Integron involvement in environmental spread of antibiotic resistance

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Bacterial evolution has largely been shaped by the high plasticity of bacterial genomes, leading to their adaptation to most ecosystems. This ability to exchange and rearrange genomic sequences to gain new traits has been extensively demonstrated with the bacterial resistance to antibiotics. Today, the increasing rate of antibiotic resistant bacteria is a major public health issue (Davies and Davies, 2010). During the last decade, several studies have underlined the environmental resistome as a source of resistance genes of clinical interest (D’Costa et al., 2006; Aminov and Mackie, 2007; Martínez, 2008; Wright, 2010). While mutation events contribute to the bacterial adaptation, horizontal gene transfer seems to be the main cause of the rapid proliferation of antibiotic-resistance genes across a wide diversity of bacteria. Much of this horizontal gene transfers have been shown to occur in the environment (Aminov, 2011). Nevertheless, the diversity of mobile genetic elements currently described (Wozniak and Waldor, 2010; Bertels and Rainey, 2011), shows that beyond horizontal gene transfer, the loss and acquisition of functional modules are an important part in the processes of rapid bacterial adaptation and development of resistance. Integrons are one of the genetic elements involved in the adaptation of bacteria. We address the question of the involvement of integrons in the environmental spread of antibiotic resistance. More specifically, the anthropogenic impacts, which have been shown to be involved in the antibiotic-resistance spread in the environment, and the role of integrons in this process.

INTEGRONS: GENERALITIES

Integrons are bacterial genetic elements able to promote acquisition and expression of genes embedded within gene cassettes (GCs; Stokes and Hall, 1989). The definition of an integron is based on a functional platform (also called 5′ conserved segment, 5′CS), composed of three key elements: the intI gene, a specific recombination site attI, and a promoter, P<sub>c</sub> (Hall and Collis, 1995; Boucher et al., 2007; Figure 1). The intI gene encodes an integrase protein IntI1, which belongs to the family of tyrosine recombinases (Nunes-Düby et al., 1998).

The GCs are non-replicative mobile elements, which generally couple an open reading frame (ORF) with an attC site. GCs are integrated or excised from the functional platform by a site-specific recombination mechanism catalyzed by the IntI1 integrase. Two types of recombination can occur (Figure 1): (i) between attI and attC sites, resulting in the insertion of GCs at the attI site, and (ii) between two attC sites, leading to excision of the GCs (Mazel, 2006). The GCs can be found either as a linear form, included in an integron, or as a covalently closed circular free intermediate (Collis and Hall, 1992). GCs are usually promoterless and require the P<sub>c</sub> promoter for their expression as in an operon. The consequence of this system is that the last integrated cassette is the closest to the P<sub>c</sub> promoter (Collis et al., 1993; Collis and Hall, 2004), leading to the highest level of expression in the integron.

Two major groups of integrons have been described: “chromosomal integrons” (CIs), and “mobile integrons” (MIs). CIs are...
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Located on the chromosome of hundreds of bacterial species; in silico analysis showed that 17% of sequenced bacterial genomes exhibited such genetic arrangements (Cambray et al., 2010). CIs are often described in bacteria from marine or terrestrial ecosystems, such as Vibrio spp. and Xanthomonas spp., CIs have also been termed “super-integrons” (SIs) as they can carry up to 200 cassettes that mainly encode proteins with unknown functions. CIs may also carry cassettes without functional reading frames. MIs are not self-transposable elements but are located on mobile genetic elements such as transposons and plasmids, which promote their dissemination among bacteria. MIs contain a limited number of GCs (less than 10 GCs; Naas et al., 2001; GenBank DQ112222). The GCs described to date in these MIs usually encode antibiotic-resistance determinants. MIs are therefore sometimes also called “resistant integrons” (RIs) or “multidrug resistance integrons” (MRIs).

In this review, we will focus on MIs.

**CLASSES OF MIs**

Most MIs have been described in a wide range of Gram-negative bacteria, and only sporadically in Gram-positive bacteria (Martin et al., 1990; Nesvera et al., 1998; Nandi et al., 2004; Shi et al., 2006; Xu et al., 2010; Barraud et al., 2011). Based on the aminoacid sequence of the IntI protein, five classes of MIs have been described (Cambray et al., 2010). Classes 1, 2, and 3 are the most commonly detected. Classes 4 and 5 have only been detected once (Hochhut et al., 2001; GenBank AJ277063).

Class 1 MIs have been extensively studied due to their broad distribution among Gram-negative bacteria of clinical interest and are the most reported in human and animals. They have been described to be mainly associated with functional and non-functional transposons derived from Tn402. The non-functional type is the main common structural organization described in clinical isolates, and led some authors to call these class 1 MIs, “clinical integrons” (Gillings et al., 2008c). In addition, these structures are frequently embedded in plasmids or larger transposons, such as those of the Tn3 family (Tn21, Tn1696) allowing their dispersion (Labbate et al., 2009). The intI1 gene sequence is highly conserved among MIs found in clinical isolates, while it shows variability in MIs-containing environmental isolates (Gillings et al., 2008b). Furthermore, many class 1 MIs exhibit a 3′ region usually called 3′-conserved segment (3′CS). However, some authors consider using this 3′CS to detect MIs could create a bias in detection, since some MIs lack this sequence (Betteridge et al., 2011). The 3′CS is composed of a qacEΔ1 gene, a functional deletion of the qacE gene still conferring resistance to quaternary ammonium compounds (QACs; Paulsen et al., 1993), followed by a sulI gene conferring resistance to sulfonamides, and orf5 encoding a protein of unknown function.

Class 2 MIs are the second most described group. In most class 2 MIs, the intI2 gene is interrupted by a stop codon, resulting in a truncated and non-functional protein. This results in a stable GCs array, mainly composed of the GC dfrA1 (involved in the resistance to trimethoprim), sat2 (involved in the resistance to streptothricin), aadA1 (involved in the resistance to streptomycin and spectinomycin), and orfX (unknown function; Hansson et al., 2002). However, some class 2 MIs with a different GCs array have been described, probably resulting from the ability of the integrase of class 1 MIs to catalyze recombination at the attI2 site (Biskri and Mazel, 2003; Ahmed et al., 2005; Ramirez et al., 2005, 2010; Gassama Sow et al., 2010). Class 2 MIs are almost always associated with the Tn7 transposon and their derivatives, hence promoting their dissemination. Two class 2 MIs have been described recently with a functional integrase, one containing nine GCs encoding unknown function and the second one harbored the dfrA14 GC followed by a second novel GC in which a lipoprotein signal peptidase gene has been predicted (Barlow and Gobius, 2006; Márquez et al., 2008).

Only few class 3 MIs have been described. Although their role in clinical antimicrobial resistance is less important, environmental ecosystems could harbor an important pool of these elements (see below).

**MIs DISSEMINATION AND THEIR INVOLVEMENT IN ANTIBIOTIC RESISTANCE**

Antibiotic pressure has probably played an important role in the MIs selection and dissemination in bacteria. More than 130 GCs conferring resistance to antibiotics and more than 60 GCs of unknown functions have been described in MIs (Partrid et al., 2009). Genes involved in resistance to almost all antibiotic families are embedded in GCs, including beta-lactams, aminoglycosides, trimethoprim, chloramphenicol, fosfomycin, macrolides, lincosamides, rifampicin, and quinolones. In addition, the qac GCs, encoding resistance determinants to antiseptics of the QACs family, are commonly found in MIs. Studies have suggested that MIs were more prevalent in bacterial communities subjected to direct or indirect antibiotic pressure in clinical, agricultural, and
environmental settings (Skurnik et al., 2005; Daikos et al., 2007; Barlow et al., 2009; Luo et al., 2010; Kristiansson et al., 2011). Other factors, such as QACs or heavy metals have also been shown to be involved in the MIs dissemination, and thus probably playing a role in their spread before the antibiotic era (see below). More generally, it has been shown, when studying animal fecal E. coli, that human activity in the near vicinity increased the prevalence of MIs in these bacteria (Skurnik et al., 2006). Concerning the role of the antibiotic selective pressure, no published studies have demonstrated the direct in vivo selection of resistance through the acquisition of an integron. One study has demonstrated the in vivo selection of resistance through a rearrangement of the GCs array within a class 1 MI under antibiotic selective pressure (Hocquet et al., 2011).

Recent in vitro studies have shown that antibiotics are able to induce integrase transcription, both in CIs and MIs, via the SOS response. The SOS response is a global regulatory network controlled by the transcriptional repressor LexA and induced by stress leading to direct or indirect DNA damage, such as damage resulting from exposure to some widely used antibiotics (fluoroquinolones, beta-lactams, trimethoprim, aminoglycosides; Guerin et al., 2009; Baharoglu et al., 2010; Cambray et al., 2011). The activation of the SOS response in bacteria results in integrase overexpression, which leads to the raise of GCs recombination events.

Clinical, veterinary, and environmental surveys have shown that bacteria harboring MIs are often associated with multidrug-resistant (MDR) phenotypes (Bass et al., 1999; Leverstein-van Hall et al., 2003; Biyela et al., 2004; Nijssen et al., 2005; Laroche et al., 2009). However, the MDR profile could not be linked only to the antibiotic-resistance GCs carried by the MIs, but also to other resistance genes located on MIs-containing plasmids and transposons. This way, MIs could be co-selected with the plasmid- and/or transposon-associated antibiotic-resistance genes (Laroche et al., 2009; Li et al., 2010). For example, co-selection of class 1 MIs on plasmids harboring a tet gene (involved in the tetracycline resistance) in oxytetracycline-contaminated environments has been reported (Li et al., 2010).

The link between MIs and antibiotic resistance is still controversial since several studies present divergent conclusions (Hoyle et al., 2006; Smith et al., 2007). Furthermore many data have to be interpreted with caution. Indeed, biases in the study of links between MIs and antibiotic resistance could be generated by the selective choice of antibiotic-specific resistant strains, leading to misinterpretation. Finally, this relationship between MIs and antibiotic resistance has mainly been studied in bacteria of clinical or veterinary interest, such as those within the family Enterobacteriaceae.

Otherwise, the environment contains a wide range of bacterial species and cultivation methods only permit the isolation of a small fraction (around 1%; Amann et al., 1995). Techniques based on the study of the metagenome have thus been developed to avoid this limitation. The combination of culturing and metagenomics approaches on environmental ecosystems has highlighted the roles of MIs in antibiotic-resistance dissemination. Tables 1 and 2 present an extensive list of the studies that have quantified the occurrence of MIs in the environment, using either cultivation methods (Table 1) or cultivation independent methods (Table 2).

Genetic methods presented in this review quantify the abundance of integrase genes in the total DNA from different ecosystems. In order to normalize the quantity of gene to the total bacterial communities, most authors have used quantification of the ubiquitous bacterial 16S rRNA encoding genes. By dividing the abundance of integrase genes by the number of 16S rRNA genes, authors were able to demonstrate relative abundance. This ratio corresponds to the integrase genes proportion in the total bacterial communities. However, some authors have multiplying the ratio by the average number of copies of the 16S rRNA encoding genes per bacteria; which is approximately four (Klappenbach et al., 2001), and other authors present their results as percentages. In order to integrate all relative abundance data from diverse studies, results have been normalized to the same ratio for the purpose of this review and the relative abundance corresponds to the percentage of MIs per bacterial cell (Table 2).

**MIs IN THE ENVIRONMENT**

There is growing evidence that the environment plays a role in the spread of antibiotic resistance among pathogenic strains. Many questions have been raised concerning the impact of the release of antibiotics and antibiotic-resistant bacteria on the environment or on human and animal health (Aminov, 2010). The distribution of MIs, and especially the class 1 MIs, in the environment is a growing focus of attention, as illustrated by the recent publications presented in Tables 1 and 2.

MIs have been described in a wide range of natural ecosystems, both aquatic (e.g., lakes, rivers, estuaries) and terrestrial. However, their distribution has been investigated mainly in human-impacted environments such as amended soils and aquatic ecosystems influenced by urbanization, agriculture, aquaculture, industrial waste, and even in indoor and outdoor dust.

**MIs OCCURRENCE IN “NATURAL” ENVIRONMENTS**

Different authors have investigated the occurrence of class 1 MIs in ecosystems considered to be untouched or barely affected by anthropogenic influence, these are often termed “reference sites” and correspond in Tables 1 and 2 to the “clean area.”

Only a few teams have studied MIs abundance in soils. Gaze et al. (2011) reported a class 1 MIs relative abundance of 0.00576% (Table 2) by a metagenomic approach in soils with no history of organic amendment, whereas the same authors previously found no class 1 MIs in the bacterial culturable fraction, which was composed of Bacillaceae, Paenibacillaceae, and Pseudomonadaceae (Gaze et al., 2005). In a study on forest soils, 11 out of 24 isolated Enterobacteriaceae strains (45%) were found to contain class 1 MIs, but these MIs harbored no GCs (Srinivasan et al., 2008).

In aquatic environments, Wright et al. (2008) and Hardwick et al. (2008) found, using metagenomics approaches, a relative class 1 MIs abundance recovery from 0.02 to 4%, in estuarine and stream water/sediments/biofilms, and 2.65% in creek sediments (Table 2). Using cultivation-dependent methods, class 1 MIs were found in lake sediments, with a prevalence of 1–4% (Stokes et al., 2006; Gillings et al., 2008). Some studies investigated the GCs content of class 1 MIs. More often, one to three GCs were present, mainly encoding unknown function. GCs implied in the resistance to QACs (qac alleles) were also frequently described and
Table 1 | Bacterial prevalence of class 1 and 2 MIs in different ecosystems (results from cultivation-dependent studies).

| Ecosystem                  | Sample                  | Class 1 MIs% (n) | Class 2 MIs% (n) | Taxonomic affiliation    | Reference                           |
|----------------------------|-------------------------|------------------|------------------|--------------------------|-------------------------------------|
| Clean area                 | Lake Sediment           | 2.1 (n = 192)    | –                | NS                       | Stokes et al. (2006)                |
|                            | 1–3 (n = 192)           |                  |                  |                          | Gillings et al. (2008a)             |
| Soil/lake                  | Sediment                | 2–4 (n = 200)    | –                | NS                       | Stokes et al. (2006)                |
| Soil                       | Forest soil             | 45.8 (n = 24)    | –                | Enterobacteriaceae       | Srinivasan et al. (2008)           |
| Agricultural land          | 0 (n = 262)             |                  |                  | NS and QACsR             | Gaze et al. (2005)                  |
| Drinking water source      | 0 (n = 436)             |                  |                  | E. coli                  | Laroc et al. (2010)                 |
| Karst                      | US from the WWTP        | 0 (n = 75)       | –                | NS                       | Li et al. (2009)                    |
| Anthropogenic impacted***  | DS from the WWTP        | 3 (n = 65)       | –                | E. coli                  | Oberlé et al. (2012)               |
|                            | 14 (n = 322)            |                  |                  |                          | Li et al. (2009)                    |
|                            | 9.1 (n = 163)           |                  |                  | NS                       | Koczura et al. (2012)              |
|                            | 86.2 (n = 87)           |                  |                  |                          | Li et al. (2010)                    |
|                            | 58.1 (n = 43)           |                  |                  | MDR                      | Biyela et al. (2004)               |
|                            | 27.7 (n = 65)           |                  |                  | Enterobacteriaceae       | Ozgumus et al. (2009)              |
|                            | 13 (n = 32)             |                  |                  | Enterobacteriaceae R     | Guo et al. (2011)                   |
|                            | 44 (n = 313)            |                  |                  | Enterobacteriaceae R     |                                     |
|                            | 21 (n = 14)             |                  |                  | MDR                      |                                    |
|                            | 8.9 (n = 279)           |                  |                  | E. coli                  |                                    |
|                            | 29.6 (n = 54)           |                  |                  | E. coli                  |                                    |
|                            | 76 (n = 183)            |                  |                  | MDR                      |                                    |
|                            | 23 (n = 87)             |                  |                  | Enterobacteriaceae R     |                                    |
|                            | 22.7 (n = 65)           |                  |                  | Enterobacteriaceae       |                                     |
|                            | 48.4 (n = 184)          |                  |                  | Enterobacteriaceae R     |                                    |
|                            | 6 (n = 50)              |                  |                  | Enterobacteriaceae       |                                     |
|                            | 36 (n = 50)             |                  |                  | Enterobacteriaceae       |                                     |
| Hospital                   | wastewater              | 54.9 (n = 302)   | –                | Enterobacteriaceae R     |                                    |
|                            | 48.4 (n = 184)          |                  |                  | Enterobacteriaceae R     |                                    |
|                            | 36 (n = 50)             |                  |                  | E. coli                  |                                     |
|                            | 15.1 (n = 643)          |                  |                  | E. coli                  |                                     |
|                            | 11.5 (n = 174)          |                  |                  | Enterobacteriaceae       |                                     |
|                            | 3.7 (n = 378)           |                  |                  | Enterobacteriaceae R     |                                    |
|                            | 10 (n = 61)             |                  |                  | Enterobacteriaceae R     |                                    |
| Retirement home WWTP       | wastewater              | 4.6 (n = 131)    | 0 (n = 131)      | E. coli                  | Oberlé et al. (2012)               |
|                            | Raw effluent            | 40 (n = 94)      | 2 (n = 94)       |                          |                                     |
|                            | 74 (n = 95)             |                  |                  | Enterobacteriaceae       |                                     |
|                            | Treated effluent        | 6 (n = 50)       | 0 (n = 50)       | E. coli                  |                                     |
|                            | Activated sludge        | 15.1 (n = 643)   | 1.4 (n = 279)    | E. coli                  |                                    |
|                            | Raw effluent            | 74 (n = 95)      | 0 (n = 95)       | E. coli                  |                                    |
|                            | Treated effluent        | 40 (n = 94)      | 2 (n = 94)       |                          |                                     |
|                            | Activated sludge        | 74 (n = 95)      | 0 (n = 95)       |                          |                                     |
|                            | Raw effluent            | 36 (n = 50)      | 0 (n = 50)       |                          |                                     |
|                            | Treated effluent        | 40 (n = 94)      | 2 (n = 94)       |                          |                                     |
|                            | Activated sludge        | 7 (n = 35)       | 6 (n = 35)       |                          |                                     |
|                            | Raw effluent            | 74 (n = 95)      | 0 (n = 95)       |                          |                                     |
|                            | Treated effluent        | 40 (n = 94)      | 2 (n = 94)       |                          |                                     |
|                            | Activated sludge        | 74 (n = 95)      | 0 (n = 95)       |                          |                                     |

(Continued)
on the bacterial chromosome (Stokes et al., 2006; Gillings et al., lacking resistance genes in environmental samples and located of a qacE mobile functions of a to the association of an "ancient" chromosomal class 1 MI with spread of class 1 MIs among pathogenic bacteria. These events led recombination events, which facilitated the tion of evolutionary recombination events, which facilitated the emergence of evolutionary models was proposed and is now well docu-
emissions, and other rearrangements finally shaped the 3′ CS and antibiotic-resistance GCs. Nevertheless, a class 1 MI found in a Pseudomonas isolate recently recovered from 15,000- to 40,000-years-old Siberian permafrost with all the characteristics of a typical clinical class 1 MI, i.e., 5′CS and 3′CS, an antibiotic resistant GC (aadA2 encoding resistance determinants to streptomycin and spectinomycin), localization on a mobile element (Tn5045 transposon), contradicts this hypothesis (Petrova et al., 2011).

**ANTHROPOGENIC IMPACT ON MIs DISTRIBUTION**

**Rivers, seas, and lakes**
Water is the main vector of pollutants in the environment and thus has received most attention. Furthermore, water bodies have been underlined as ideal vectors for the antibiotic-resistance

Table 1 | Continued

| Ecosystem | Sample | Class 1 MIs% (n) | Class 2 MIs% (n) | Taxonomic affiliation | Reference |
|-----------|--------|-----------------|-----------------|----------------------|-----------|
| Raw effluent | 20.4 (n = 54) | – | LF Enterobacteriaceae and Aeromonas spp. | Ma et al. (2011a) |
| Treated effluent | 38.9 (n = 54) | – | – | – | | |
| Activated sludge | 30.9 (n = 81) | – | – | – | | |
| Raw effluent | 10 | – | E. coli** | Ferreira da Silva et al. (2007) |
| Treated effluent | 9.6 | – | – | – | | |
| Raw effluent | 19.1 (n = 204) | 4.9 (n = 204) | E. coli** | Figueira et al. (2011) |
| Treated effluent | 22.3 (n = 117) | 4.3 (n = 117) | – | – | | |
| Raw effluent | 16.4 (n = 49) | 0 (n = 49) | E. coli | Oberié et al. (2012) |
| Treated effluent | 8.5 (n = 49) | 2 (n = 49) | – | – | | |
| Treated effluent | 14 (n = 179) | – | NS | Li et al. (2009) |
| Treated effluent | 97.4 (n = 189) | – | – | – | | |
| Activated sludge | 33 (n = 109) | – | LF | Zhang et al. (2009b) |
| Reed bed | Activated sludge | 1 (n = 193) | – | Enterobacteriaceae** | Diaz-Mejía et al. (2008) |
| GWTP | Sediment | 14.9 (n = 127) | – | NS and QACsR | Gaze et al. (2005) |
| Soil | AC biofilm | 30 (n = 192) | – | NS | Gillings et al. (2008a) |
| | | 6.6 (n = 500) | 10.2 (n = 500) | NS + antibioticR | Byrne-Bailey et al. (2010) |
| | | 6.6 (n = 213) | – | tetR strains | Agero and Sandvang (2005) |
| Soil/manured soil | Manured soil | 89.3 (n = 56) | – | Enterobacteriaceae | Srinivasan et al. (2008) |
| Compost | Soil/pig slurry | 6.2 (n = 531) | 9.6 (n = 531) | NS + antibioticR | Byrne-Bailey et al. (2009) |
| Urban dust | Indoor | 5.6 (n = 136) | – | – | – | | |
| | outdoor | ≈2 (n = 183) | – | E. coli ** | Heringa et al. (2010) |
| | Outdoor | ≈15 (n = 116) | – | E. coli sulR | Diaz-Mejía et al. (2008) |

n, Number of isolated strains; LF, lactose fermenting; GWTP, ground water treatment plant; AC, activated carbon; NS, non-selective; US and DS, upstream (US) or downstream (DS) from the WWTP discharge in the receiving river; MDR, multidrug resistant; *coliform, Pseudomonas-like and Vibrio-like; **the taxonomic affiliation is based on 16S rRNA gene sequencing; ***impacted environment by urban and/or agricultural activities (sewage/industries/WWTP/animal husbandaries facilities/lakespools/organic amendment); †the WWTP specifically treated effluents from a penicillin production facilities; ‡the WWTP specifically treated effluent from an oxytetracycline production facilities; ′prevalence comprise both class 1 and 2 MIs; QACsR, quaternary ammonium compounds resistant strains; Enterobacteriaceae and Aeromonas spp.* refer to selected strains resistant to at least one antibiotic; ampr, ampicillin resistant, sulR, sulfonamide resistant, tetR, tetracycline resistant; ′′≈′′: values have been extracted from graph.

antibiotic-resistance GCs were rarely found (Gillings et al., 2008c, 2009a).

**ENVIRONMENTAL SOURCE OF MIs**
The class 1 MIs are ubiquitous elements naturally occurring in the environment, and different studies suggest that these elements emerged from ancestral environmental CIs (Rowe-Magnus et al., 2001; Mazel, 2006). Following the discovery of several class 1 MIs lacking resistance genes in environmental samples and located on the bacterial chromosome (Stokes et al., 2006; Gillings et al., 2008a), an evolutionary model was proposed and is now well documented (Gillings et al., 2008a; Labbate et al., 2009; Cambray et al., 2010; Stokes and Gillings, 2011). This model involves a succession of evolutionary recombination events, which facilitated the spread of class 1 MIs among pathogenic bacteria. These events led to the association of an “ancient” chromosomal class 1 MI with mobile functions of a Tn402-like transposon, and the acquisition of a qacE and sul1 genes. During this evolution, deletions, insertions, and other rearrangements finally shaped the 3′ CS of current class 1 MIs found in clinical isolates, as well as their inclusion in larger mobile platforms (plasmids and transposons), resulting in the spread of these elements among a broad range of bacteria, including pathogenic species (Gillings et al., 2008a; Labbate et al., 2009). Finally, it has been suggested that the class 1 MIs were probably widely distributed in Proteobacteria before the antibiotic era (Stokes and Gillings, 2011). These authors suggested that these class 1 MIs were unlikely to have GCs encoding antibiotic resistant determinants, and that they further evolved by acquisition of the 3′CS and antibiotic-resistance GCs. Nevertheless, a class 1 MI found in a Pseudomonas isolate recently recovered from 15,000- to 40,000-years-old Siberian permafrost with all the characteristics of a typical clinical class 1 MI, i.e., 5′CS and 3′CS, an antibiotic resistant GC (aadA2 encoding resistance determinants to streptomycin and spectinomycin), localization on a mobile element (Tn5045 transposon), contradicts this hypothesis (Petrova et al., 2011).
**Table 2** | Concentration and relative abundance of class 1 MIs in total community DNA from different ecosystems.

| Samples                              | Characteristics                  | Class 1 MIs (int/l·L⁻¹ or g⁻¹) | Class 1 MIs relative abundance given by authors | Relative abundance recalculated (%) | Reference |
|--------------------------------------|----------------------------------|--------------------------------|-------------------------------------------------|----------------------------------|-----------|
| Clean area a                         | River/lake                       | Waters                         | ≈10⁴                                            | ≈0.01 c                          | 4         | Wright et al. (2008) |
|                                      | Sediments                        | ≈10³–10⁴**                     | ≈0.001 c                                        |                                   | 0.4       |                      |
|                                      | Biofilm                          | ≈10⁻¹–10⁴**                    | ≈0.0001 c                                       |                                   | 0.04      |                      |
|                                      | Sediments                        | –                              | 2.65 (max = 8) d                               | 2.65 (max = 8)                    | Hardwick et al. (2008) |
|                                      | Creek, GWTP, and pond biofilms   | –                              | 4.5 (min = 1/max = 9) d                         | 4.5 (min = 1/max = 9)             | Gillings et al. (2008c) |
| Estuary                              | Waters                           | ≈10³–10⁴**                     | ≈0.0005–0.0001 c                               | 0.02–0.04                        | Wright et al. (2008) |
|                                      | Sediments                        | –                              | 0.0036 c                                       | 0.00576                          | Gaze et al. (2011) |
| Soil                                 | Anthropogenic-impacted area b    | Waters                         | Industrial polluted                             | ≈10⁻¹–10⁴ d                      | 0.0001 c  |                      |
|                                      | Sediments                        | –                              | 0.001 or 0.005 c                               | 0.0001 c                         | 0.04      |                      |
|                                      | Waters microcosms                | Initial (day 0)                | ≈10⁴                                            | ≈0.0001 c                        | 0.04      |                      |
|                                      | incubated at 23°C                | Cadmium, 0.1 mM                | ≈10⁻¹–10⁵                                      | ≈0.001 c                         | 0.4       |                      |
|                                      | during 7 days                    | Tetracycline, 30 mg L⁻¹        | ≈10⁻¹–10⁵                                      | ≈0.01 c                          | 4         |                      |
|                                      | Control                          | –                              | ≈10⁻¹–10³                                      | ≈0.0001 c                        | 0.04      |                      |
|                                      | Sediments                        | –                              | ≈1.5 (max = 4.294 for the sewage input) f       | 6 (max = 17)                     | Rosewarne et al. (2010) |
|                                      | Waters                           | Agricultural/clean area         | –                                               | ≈0.1 (mini = 0.02) f             | 0.4       | Luo et al. (2010)   |
|                                      | Sediments                        | Urban and agricultural influenced | ≈10⁻¹–10⁶                                      | ≈0.000005–0.005 c                | 0.002–2   | Zhang et al. (2009a) |
|                                      | River Waters                     | Urban and industrial polluted  | ≈10⁵                                            | between 10³ and 10⁵ g             | –         |                      |
|                                      | Lake Waters                      | Urban and industrial polluted  | ≈10⁶                                            | between 10³ and 10⁴ g             | –         |                      |
|                                      | Lake Sediments                   | Urban and industrial polluted  | ≈10¹¹                                          | between 10³ and 10⁴ g             | –         |                      |
|                                      | River Water                      | DS of a sewage output          | ≈1–6 × 10⁶                                     | ≈0.0005–0.005 c                  | 0.2–2     | Lapara et al. (2011) |
|                                      | River sediment                   | DS of a sewage output          | 2.4–2.5 × 10⁶                                 | –                                 | 0.05      |                      |
|                                      | Lake Water                       | Far DS of a sewage output      | ND                                              | ND                                | ND        |                      |
|                                      | Lake sediment                    | Far DS of a sewage output      | 4.9–7.7 × 10⁶                                 | –                                 | 0.02      |                      |
| Estuary                              | Waters                           | Industrial polluted            | ≈10⁴                                            | ≈0.001 c                         | 0.4       | Wright et al. (2008) |
| Environment          | Type              | 16S rRNA Copies (copies·g⁻¹) | Integron Copies (copies·g⁻¹) | Relative Abundance (%) |
|----------------------|-------------------|-----------------------------|-----------------------------|------------------------|
| Sediments            | Industrial polluted | ≈10⁴**                     | ≈0.0001 c                   | 0.04                   |
| WWTP                 | Raw effluent      | 2.04 × 10¹⁰                | 1.46 × 10⁵ g                |                         |
|                     | Treated effluent  | 1.20 × 10⁹                 | 1.48 × 10⁵ g                |                         |
|                     | Activated sludge  | 2.49 × 10¹²                | 1.17 × 10⁶ g                |                         |
|                     | Raw effluent      | ≈10¹¹ and 10¹²             | ≈10⁶ g                      |                         |
|                     | Treated effluent  | ≈10⁶                        | ≈10⁵ and 10⁶ g              |                         |
|                     | Activated sludge  | ≈10⁹                        | ≈10⁴ g                      |                         |
|                     | Digested sludge   | ≈10⁶ and 10¹¹              | ≈10¹ and 10² g              |                         |
|                     | Treated effluent  | ≈1.8 × 10⁷                 | 0.009 c                     | 3.6                    |
| Sludges              | Sludges           | 5.13 × 10⁸**               | ≈0.01 c                     | 4                      |
|                     | Digested sludge   | QACs + ATB polluted        | –                           | 1.616                  |
|                     | Treated sludges   | limed and dewatered        | –                           | 0.896                  |
|                     | Reed bed cores    | QACs polluted              | –                           | 1.04                   |
| GWTP                 | Biofilter         | Raw influent               | 8.0 and 9.28 × 10⁴         | 2870 and 309.3 g       |
|                     | Treated effluent  | 1.29 and 1.39 × 10⁴        | 194.9 and 1774 g           |                         |
|                     | Biofilms          | ND                         | 856.9 and 823 g            |                         |
| Soil                 | Pig slurry        | Initial                    | –                           | 0.0002 e               |
|                     | amendment         | 1 day PA                   | –                           | 0.01 e                 |
|                     | experiments       | 21 day PA                  | –                           | 0.008 e                |
|                     |                    | 90 day PA                  | –                           | 0.003 e                |
|                     |                    | 289 day PA                 | –                           | 0.004 e                |
|                     |                    | 1 month PA                 | –                           | 0.36 e                 |
|                     |                    | 12 month PA                | –                           | 0.02 e                 |
|                     |                    | 24 month PA                | –                           | 0.01 e                 |
| Animal waste         | Pig slurry        | Antibiotic (tylosin)       | –                           | 0.21 e                 |
|                     |                    | treated pig               | –                           | 0.21 e                 |

The relative abundance was calculated using the formula: \((\text{intI}/16S) \times 4 \times 10^9\), with four being the average number of copies of the gene encoding 16S rRNA per bacterial cell, according to the ribosomal RNA database (Klappenbach et al., 2001). **The results are expressed as copies·g⁻¹.

*Represent natural environments without hospital proximity. WWTP: agriculture or animal husbandries facilities or no historical organic amendment practice; + impacted environment by urban and/or agricultural activities (sewage/industrial/WWTP/animal husbandries facilities/fishponds/organic amendment); ` the relative abundance of integron was calculated per 16S rRNA encoding gene (intI/16S); † the relative abundance of integron was calculated per percent of bacterial cells (intI/16S) \times 10^9; ‡ the relative abundance of integron was calculated per percent of bacterial cells (intI/16S); § the relative abundance of integron was calculated per percent of 16S rRNA encoding gene (intI/16S); ¶ the relative abundance of integron was calculated per percent of total extracted DNA, PA, post-application; ND, not detected; GWTP, ground water treatment plant; QACs, quaternary ammonium compounds; ATB, antibiotic; DS, downstream; CAS, conventional activated sludge system; "≈": values have been extracted from graphs.

Zhang et al. (2009a) | Lapara et al. (2011) | Ma et al. (2011b) | Ghosh et al. (2009) | Diehl and Lapara (2010) | Byrne-Bailey et al. (2010) | Gaze et al. (2011) | Byrne-Bailey et al. (2010) |
dissemination (Lupo et al., 2012). Indeed, compared to the “natural” waters previously described, the prevalence of class 1 MIs-containing strains is higher in known polluted waters (Table 1). The variation of results observed among studies may depend on many factors, such as the selected bacterial species, the applied culture method (selective or not selective), as well as the sample characteristics (e.g., sediment or water, occurrence of rain events before sampling, close location of a wastewater discharged site). Using metagenomic approaches, urban and agricultural activities were positively associated with class 1 MIs. High concentrations of class 1 MIs were found in a Chinese river located in an urban and agriculturally influenced region, with around $10^7$–$10^8$ copies L$^{-1}$ and $10^3$–$10^4$ copies g$^{-1}$ of sediment (Luo et al., 2010), whereas in a clean area, concentrations of class 1 MIs were found to be around $10^3$ copies L$^{-1}$ and $10^3$–$10^4$ copies g$^{-1}$ of sediments (Wright et al., 2008). Zhang et al. (2009b) found a significant enrichment of class 1 MIs into the Yangtze river along its course through the Nanjing city, highlighting the impact of urban areas on rivers. Also, the relative abundance of class 1 MIs has been strongly correlated with the contribution of treated sewage output flow in the receiving river sediment (Rosewarne et al., 2010). This has been confirmed in a recent study carried out by Lapara et al. (2011), underlining the role of the wastewater treatment plant (WWTP) in the dissemination of class 1 MIs in the environment. Otherwise, fish farming has been shown to significantly elevate the prevalence of class 1 MIs in motile Aeromonads in river waters. The MIs identified contained dfr GCs encoding trimethoprim determinants, and their occurrence correlated with the administration of combined sulfonamide/trimethoprim drugs in freshwater fish farms (Schmidt et al., 2001). In polluted estuaries, the prevalence of class 1 MIs appears to be less important than in the aquatic ecosystems previously described, with values ranging between 2.7 and 14.7% (Laroche et al., 2009). Nevertheless, it has been observed that in anthropogenically impacted estuaries the relative abundance was around 10 times more than in an unpolluted reference estuary (Wright et al., 2008; Table 2). The authors did not show any influence of the tide, the relative MIs abundance being similar during ebb or flood tides.

Studies involving effluents of factories which produce antibiotics showed that antibiotic production could have an effect on the prevalence of MI-containing bacteria in the receiving river (Li et al., 2009, 2010). In these two studies, the impact differed according to the industry production, although the effluent treatment processes were equivalent in the two industries (anaerobic digestion following by activated sludge process without disinfection step). Indeed, the penicillin production effluents elevated the prevalence of class 1 MIs-harboring strains in the river, from 0% upstream of the discharge to 9.1% after the treated effluent was discharged whereas the oxytetracycline production effluents elevated the MIs prevalence in the river from 3% upstream of the discharge to 86.2% downstream (Li et al., 2009, 2010). Moreover, the authors suggested that some Pseudomonas sp. and Bacillus sp. isolates harbored simultaneously up to seven different class 1 MIs per bacteria, from the effluent of the oxytetracycline factory, as well as in the receiving river. In comparison, the bacteria from upstream of the WWTP harbored only one class 1 MI. More recently in a metagenomics study, authors observed a 6.7-fold enrichment of class 1 MIs in river sediments downstream of a treated WWTP effluent discharge point from an antibiotic production complex (Kristiansson et al., 2011).

However, the impact of anthropogenic activities is not limited to antibiotic pressure alone, since similar observations have been made in environments without sources of antibiotics input. An Australian study has correlated the rise of the relative abundance of class 1 MIs with environmental parameters (Hardwick et al., 2008). When the environmental conditions were more stressful to the bacteria, the relative abundance of class 1 MIs was higher. Industrial activities (mainly resulting in heavy metal contamination) also have been shown specifically to contribute to the increase of class 1 MIs relative abundance (Wright et al., 2008; Rosewarne et al., 2010). It has been shown that adding tetracycline or cadmium to a water stream in microcosm experiments increased the MIs relative abundance by a factor of between 10- and 100-fold (Wright et al., 2008). The co-selection of resistance genes with heavy metal such as mercury resistance has been previously described (Aminov and Mackie, 2007). Class 1 MIs have been described on the Tn21 transposon which also contains a mercury resistance operon (Liebert et al., 1999). Antiseptic agents as QACs have also been shown to be associated with a higher prevalence of class 1 MIs (Gillings et al., 2008c). In QACs contaminated reed bed, it was shown that 95% of the isolated strains with class 1 MIs harbored a qac gene (Gaze et al., 2005). Heavy metals and QACs are thus probably involved in MIs dissemination and may have contributed to the MIs selection before the antibiotic era (Stokes and Gillings, 2011).

In anthropogenic-impacted waters, an important diversity of GCs has been recovered (Rosser and Young, 1999; Roe et al., 2003; Henriques et al., 2006; Taviani et al., 2008; Laroche et al., 2009; Li et al., 2009; Ozgumus et al., 2009; Verner-Jeffreys et al., 2009; Kumar et al., 2010; Rosewarne et al., 2010; Chen et al., 2011). Resistance to almost all families of antimicrobials has been recovered with various GCs: aad, aac (confering resistance to aminoglycosides); bblaCARR-2, blaOXA, bblaT (confering resistance to beta-lactams); dfr (confering resistance to trimethoprim); catB (confering resistance to chloramphenicol); ereA (confering resistance to erythromycin); arr (confering resistance to rifampicin); and qac (confering resistance to QACs). Moreover, GCs with unknown function have been also commonly found. Several studies have characterized the total pool of integron GCs from environmental samples by using a PCR approach targeting only the GCs (attC sites) and not the integrate genes. They showed a huge GCs diversity mostly encoding unknown functions, and underlined the effect of both environmental and anthropogenic conditions on the GCs pool composition (Koenig et al., 2008, 2009, 2011; Huang et al., 2009; Elsaeed et al., 2011). Anthropic activity thus increases the prevalence of class 1 MIs in microbial communities. These anthropogenic environmental changes result in an increase in transferable genetic elements potentially harboring resistance genes, and an ability to capture new resistance genes from autochthonous hosts. Antibiotic-resistance genes located in mobile genetic elements (plasmids, transposons, integrons) have been suggested to be “genetic pollutants” representative of human activities (Martinez, 2009a). Moreover, anthropogenic stresses has been suggested to facilitate the possible transfer of chromosomal...
Wastewater treatment plants are the interface between human waste and both the aquatic and soil environments (Cattoir et al., 2008; Picão et al., 2008; Martinez, 2009a,b; Rahube and Yost, 2010).

Class 2 MIs are less prevalent than class 1 in polluted waters (0–7.4%), Table 1. In a culture-independent method survey, the low relative abundance rate of class 2 MIs from river has been underlined (Luo et al., 2010). These results suggest that their role in aquatic ecosystems is probably minor.

**Sewage and wastewater treatment plants**

Wastewater treatment plants are the interface between human waste and both the aquatic and soil environments (Figure 2). They collect effluents from diverse sources (such as hospital, private household, industries, animal husbandries), which contribute to the final ecosystem of the WWTP. These include the organics, chemicals, and microbiological wastes. Finally the WWTP ecosystem constitutes a “broth” where each element interacts with each other under a physical and chemical constraint resulting mainly in an organic degradation in the aqueous and solid phase. Microorganisms are key to the process resulting in organic and chemical degradation or transformation. The bacterial communities are organized in free biofilm entities (called bacterial flocs), which constitute the total biomass (the sludge). As suggested by many authors, the high bacterial density, due to the nutritional richness, indicates that WWTP are hot spots for horizontal gene transfers.

As previously described in the aquatic ecosystems, the low prevalence of class 2 MIs in WWTP suggests that their role is probably minor. Although less than 10 publications have reported class 3 MIs, 2 of them have been described in *Delftia sp. (D. acidovorans and D. tsuruhatensis)* isolated from activated sludge (Xu et al., 2007). These class 3 MIs contained GCs of unknown function. Moreover, using molecular approach, class 3 MIs were detected in effluents from an urban WWTP and a slaughter house WWTP (Moura et al., 2010). These findings suggest that even if class 3 MIs play a minor role in clinical microbiology, their role in the environment is probably more extensive.

The analysis of GCs content from wastewater ecosystems showed a huge diversity of genes encoding antibiotic resistance: resistances to aminoglycosides with *aad, aacA* GCs; to beta-lactams with *blaOXA*, *blaVIM-2*, *blaIMP*, *blaP1*, *blaGES-5*, and *blaGES-7* GCs; to trimethoprim with *dfr* gene GCs; to chloramphenicol with *cat* and *cml* GCs; to erythromycin with *ereA* and *estX* GCs; torifampicin with *arr* GCs; and to quinolones with *qnrVCA* GC (Tennstedt et al., 2003; Ferreira da Silva et al., 2007; Moura et al., 2007, 2012; Taviani et al., 2008; Li et al., 2009, 2010; Pellegrini et al., 2009, 2011; Zhang et al., 2009b, Xia et al., 2010; Girlich et al., 2011; Guo et al., 2011; Ma et al., 2011a; Scotta et al., 2011). A molecular approach describing the global pool of GCs in WWTP have shown a great diversity of GCs, mainly encoding for determinants implied in metabolic functions or unknown functions, suggesting the wide potential reservoir of GCs in WWTP (Moura et al., 2010).

**Efficiency of WWTP process to remove MIs**

While the WWTP reduced the bacterial load, it appears that the treatment is inefficient to remove both antibiotic resistant bacteria (Novo and Manaia, 2010; Luczkiewicz et al., 2010), and MIs-harboring bacteria.

As presented in Tables 1 and 2, the prevalence or relative abundance of MIs after the activated sludge process is not reduced, and is even often higher than in the raw effluent (Ferreira da Silva et al., 2007; Moura et al., 2007; Figueira et al., 2011; Ma et al., 2011a). These authors often concluded that activated process can remove bacteria, but do not reduce significantly the bacteria harboring class 1 MIs. When using abundance normalized to the total...
DNA amount, same observation have been done (Zhang et al., 2009a), however in another study, these authors found that the effluent treatment process decreased the MIs rate (Zhang et al., 2009b). Nevertheless, normalization to DNA amount is critical as total community DNA usually contains DNA of non-bacterial origin. The removal of bacteria bearing antibiotic-resistance genetics elements by the WWTP is a new challenge for the future. Several studies have investigated the efficiency of different advanced processes such as UV treatment, membrane biological reactors, and chlorination, to remove bacteria carrying antibiotic-resistance genes (Auerbach et al., 2007; Garcia et al., 2007; Kim et al., 2010; Huang et al., 2011; Munir et al., 2011), but no studies have examined the effects on MIs. Recently, hospital effluents were shown to be potential sources of dissemination of MIs in the sewage network (Guo et al., 2011). Oberlé et al. (2012) noted a decrease of the prevalence of class 1 MIs introduced in soil (Heuer and Smalla, 2007; Binh et al., 2009; Byrne-Bailey et al., 2010), see Table 2. Moreover, studies on sewage sludge and pig slurry amendment showed that even if the prevalence of class 1 MIs decreased after the particular amendment (2 years and 10 months, respectively), the prevalence was still higher than in control soils without amendment (around 100 times more; Byrne-Bailey et al., 2010; Gaze et al., 2011). Some authors studied the GCs array of class 1 MIs introduced in soil via manure amendment and mainly found streptomycin and spectinomycin resistance aadaA GCs (Heuer and Smalla, 2007; Binh et al., 2009). Class 2 MIs have been also identified from amended soils with relative high rates (Byrne-Bailey et al., 2009, 2010; Rodríguez-Minguela et al., 2009). The high antibiotics consumption in some animal husbandries, and their systemic application as food additives in the past, had probably significantly contributed to MIs dissemination in amended soils. Tschäpe (1994) showed that the streptomycin usage as food additive contributed to the dissemination of sat genes in amended soils via mobile genetic structures, such as the Tn7 transposon carrying a class 2 MI usually bearing a streptothricin-resistant sat2 GC.

Animal wastes (e.g., manure, poultry litter, and slurry) are the main vectors of MIs dissemination in soil. As recently reviewed by Heuer et al. (2011), only a few studies have investigated the reduction of some resistance genes following different processes, such as storage, composting, and anaerobic digestion (Chen et al., 2007, 2010; Heuer et al., 2008). Only composting was efficient in reducing the prevalence and absolute amount of erythromycin resistance genes (Chen et al., 2007). Concerning the MIs, one study reported that after 57 days of storage of manure at 20°C, the class 1 MIs GCs array electrophoresis gel profiles were almost identical to that at the beginning of the experiment; however the GCs contents was not investigated (Heuer et al., 2008).

**ROLE OF THE FOOD CHAIN**

The food chain probably also takes place in the transit of MIs from the environments to the human. Indeed, bacteria harboring MIs have been recovered from a variety of aquatic living organisms, such as in prawns, with an Enterobacter cloacae harboring a class 1 MI (Gillings et al., 2009b); or in Corbicula with a class 1 MIs relative abundance of 4% (Wright et al., 2008); and in oysters where the uncommon class 3 MIs prevailed (Barkovskii et al., 2010). Transfers of MIs between animals and human occur and have been well reviewed by Stokes and Gillings (2008); and in oysters where the uncommon class 3 MIs prevailed (Barkovskii et al., 2010). Transfers of MIs between animals and human occur and have been well reviewed by Stokes and Gillings (2011). Class 1 MIs have been also reported from biofilms of drinking water supplies (Tables 1 and 2; Gillings et al., 2008a; Zhang et al., 2009a). All these results underline the link, via the food chain, between the environmental MIs and the human or animal MIs.

**CONCLUSION**

As described in this review, MIs are efficient tools for bacterial adaptation and play a significant role in antibiotic resistance. Environmental studies demonstrated that anthropogenic impact lead to enrichment of class 1 MIs. More specifically, all factors leading to bacterial stress, such as antibiotics, QACs, or high concentrations of heavy metals resulted in the selection of class 1 MIs-harboring bacteria. Several hot spots of class 1 MIs dissemination have been identified, as agricultural manure amendment, WWTP, or industrial effluents. While these wastes are treated in varying degrees before their discharge, it appears that the current processes are inefficient to reduce MIs dissemination. This uncontrolled dissemination of MIs in the environment could represent a risk for human health.

**ACKNOWLEDGMENTS**

This work was supported by the regional council of Limousin. The authors wish to thank Colin Hunter and William Rawlinson for their help in critical reading of the manuscript.
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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 November 2011; accepted: 13 March 2012; published online: 09 April 2012.

Citation: Stalder T, Barraud O, Casella M, Dagot C and Ploy M-C (2012) Integron involvement in environmental spread of antibiotic resistance. Front. Microbiol. 3, 119. doi: 10.3389/fmicb.2012.00119

This article was submitted to Frontiers in Antimicrobials, Resistance and Chemotherapy, a specialty of Frontiers in Microbiology.

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