION

Evolution is a natural trend of complex systems, and might be accelerated by changing and stressful conditions. The Anthropocene is the current human-dominated geological epoch where nature is changed and stressed by the action of humans (Zalasiewicz et al., 2011; Biermann et al., 2012). Industrial antibiotics are a paradigmatic example of substances exerting a powerful effect of anthropogenic origin on the bacterial communities of the microbiosphere. Not only most of these substances are unspecifically killing bacterial organisms, and selecting for resistance, but directly influence the mechanisms of genetic variation (mutation, recombination, transposition, modularization, gene transfer; Baquero, 2009; Gilling and Stokes, 2012).

Such effects on microorganisms will be further enhanced by a diversity of other anthropogenic effects as the release of biopharmaceuticals, biocides, heavy metals, industrial and agricultural residues, and plastic materials or changes in the environmental conditions. The mixing of bacterial populations (human organisms with other human, animal, or environmental organisms), that makes the emergence and spread of resistance possible is also favored by poor sanitation, facilitating contact of human or animal sewage with the soil. Some of these effects might escalate with other anthropogenic effects as destruction of diversity in food animals (Baquero, 2012) or even global warming (Baquero et al., 2008; Baquero, 2009; Balbas et al., 2013). The fight against antibiotic resistance should focus not only on acting on its appropriate usage in human and veterinary medicine, but by considering possible initiatives at ecological and evolutionary levels, as eco-geo drugs and strategies (Baquero et al., 2011) in accordance with the environmental distribution of bacterial organisms (Tamames et al., 2010), in the scope of progressing toward a protective and restorative planet medicine (Baquero, 2009).

ACKNOWLEDGMENTS

The authors’ work was sponsored by grants from the European Union (PAR-241476 and EviTAR-282094), the Instituto de Salud Carlos III – Ministry of Economy and Competitiveness of Spain (FIS-PS09-02381, FIS-P10-02588, P11-01521, and the Regional Government of Madrid in Spain (FRCOMPCT-52010/BMD2411). The authors are also grateful to the Spanish Network for the Study of Plasmids and Extrachromosomal Elements (REDDEEX) for encouraging and funding cooperation among Spanish microbiologists working on the biology of mobile genetic elements (grant RTI 2012-0079-E/BMC, Spanish Ministry of Science and Innovation).
REFERENCES

Aagaard, R., Clausen, D., and Lambart, A. (2006). Atlantic fish in a dynamic metapopulation: founder effects vs selection effects. Fish Shellfish Immunol. 20, 105–117.

Aagaard, R., Lambart, A., and Clausen, D. (2011). Ecological speciation in dynamic landscapes. J. Evol. Biol. 24, 265–277.

Allen, H. K., Looft, T., Rayes, D. O., Humphrey, S., Lertea, U. L., Alli, D., et al. (2011). Antibiotics in leaf indole prohormones in rice foliar microflora. Mol. Syst. Biol. 7, 5026–e00211.

Allen, H. K., Moe, L. A., Boldtrom, J., Guerard, A., and Handelman, J. (2009). Functional metagenomes reveals diverse beta-lactamases in a remote Alaskan soil. ISME J. 3, 245–259.

Allison, S. D., and Martiny, J. B. H. (2008). Collaborative paper: resistance, redundancy and redundancy in microbial communities. Proc. Natl. Acad. Sci. U.S.A. 105, 11512–11519.

Almeida, H., and Guedes, J. E. (2006). Integration sequenced dideoxynucleotide: a recently rediscovered enzyme. Fungal Microb. 14, 256–242.

Almazán-Gómez, C., Wiegand, I., Olivas, J., Hansøy, R. E. W., and Martínez, J. L. (2011). The intrinsic resistance of Pseudomonas aeruginosa to β-lactams. Virulence 2, 144–146.

Almiron, R. I. (2009). The role of antibiotics and antibiotic resistance in nature. Microbe 11, 296–298.

Almiron, R. I., and Macleod, B. L. (2007). Evolution and ecology of antibiotic resistance genes. FEMS Microb. Lett. 271, 147–161.

Andrade, L. N., Curiao, T., Ferreira, Alonso, H., and Gready, J. E. (2011). Dissemination of β-lactam resistance to animal and wildlife reservoirs through hospital, farm, and public water systems. Proc. Natl. Acad. Sci. U.S.A. 108, 6500–6514.

Antia, A. E., Bush, M., and Edwards, R. A. (2013). Transposons are the most abundant, most ubiquitous genes in nature. Nucleic Acids Res. 38, 4207–4217.

Bakkeren, Z., and Mand, D. (2011). Vibrio cholerae utilizes SOS and immunity in response to a wide range of antibiotics: a route towards resistance. Antonie Van Leeuwenhoek 99, 745–751.

Babaláš, J., Rollal, A. B. A., Funka, R. A., McAfee, T. F., and Ziese, L. (2013). Implications of global climate change for the assessment and management of human health risks of chemicals in the natural environment. Environ. Toxicol. Chem. 52, 62–78.

Ballou, J. C., Longo, J. M., Clímaco, E. C., and Hall, R. M. (2011). Distribution of the BlaTEM gene and blaTEM-containing transposons in common Escherichia coli. Antonie Van Leeuwenhoek 99, 731–736.

Baquero, F., and Nembela, C. (2012). The microbiome as a human organ. Clin. Microbiol. Infect. 18, 2–4.

Baquero, M.-R., Galán, J. C., Del Carmen Tartiere, M., Cantón, R., Coque, T. M., Martínez, J. L., et al. (2008). Increased mutation frequencies in Escherichia coli isolates harboring extended-spectrum beta-lactamases. Antonie Van Leeuwenhoek 94, 4754–4776.

Barlow, M., and Hall, B. G. (2012). Origin and evolution of the AmpC beta-lactamases of Citrobacter pa seols. Antonie Van Leeuwnhoek 94, 1195–1202.

Barlow, M., and Hall, B. G. (2012). Evolution of the AmpC beta-lactamases of Citrobacter pa seols. Antonie Van Leeuwenhoek 94, 1195–1202.

Barlow, M., and Hall, B. G. (2012). Evolution of the AmpC beta-lactamases of Citrobacter pa seols. Antonie Van Leeuwenhoek 94, 1195–1202.

Baquero, F., Coque, T. M., and de la Cruz, F. (2011). Ecology and evolution as targets: the need for novel eco-eco drugs and strategies to fight antibiotic resistance. Antonie Van Leeuwenhoek 99, 3649–3661.

Baquero, F., Martínez, L.-J., and Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments. Curr. Opin. Biotecnol. 19, 266–267.

Baquero, F., and Sepúlveda, M. C. (1997). Selective compartments for resistant microorganisms in antibiotic gradients. Bioenerg. Biofuel 5, 715–736.

Baquero, F., and Nembela, C. (2012). The microbiome as a human organ. Clin. Microbiol. Infect. 18, 2–4.

Baquero, M.-R., Galán, J. C., Del Carmen Tartiere, M., Cantón, R., Coque, T. M., Martínez, J. L., et al. (2008). Increased mutation frequencies in Escherichia coli isolates harboring extended-spectrum beta-lactamases. Antonie Van Leeuwenhoek 94, 4754–4776.

Barlow, M., and Hall, B. G. (2012). Origin and evolution of the AmpC beta-lactamases of Citrobacter pa seols. Antonie Van Leeuwenhoek 94, 1195–1202.

Becker, J., Eisenhauer, N., Scheu, S., and Josset, A. (2012). Increasing antagonistic interactions cause bacterial communities to collapse at high diversity. Ecol. Lett. 15, 468–478.

Benedetti, K., and Davies, J. (1973). Aminoglycoside antibiotic-inactivating enzymes in actino mycetes similar to those present in clinical isolates of antibiotic-resistant bacteria. Proc. Natl. Acad. Sci. U.S.A. 70, 2228–2230.

Bertola, F., and Ramsey, P. B. (2011). Within-genome evolution of REFPINs: a new family of marine mobile DNA in bac tera. FEMS Genet. 7:e1002132. doi: 10.1103/journal.pgen.1000498.

Binani, A. K., Liu, X., Djordjevic, B. R., and Hall, B. G. (2010). Transposons related to Tn916 in IncFII plasmids in multiple antibiotic resistant Salmonella enterica serovar Typhimurium from Australian animals. Microbes. Res. 166, 197–202.

Cantón, R., and Coque, T. M. (2004). The CTX-M beta-lactamase pan dom. Curr. Opin. Microbiol. 7, 606–675.

Cantón, R., Mira, N., Sánchez, M., and Baquero, F. (1999). MIC distribution and in vivo effect of LE35328: a study of vancomycin-susceptible and Vanc-type and Vanc-type enterococci obtained from intensive care unit patient surveillance cultures. Clin. Microbiol. Infect. 5, 554–559.

Carroll, A. (2009). Persistence plasmid families in Enterobacteriaceae. Antonie Van Leeuwenhoek 93, 2227–2238.

Carroll, A., Esguerra, L. E., and Suenza, V. (2005). A genomic population gen etics analysis of the pathogenic enteric ecytole infections island in Escherichia coli: the search for the unit of selection. Proc. Natl. Acad. Sci. U.S.A. 102, 1542–1547.

Chambers, H. F., and DeLeo, F. R. (2009). Waves of resistance: Staphylococcus
transfer of transposons, integrases, and gene cassettes between bacterial species. PLoS Pathog. 8:e1002857. doi: 10.1371/journal.ppat.1002857

Doreljargal, W. F. and Zhuchenyaev, O. (2010). Metagenomics and the units of biological organization. Front Microbiol 60: 102–112.

Doyle, M., Fookes, M., Ivens, A., Mangan, M. W., Watson, J., and Dveyman, C. J. (2007). An H-NS-like histone protein aids horizontal DNA transmission in bacteria. Science 315, 251–252.

Doyle, M. J., and O’Malley, A. M. (2007). Metagenomics and biological ontol. ety. Philos. Trans. R. Soc. B. 362: 854–866.

Fajardo, A., and Martinez, J. L. (2008). Antibiotics as signals that trigger specific bacterial responses. Gov. Open. Biol. 11: 164–167.

Fajardo, A., Martínez-Martín, N., Cadillo, M., Galán, J. C., Ouyeld, B., Matinós, S., et al. (2008). The neglected intrinsic resistomes of bacterial pathog. rns. PLoS ONE 3:e1619. doi: 10.1371/journal.pone.0001619

Faul, K., and Raes, J. (2012). Microbial interactions: from networks to models. Nat. Rev. Microbiol. 10: 526–539.

Faul, K., Sahinpongoussi, I. F., Izard, J., Segura, N., G sau, D., Raes, J., et al. (2012). Microbial co-resistance relationships in the human microbiome. PLoS Comput. Biol. 8:e1002800. doi: 10.1371/journal.pcbi.1002800

Fernández-Alvarino, C., Singer, R. S., and Johnson, T. J. (2011). Comparative genomics of multidrug resistance encoding IncI/C plasmids from commensal and pathogenic Escherichia coli from multiple animal sources. PLoS One 6:e22413. doi: 10.1371/journal.pone.0022413

Fondl, M., and Farf, R. (2010). The horizontal flow of the bacte. rial resistant genes in people with nosocomial enterococcal infection. Int. J. Evol. Biol. 2012:5228–5242.

Forssberg, K. I., Reyes, A., Wang, B., Schiödt, E. M., Sommer, M. O. A., and Dantas, G. (2012). The shared antibiotic resistosome of soil bacteria and human pathogens. Science 337, 1107–1111.

Froushi, F., Guinane, C. M., Hussey, S., Wall, R., Ryan, C. A., Dempsey, E. M., et al. (2012). High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microorganisms following parental antibiotic treatment with ampicillin and gentamicin. Antonie. nsc. Biotechnol. Lett. 36, 501–506.

Froushi, F., Guinane, C. M., Hussey, S., Wall, R., Ryan, C. A., Dempsey, E. M., et al. (2012). High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microorganisms following parental antibiotic treatment with ampicillin and gentamicin. Ant. l. 36, 501–506.

Froushi, F., Guinane, C. M., Hussey, S., Wall, R., Ryan, C. A., Dempsey, E. M., et al. (2012). High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microorganisms following parental antibiotic treatment with ampicillin and gentamicin. Ant. l. 36, 501–506.

Froushi, F., Guinane, C. M., Hussey, S., Wall, R., Ryan, C. A., Dempsey, E. M., et al. (2012). High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microorganisms following parental antibiotic treatment with ampicillin and gentamicin. Ant. l. 36, 501–506.

Froushi, F., Guinane, C. M., Hussey, S., Wall, R., Ryan, C. A., Dempsey, E. M., et al. (2012). High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microorganisms following parental antibiotic treatment with ampicillin and gentamicin. Ant. l. 36, 501–506.

Froushi, F., Guinane, C. M., Hussey, S., Wall, R., Ryan, C. A., Dempsey, E. M., et al. (2012). High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microorganisms following parental antibiotic treatment with ampicillin and gentamicin. Ant. l. 36, 501–506.
Guglielmini, J., Quintais, L., García-Guerin, E., Cambray, G., Sanchez-Graessler, J., Qin, Y., Zhong, H., Hamprecht, A., Poirel, L., Göttig, S., Hallatschek, O. (2012). Bacteria evolve. Front. Microbiol. 3, 127–132.

Humphrey, R., Thomson, N. H., Thomas, C. M., Broks, K., Sanders, M., Dufour, A. A., et al. (2012). Fitness of Escherichia coli strains carrying expressed and partially silent IncN and IncP plasmids. BMC Microbiol. 12, 53. doi: 10.1186/1471-2180-12-53

Jeffery, I. R., Glasson, M. J., T’Oile, P. W., and Shanahan, F. (2012). Categorization of the gut microbiota: ecometatypes or metagenomes? Nat. Rev. Microbiol. 10, 591–592.

Jones, R. V. (2010). The human gut mobile metagenome: a metaspace perspective. Gut Microbes 1, 415–413.

Kholodii, G., Minlma, S., Petters, M., and Minakukina, S. (2013). Tn5000 from the Siberian permafrost is most closely related to the ancestor of Tn21 prior to integron acquisition. FEMS Microbiol. Lett. 230, 235–237.

Kondrashov, F. A., Rogozin, I. B., and Koonin, E. V. (2003). The use of CRISPR in type I and type III systems. Curr. Opin. Microbiol. 6, 363–369.

Lewontin, R. C. (1970). The units of selection. Am. Zool. 1, 1–18.

Ley, R. K., Turnbaugh, P. J., Klein, S., and Gordon, J. I. (2006). Microbial ecology: human gut microflora associated with body mass index. Nature 444, 1022–1023.

Li, J. V., Asensio, H., Buetin, M., Kimura, J., Iida, C., Le Roux, C. W., et al. (2011). Metabolic surgery profoundly influences gut microbial-host metabolic cross-talk. Gut 60, 1214–1223.

Lobbert, C. A., Hall, R. M., and Summers, A. O. (1999). Transposon Tn21. Flagship of the flotting mobile element. Mol. Microbiol. 36, 507–512.

Liu, Y., Yu, X., Voon, Y., and Horste, C. J. (2010). A brief overview of Escherichia coli O157:H7 and its plasmid O157. J. Microbiol. Biotechnol. 20, 5–14.

Linares, J. F., Gristianou, I., Baquero, F., and Martinez, J. I. (2006). Antibiotic resistance in pathogenic protozoa. Int. J. Antimicrob. Agents 28, 5–14.

Lloyd, E. (2008). “Units and levels of selection.” In The Stanford Encyclopedia of Philosophy, ed. N. Zalta. Online. Available from: http://plato.stanford.edu/entries/selection/ 

Loeff, T., and Allen, H. K. (2012). Cell-wall effects of antibiotics on mammalian gut microflora. Gut Microbes 3, 655–667.

Loeff, T., Johnson, T. A., Allen, H. K., Barlos, D. O., Ali, D. F., Stuhlfi, R. D., et al. (2012). In-cell antibiotic effects on the innate intestinal microflora. Proc. Natl. Acad. Sci. U.S.A. 109, 1638–1638.

Luo, C., Walk, S. T., Gordon, D. M., Feldgarden, M., Tsui, J. M., and Konstantinidis, K. T. (2011). Genome sequencing of environmental Escherichia coli expands our understanding of the ecology of the species. Proc. Natl. Acad. Sci. U.S.A. 108, 10242–10247.

Long, K. S., Davison, J. L., Xia, W., and Johann, T. J. (2012). Transmembrane swapping of pARM00822, a blactm-2-positive broad-host-range IncA/C plasmid. Appl. Environ. Microbiol. 78, 3379–3381.

Lawrence, J. G. (1997). Selfish sporulation, and speciation by gene transfer. Trends Microbiol. 5, 555–559.

Levin, B. R. (2010). Stability versus costly plasmids: population dynamics, and the conditions for establishing and maintaining CRISPR-mediated adaptive immunity in bacteria. Proc. Natl. Acad. Sci. U.S.A. 106, 10171–10177.

Lewintin, R. C. (1970). The units of selection. Am. Zool. 1, 1–18.

Ley, R. K., Turnbaugh, P. J., Klein, S., and Gordon, J. I. (2006). Microbial ecology: human gut microflora associated with obesity. Nature 444, 1022–1023.
Baquero et al. | Multi-level population biology of resistance

www.frontiern.org
March 2013 | Volume 4 | Article 13 | 13

speciation of the model bacterial species. Proc. Natl. Acad. Sci. U.S.A. 108, 7280–7285.

Lupu, A., Gaynet, S., and Benben-
denik, T. E. (2012). Origins and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. Front. Microbiol. 3:18. doi: 10.3389/fmicb.2012.00018

Maiden, M. C. (1998). Horizontal genetic exchange, evolution, and spread of antibiotic resistance in bac-
teria. Curr. Opin. Infect. Dis. 11, 512–520.

Marchi, J. R. (2010). Polyketide and fatty acid synthetase of the human gut. Appl. Environ. Microbiol. 75, 43–52.

Marchi, J. R. (2010). Shifting from a gene-centric to metabolite-centric strategy to determine the core gut microbiome. Bmg 2, 309–314.

Marraffini, L. A. and Sontheimer, E. J. (2010). CRISPR interference: RNA-directed adaptive immunity in bacte-
ria and archaea. Nat. Rev. Genet. 11, 186–198.

Martínez, J. L., Martinez, J. L. (2008). Antibiotics and metabolic regulation of antibiotic resistance. Proc. Natl. Acad. Sci. U.S.A. 95, 958–965.

Martínez, J. L., Baquero, F., Roberts, A. P., and Peters, J. E. (2009). Tn7 and IS5075-containing Tn1696::Tn1 and IS5075-

Tn21. Antimicrob. Agents Chemother. 54, 825–834.

Noriega, A., Comas, I., Baquero, F., Cantón, R., Coque, T. M., Moya, A., et al. (2010b). Evolutionary tra-
ductions of beta-lactam-CT1 clades: studying the evolution of antibiotic resistance. PLoS Pathog. 6:e1000775. doi: 10.1371/journal.ppat.1000775

Parks, A. R., and Peters, J. E. (2009). Tn7 family of immunity regions in Gram-negative bacteria: the modular structure of bacterial mobile elements, in The Horizontal Gene Pool: Bacterial Plasmids and Gene Spread, ed. C. M. Thomas (Harwood Academic Press), 135–168.

Phelps KE, 2012. doi: 10.1371/journal.

Parks, O. S., Mazzocco, I., Narvaez, I., and Tsai, A. (2009). “Gene recruiters and transporters: the modular struc-
ture of bacterial mobile elements,” in The Horizontal Gene Pool: Bacterial Plasmids and Gene Spread, ed. C. M. Thomas (Harwood Academic Press), 135–168.

Petró, M., Gorlenko, Z., and Mindlin, S. Z. (2010). Evolutionary trajectories of beta-lactamase CTX-M-1 cluster enzymes: predicting antibiotic resistance. PLoS Pathog. 6:e1000775. doi: 10.1371/journal.ppat.1000775

Quinn, J. P., and Nordmann, P. (2011). The IncP-1 plasmid back-
ground. FEMS Microbiol. Rev. 35, 96–110.

Riccardi, M. C., Monira, S., Nakamura, S., Gotoh, K., Tanaka, T., Kato, C., et al. (2010a). CRISPR interference: RNA-

neighbours and archaea. FEMS Microbiol. Lett. 52, 1257–1263.

Riccardi, M. C., Monira, S., Nakamura, S., Gotoh, K., Tanaka, T., Kato, C., et al. (2010a). CRISPR interference: RNA-

neighbours and archaea. FEMS Microbiol. Lett. 52, 1257–1263.

ROSCHTEIN, A. (1999). “Intragene-

conflict,” in Levels of Selection in Evolution, ed. L. D. Koller (Princeton University Press), 121–132.

Partridge, S. R. (2011). Analysis of antibiotic resistance regions in Gram-
negative bacteria. FEMS Microbiol. Rev. 35, 820–835.

Partridge, S. R., and Hall, B. R. (2004). Complex multiple antibiotic and mercury resistance region derived from the v-rat of NEI (R308). Antimicrob. Agents Chemother. 48, 4250–4255.

Partridge, S. R., and Hall, B. R. (2003). The IS2111 family members Bgi322 and Bgi3077 have subtermi-
nal inverted repeats and target the transposase of Tn21 transposon. J. Bacteriol. 185, 6703–6714.

Partridge, S. R., and Isoldi, J. B. (2012). Genetic Contexts of MaDNM1. Antimicrob. Agents Chemother. 56, 6955–6967.

Perl, L., Battel, R. C., Artem, S., Madrid, C., Bulabolsi, C., and Juarez, A. (2011). Antibiotics shaping bacterial genome: deletion of an 191 flanked virulence determinant upon exposure to subinhibitory antibiotic concentrations. PLoS ONE 6:e27606. doi: 10.1371/journal.pone.0027606

Perret, G. G., Gonzalez, A., and Buck-
lind, A. (2007). Source-sink dynam-
ics shape the evolution of antibiotic resistance and its pleiotropic fitness cost. Proc. Natl. Acad. Sci. U.S.A. 274, 2351–
2356.

Petró, M., Gorlenko, Z., and Mindlin, S. (2009). Molecular structure and transduction of a multiple antibi-
otic resistance region of a Pse-
udomonas psychrophila phasmidsic plasmid. FEMS Microbiol. Lett. 290, 195–202.

Petró, M., Gorlenko, Z., and Mindlin, S. (2011). Transposon, a novel integron-containing antibiotic and chromate resistance transposon isolated from a fermentation bacterium. J. Bacteriol. 193, 327–332.

Penel, E., Benin, R. A., and Nord-
mann, P. (2004). Genetic support and diversity of acquired extended-spectrum β-lactamases in Gram-
negative rods. Infect. Genet. Evol. 12, 863–893.

Penel, E., Rodriguez-Martínez, J. M., Almazán, N., Dobretz-Ossenkopp, Y. J., and Nordmann, P. (2010). Characterization of DEI-
1, an integron-encoded metallo-

bacta renium (1991 to 2010). Antimicrob. Agents Chemother. 56, 3973–3976.

Novais, C., Flettis, A. R., Silveira, E., Baquero, F., Peire, L., Roberts, A. P., et al. (2012a). A set (M) hybrid from CTn5000 and CTn916 recom-
mobilization. Microbiology 158, 2718–
2711.

Novais, A., Ferrié, J., Silva, E., Baquero, F., Peire, L., Roberts, A. P., et al. (2012b). A set(M) hybrid from CTn5000 and CTn916 recom-
mobilization. Microbiology 158, 2718–
2711.

Novais, A., Peire, L., Peires, C., Pires, J., Montenegro, C., Domínguez, C., et al. (2012). Diversity and Coexistence in the core gut microbiome. Microbiol. 158, 2718–
2711.

Novais, C., Flettis, A. R., Silveira, E., Baquero, F., Peire, L., Roberts, A. P., et al. (2012a). A set(M) hybrid from CTn5000 and CTn916 recom-
mobilization. Microbiology 158, 2718–
2711.

Novais, A., Peire, L., Peires, C., Pires, J., Montenegro, C., Domínguez, C., et al. (2012a). Diversity and Coexistence in the core gut microbiome. Microbiol. 158, 2718–
2711.

Novais, A., Peire, L., Peires, C., Pires, J., Montenegro, C., Domínguez, C., et al. (2012a). Diversity and Coexistence in the core gut microbiome. Microbiol. 158, 2718–
2711.

Novais, A., Peire, L., Peires, C., Pires, J., Montenegro, C., Domínguez, C., et al. (2012a). Diversity and Coexistence in the core gut microbiome. Microbiol. 158, 2718–
2711.

Novais, A., Peire, L., Peires, C., Pires, J., Montenegro, C., Domínguez, C., et al. (2012a). Diversity and Coexistence in the core gut microbiome. Microbiol. 158, 2718–
2711.

Novais, A., Peire, L., Peires, C., Pires, J., Montenegro, C., Domínguez, C., et al. (2012a). Diversity and Coexistence in the core gut microbiome. Microbiol. 158, 2718–
2711.

Novais, A., Peire, L., Peires, C., Pires, J., Montenegro, C., Domínguez, C., et al. (2012a). Diversity and Coexistence in the core gut microbiome. Microbiol. 158, 2718–
2711.

Novais, A., Peire, L., Peires, C., Pires, J., Montenegro, C., Domínguez, C., et al. (2012a). Diversity and Coexistence in the core gut microbiome. Microbiol. 158, 2718–
2711.

Novais, A., Peire, L., Peires, C., Pires, J., Montenegro, C., Domínguez, C., et al. (2012a). Diversity and Coexistence in the core gut microbiome. Microbiol. 158, 2718–
2711.
Pupa, O., Haukka-Coro, E., Laran- dan, G., Martin, W., and Dagon, T. (2011). Directed networks reveal genomic barriers and DNA repair bypasses to lateral gene transfer among prokaryotes. Genome Biol. 21, 480–489.

Post, V., and Hall, M. R. (2009). Insertion sequences in the D1717 family that target the rphc recombination sites of integron-associated gene cassette families. FEMS Microbiol. Lett. 287, 182–187.

Petten, A., Nordin, P., Rendinaed, E., Ingrouille, P., and Pöld, L. (2013). A mosaic transposon encoding OXA- 48 and CTX-M-15 within pathovar resistance. J. Antimicrob. Chemother. 68, 476–477.

Qu, J., and Lam, J. C. (2011). Catalytic pathway for 2-nitroimidazole amides of a novel nitroreductase that also confers drug resistance. Environ. Microbiol. 13, 1010–1017.

Quinteria, S., Novoa, C., Arantes, P., Campos, J., Frestan, A. B., Abulkheir, A. et al. (2011). Targeting antibiotic resistance along the Silk Road. Clin. Microbiol. Infect. 17, e38–e40.

Rankin, D. J., Gatty, S. E. M., Novoa, C., Teusch, M., Tiedke, F., Rocha, E. P. C., and Brown, J. C., and Top, E. M. (2011). Genomics of Inc-F1 antibiotic resistance plasmids isolated from wastewater treat- ment plants provides evidence for a widely accessible drug resistance gene pool. FEMS Microbiol. Rev. 35, 449–472.

Saban, D., Van der Ancker, G. A., Rogers, I. M., Thomas, C. M., Brown, C. J., and Top, E. M. (2011). Broad-host-range plasmids from agricultural soils have Inc-F1 backbones with diverse accessory genes. Appl. Environ. Microbiol. 77, 7795–7805.

Santacruz, A., Collado, M. C., García- san Millán, A., Heilbron, K., and W . (2012). Is the pan-genome also a site with-gene-flow. Proc. Biol. Sci. 279, 2907–2916.

Santos-Silva, R. F., Mendonça, S. C. M., Pinhassi, J., et al. (2011a). Bacterial cooperation and私立er networks reveal that also confers drug resistance. FEMS Microbiol. Lett. 35, 707–715.

Sjölund, M., Bonnedahl, J., Hernan- dez, J., Bengtsson, S., Cederbrant, G., Cordero, O. X., David, L. A., and Alm, E. J. (2011). Ecology drives a global network of gene exchange connecting the human microbiome. Nature 480, 244–247.

Smith, J. (2012). Tragedy of the com- mons among antibiotic resistance plasmids. Evolution 66, 1289–1274.

Smyrnitis, M., Synploska, M., Zaloukis, P., Vounrymbit, P., Maj, A., Plaschke, A., and Brun, H. M. (2009). Horizontal gene transfer and mobile genetic elements in marine systems. Methods Mol. Biol. 552, 435–453.

Sommer, M. O. A., Dantas, G., and Church, G. M. (2009). Functional characterization of the antibiotic resistance reservoir in the human microbiota. Science 325, 1288–1291.

Sorensen, V., Povenska, J., and Suhailkhd, E. (2012). Novel vari- ants of AbiV resistance islands with a common backbone in Acinetobacteriovoslavi isolates of European clonal II. FEMS Microbiol. Lett. 36, 1969–1973.

Sorrell, J., Brown, E. S., and Reimann, J., Gill, J., et al. (2011). Major families of multifunctional plasmids from geographically and epide- miologically diverse staphylococci. G 7, 381–391.

Shi, P., Ji, S., Zhang, X.-X., Zhang, Y., Cheung, A., and Li, A. (2011). Metagenomic insights into informa- tion effects on microbial antibiotic resistance in drinking water. Water Res. 47, 111–120.

Shin, K. F., Mendonça, S. C. M., Carrillo, M., Reus, A. M., Geraldo, I., Trindade, S., et al. (2011). Peritoneal sepsis outcomes between conjugative plasmids and drug-resistant non-communicable disease. Pediatr. Infect. Dis. J. 30, 1032–1038.

Skippington, E., and Ragan, M. A. (2009). Functional genomics of Inc-F1 backbones with diverse accessory genes. Appl. Environ. Microbiol. 75, 10119–10126.

Sokol, T., Barrauld, O., Casadou, M., Dupré, C., and Poy-M. C. (2012). Lateral genetic transfer and mobile genetic elements in marine systems. Methods Mol. Biol. 552, 435–453.

Sommer, M. O. A., Dantas, G., and Church, G. M. (2009). Functional characterization of the antibiotic resistance reservoir in the human microbiota. Science 325, 1288–1291.

Staldal, T., Barrauld, O., Casadou, M., Dupré, C., and Poy-M. C. (2012). Lateral genetic transfer and mobile genetic elements in marine systems. Methods Mol. Biol. 552, 435–453.

Stokes, H. W., Martinez, E., Roy Chowd- hari, P., and Djordjevic, S. (2012). Directed networks reveal a novel gene-capturing system of the 21st century? Microbiol. Mol. Biol. Rev. 79, 296–316.

Stern, A., G. Guo, M. A., and Walh, T. R. (2011). Combinatorial events of insertion sequences and ICE in Gram-negative bacteria. FEMS Microbiol. Rev. 35, 912–925.

Tolmasky, M. E. (1990). Sequencing and expression of ada, bla, and qnr from the multiresistance transposon Tn1545. Plasmid 29, 218–226.

Ubeda, C., Misqués, E., Knecht, E., Loz, N., Norman, R. F. R., and Pedano, J. R. (2005). Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. Microbiol. Mol. Biol. Rev. 69, 856–844.

Vallin, Y., Goubard, M. J., De Vries, L. E., Abellán, J. L., and Francis, M. P. (2012). Metagenomics and development of the gut microbiota in infants. Clin. Microbiol. Infect. 18, 21–29.

Vander, A., Cantor, R.,钒dink, Barcus, M. P., Novoa, A., Galin, C. J., Abravado, A. et al. (2009). Spread of VT-MX-14: dissemination by Inc-F plasmids disseminated among Escherichia coli plp thermophages A, B, and D in Spain. J. Antimi- crob. Agents Chemother. 53, 5206– 5212.

Via, S. (2012). Divergence hitchhiking and the spread of genomic iso- lation during ecological speciation-column: gene flow. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 367, 435–460.

Vishnivetskaya, T. A., and Kathariou, S. (2005). Pore-forming toxins conserved in Enterobacteriaceae isolates from ancient Siberian permafrost and from contemporary surface habitats. Appl. Environ. Microbiol. 71, 6954– 6962.
Baquero et al. Multi-level population biology of resistance

Wilkman, S. A. (1961). “The role of antibiotics in nature,” in Perspectives in Biology and Medicine, Vol. 4 (Chicago: University of Chicago Press), 271–287.

Wahl, T. R. (2006). Combinatorial genetic evolution of multiresistance. Curr. Opin. Microbiol. 9, 476–482.

Worren, J. H. (2011). selfish genetic elements, genetic conflict, and evolutionary innovation. Proc. Natl. Acad. Sci. U.S.A. 108, 10863–10870.

Wade, B., and Levin, B. M. (2011). Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. FEMS Microbiol. Rev. 35, 597–604.

Williams, R. J. L., Top, J., Van Schaik, W., Willems, R. J. L., Hanage, W. P., Walsh, T. R. (2006). Combinatorial.

Werren, J. H. (2011). Selfish genetic elements, genetic conflict, and evolutionary innovation. Proc. Natl. Acad. Sci. U.S.A. 108, 10863–10870.

Westbrook, E., and Levin, B. M. (2011). Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. FEMS Microbiol. Rev. 35, 597–604.

Willems, R. J. L., Hanage, W. P., Rosen, D. E., and Feil, E. J. (2011). Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. FEMS Microbiol. Rev. 35, 597–604.

Williams, R. J. L., Top, J., Van Schaik, W., Lewis, H., Bonten, M., Antia, J., et al. (2012). Restricted gene flow among hospital subpopulations of Enterococcus faecalis. Mol. Microbiol. 89, 0048-6673.

Williams, L. E. W., Wirth, J., Hilliard, V. C., and Summers, A. O. (2012). Large plasmids of Escherichia coli and Salmonella encode highly diverse arrays of accessory genes on common replicon families. Plasmid 69, 36–48.

Woodford, N., Rutten, J. F., and Livermore, D. M. (2011). Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol. Rev. 35, 726–755.

Wissniewski, R. A. F., and Waldor, M. K. (2010). Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. Nat. Rev. Microbiol. 8, 552–563.

Wright, M. S., Baker-Austin, C., Lindley, A. H., Stepanauskas, R., Stokes, H. W., and McArthur, J. V. (2008). Influence of industrial contamination on mobile genetic elements: implications for the spread of antibiotic resistance in aquatic bacterial communities. ISME J. 2, 417–428.

Yim, G., Wang, H. H., and Davies, Z. A., and Ellis, M. (2011). The Anthropocene: a new epoch of geological time? Philos. Trans. A Math. Phys. Eng. Sci. 369, 835–841.

Zhang, G., Lambert, G., Liao, D., Kim, H., Robin, K., Tseng, C., et al. (2011). Acceleration of emergence of bacterial antibiotic resistance in connected microenvironments. Science 333, 1794–1797.

Zheng, Y., Antin, B. F., Roberts, R. J., and Kauf, S. (2004). Segmentally variable gene: a new perspective on adaptation. PLoS Biol. 2, e81. doi: 10.1371/journal.pbio.0020081

Zhou, X., Devosch, J., Fox, R., Top, E. M., and Krebs, S. M. (2012). On the meaning and estimation of plasmid transfer rates for surface-associated and well-mixed bacterial populations. J. Theor. Biol. 306, 144–152.

Zhang, Y., Wozniak, R. A. F., and Waldor, M. K. (2011). Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol. Rev. 35, 726–755.

Zalasiewicz, J., Williams, M., Haywood, A., and Ellis, M. (2011). The Anthropocene: a new epoch of geological time? Philos. Trans. A Math. Phys. Eng. Sci. 369, 835–841.