The intestinal microbiota is a complex environment that hosts $10^{13}$ to $10^{14}$ bacteria. Among these bacteria stand multidrug-resistant enterobacteria (MDRE), which intestinal densities can substantially vary, especially according to antibiotic exposure. The intestinal density of MDRE and their relative abundance (i.e., the proportion between the density of MDRE and the density of total enterobacteria) could play a major role in the infection process or patient-to-patient transmission. This review discusses the recent advances in understanding (i) what causes variations in the density or relative abundance of intestinal colonization, (ii) what are the clinical consequences of these variations, and (iii) what are the perspectives for maintaining these markers at low levels.

Keywords: carriage, concentration, extended-spectrum beta-lactamases, microbiota, antibiotics

The intestinal microbiota is mostly composed of peptidoglycans, especially if eukaryotes, such as fungi, are also present. However, the intestinal microbiota plays a role of an organ in humans and provides various benefits to its host. One benefit is the barrier effect or inoculation barrier, which comprises $10^6$ to $10^7$ colony-forming units (CFU) per gram of feces.
colonicization resistance, which refers to the ability of intestinal microbiota to prevent sustainable colonization by exogenous bacteria (Duchateau et al., 1977; Volgaard and Clasen, 1994). Most ingested bacteria only transit through the digestive tube and do not colonize the patient for a significant period of time (Buck and Cooke, 1969; Cooke et al., 1971). The barrier effect mainly relies on the fact that the endogenous intestinal microbiota leaves very few niches (available nutrients and attachment sites) for use by exogenous bacteria (Volgaard and Clasen, 1994). The intestinal microbiota is also involved in the maintenance of intestinal epithelial homeostasis (Balhoff-Nahum et al., 2004) and promotes angiogenesis (Stappenbeck et al., 2002). Moreover, a link between the intestinal microbiota and metabolic disorders has been proposed; in particular, low species diversity in the intestinal microbiota has been linked to medical conditions, such as obesity, inflammatory bowel diseases, atopy, and diabetes (Ley et al., 2005; Turnbaugh et al., 2006; Rouset and Marzuman, 2009; Qin et al., 2012).

THE INTESTINAL MICROBIOTA IS A VAST REPOSITORY FOR RESISTANCE GENES

Next-generation sequencing has not yet changed the way antibiotic resistance is investigated; perhaps because the resistance genes that may be harbored by subdominant bacteria remain inaccessible to the current sequencing methods (Lagier et al., 2012b). However, the intestinal tract is a major reservoir for antibiotic-resistant bacteria, including naturally resistant bacteria and those with acquired resistance-confering genes carried on mobile genetic elements such as plasmids, conjugative transposons or integrative and conjugative elements (ICEs; Sommer et al., 2009). The diversity of the resistance genes among intestinal bacteria (i.e., the intestinal resistome) cannot be effectively assessed by conventional methods based upon culture on antibiotic-supplemented agar media because most resistant intestinal bacteria cannot be cultured using conventional methods. The use of various culture media, atmospheric conditions and mass-spectrometry identification (“culturomics”) allowed the establishment of an inventory of bacterial species, including many new intestinal microbiota species, in numbers even greater than those discovered using pyrosequencing (Lagier et al., 2012a). Thus far, this technique has not addressed the question of global antibiotic resistance of the microbiota.

Currently, the best description of the diversity of resistance genes present has been obtained using culture-independent methods (Sommer et al., 2009; Sommer et al., 2009). Cloned the metagenome of feces samples into susceptible E. coli and plated it on agar media supplemented with various antibiotics. When applied to an overnight aerobic culture of feces, 95% of the identified genes had >90% nucleic identity with sequences in GenBank found in pathogenic bacteria. Indeed, the genes identified in the aerobic fraction had been reported, repeatedly, to occur on mobile genetic elements (NAC728; M. blautii, aac(3)-Ib, aac(6′)-Ib, blat-ace2) found in pathogenic bacteria, such as enterobacteria. Conversely, when applied to the feces metagenome with no previous aerobic culture, the average shared identity dropped to 60.7%. Thus, the intestinal resistome can be divided into (i) a "resident" resistome, composed of the resistance genes naturally present in permanent members of the intestinal microbiota, such as the beta-lactamase gene from Bacteroides spp., and (ii) a variable resistome, composed of exogenous genes present in transient bacteria or acquired by lateral transfer (Wellington et al., 2013). Enrichment of the variable resistome comes from ingestion of resistant bacteria through food (Ruimy et al., 2010) or fecal periar (Tangden et al., 2010). Although exogenous bacteria may not colonize because of the intestinal microbiota barrier effect (also called "resistance to colonization"), their resistance genes can be transferred to resident bacteria through horizontal gene transfer during transit (Duval-Allah et al., 1980).

MULTIDRUG-RESISTANT ENTEROBACTERIA: A FOCUS ON BETA-LACTAMS

Antibiotics have been extensively used since the 1950s with a parallel increase in the proportion of resistant bacteria (Clatworthy et al., 2007). Indeed, there are no more or fewer bacteria since antibiotics were initiated; yet, there are more resistant bacteria. Between 1950 and 1980, the continuous discovery of new and more potent antibiotics has conferred to medicine a constant advantage over the rise of bacterial resistance. As long as new antibiotics were made available on a regular basis, resistance was not a real problem because clinicians always had drugs to which bacteria were susceptible to treat patients. Still, resistance never slowed down and benefited from extensive international exchanges to spread worldwide (MacPherson et al., 2009). Meanwhile, the pipeline of new, effective antibiotics has nearly ceased (Spittell et al., 2004). The efficacy of beta-lactams, the most widely used antibiotic family worldwide, is now challenged by the spread of the so-called “bad bugs,” e.g., enterobacteria, Pseudomonas aeruginosa and Acinetobacter baumanii that produce wide-spectrum beta-lactamases (Table I; Peterson, 2009). Until the early 2000s, such resistant bacteria were isolated quasi-exclusively in healthcare structures and did not affect community patients. Successful interventions to control their spread in healthcare structures have been developed and are now part of usual care (Lucet et al., 1999). Yet, the situation has dramatically changed with the emergence and dissemination in the community of enterobacteria that produce CTX-M — type extended-spectrum beta-lactamases (ESBL; Pittout and Laupland, 2008). Occurrence of CTX-M beta-lactamases is especially prominent in developing countries, maybe because of uncontrolled antibiotic consumption and suboptimal hygienic living conditions (Rupke et al., 2009; Woertz et al., 2010).

Therapeutic options for patients infected by ESBL-producing enterobacteria (ESBL-E) remain limited to a few antibiotics, including carbapenems. Thus, the rise of ESBL-E fuels a cycle of increased carbapenem consumption. This cycle leads to the dissemination of carbapenem-resistant enterobacteria (CRE). Carbapenem resistance in enterobacteria occurs through either porin loss (Skurnik et al., 2010) or carbapenem-hydrolyzing enzyme (Carbapenemases), (Quinlan and Bush, 2007; Nordmann et al., 2009; Kumaarsamy et al., 2010; Poirel et al., 2012). There are two main concerns regarding CRE. First, carbapenemase are derivatives of class A, B, and D beta-lactamases, and some have been repeatedly recovered from patients with no recent history of hospitalization or travel abroad (Vaux et al., 2011) or in the community

http://www.ncbi.nlm.nih.gov
Table 1 | Main acquired beta-lactamases produced by Gram-negative bacilli from the intestinal microbiota.

| Name of beta-lactamase | Type of enzyme | Beta-lactam spectrum of hydrolysis | First-line alternative drugs | Bacterial hosts | Presence in community | Prevalence |
|------------------------|---------------|-----------------------------------|-----------------------------|----------------|-----------------------|------------|
| CTX-M gp 1             | ESBL          | PEN, CEPH, ATM                     | CBP, TGC, COL               | Ent, Psa, Acinetobac | Yes                    | Very high  |
| CTX-M gp 2             | ESBL          | PEN, CEPH, ATM                     | CBP, TGC, COL               | Ent, Psa                    | Yes                    | Very high  |
| CTX-M gp 25            | ESBL          | PEN, CEPH, ATM                     | CBP, TGC, COL               | Ent                      | Yes                    | High       |
| CTX-M gp 8             | ESBL          | PEN, CEPH, ATM                     | CBP, TGC, COL               | Ent                      | Yes                    | High       |
| CTX-M gp 9             | ESBL          | PEN, CEPH, ATM                     | CBP, TGC, COL               | Ent, Psa, Acinetobac      | Yes                    | Very high  |
| TEM-type ESBL          | ESBL          | PEN, CEPH, ATM                     | CBP, TGC, COL               | Ent, Psa, Acinetobac      | Yes                    | Very high  |
| IMP gp 1 and 2         | CP            | PEN, P + 1, CEPH, CBP              | TGC, ATM, COL               | Ent, Psa, Acinetobac      | No                     | Low        |
| KPC                    | CP            | PEN, CEPH, ATM, CBP                | TGC, COL                    | Ent, Psa                    | No                     | High       |
| NDM-1                  | CP            | PEN, P + 1, CEPH, CBP              | TGC, ATM, COL               | Ent, Psa, Vibrio           | Yes                    | High       |
| Diaz-4                 | CP            | PEN, P + 1, CBP                    | CEPH, TGC, COL              | Ent                      | Yes                    | High       |
| VIM gp 1 and 2         | CP            | PEN, P + 1, CEPH, CBP              | TGC, ATM, COL               | Ent, Psa, Acinetobac      | No                     | Low        |
| Cit-group AmpC (CMV2)  | AmpC          | PEN, P + 1, CEPH                   | TGC, ATB, CBP               | Ent, Psa, Acinetobac, Psa, | Yes                    | High       |
| Other plasmidic AmpC   | AmpC          | PEN, P + 1, CEPH                   | TGC, ATB, CBP               | Ent, Psa, Acinetobac, Psa, | No                     | Low        |

ESBL, extended-spectrum beta-lactamase; PEN, penicillins; CEPH, cephalosporins; ATM, aztreonam; CBP, carbapenems; TGC, tigecycline; COL, colistin; Ent, Enterobacteriaceae; Psa, Pseudomonas aeruginosa; Acinetobac, Acinetobacter baumannii; CP: carbapenemase; P + 1, penicillin + class A beta-lactamase inhibitor; AmpC, cephalosporinase.

Antibiotics as a cause of variations in the intestinal density of colonization of resistant bacteria

Quantitative impact: Loss of diversity

Antibiotic effects on the intestinal microbiota depend on (i) the colonic concentrations of the antibiotic (luminal and mucosal) and/or its active metabolites and (ii) the activity of these concentrations on the bacterial species present. The growth of susceptible bacteria will either be impeded (bacteriostatic effect), or they will be killed (bactericidal effect; Dethlefsen et al., 2008; Antonopoulos et al., 2009). Thus, the extent and persistence of the impact of antibiotics on the intestinal microbiota is highly drug-dependent (Nord et al., 1984; Taur et al., 2012). Even antibiotics of the same family and spectrum of activity can have a very different impact depending on their rate of intestinal excretion (Beaucham et al., 1986; Michal-Hamzchour et al., 1998). Using next-generation sequencing, Dethlefsen and Feltman (2011) precisely observed the fecal diversity of three healthy subjects during 300 days, during which they received 2 x 5-day courses of ciprofloxacin, at days 60 and 250. Ciprofloxacin caused a loss of diversity and a shift in community composition occurring within 3–4 days of drug initiation. This effect was somewhat surprising because most of the microbiota is composed of anaerobes that are weakly susceptible to ciprofloxacin (Nord and Edlund, 1989). However, concentrations of ciprofloxacin that accumulate in the colon during treatments (Fanth et al., 2009) are so high that they most likely overcame their minimal inhibitory concentrations. The perturbation created by antibiotic use took weeks to be resolved; furthermore, the composition of the intestinal microbiota remained altered from its initial state.

In newborns treated by a combination of ampicillin and gentamicin, the Actinobacteria and Firmicutes phyla, comprising bacteria with potential benefit (Bifidobacterium and Lactobacillus) were replaced by Proteobacteria, including Enterobacteriaceae (Fouhy et al., 2012). This effect could be of importance considering that Proteobacteria are enriched with mobile genetic elements, including antibiotic resistance encoding genes (Baquero et al., 2013). The increase of Proteobacteria was persistent after 8 weeks. Indeed, in patients undergoing allogeneic hematopoietic stem cell transplantation, metronidazole (an antibiotic with broad-spectrum activity against anaerobes) strongly reduced the diversity of the intestinal microbiota (Taur et al., 2012). In mice receiving a combination of amoxicillin, metronidazole, and bismuth, the composition of the intestinal microbiota was altered, but the perturbation was resolved within 2 weeks; in contrast, resolution took approximately 6 weeks for mice treated with cefoperazone, a wide-spectrum cephalosporin (Antonopoulos et al., 2009). The resilience of the composition of the intestinal microbiota is thus likely to be different according to the type of antibiotic given.
QUALITATIVE IMPACT: LOSS OF THE BARRIER EFFECT

As described above, the barrier effect is mainly exerted by anaerobes (Ducluzeau et al., 1977; Voilaard and Claesner, 1994). Thus, antibiotics with high activity against anaerobes, such as clindamycin, potently affect the capacity of the microbiota to prevent colonization by exogenous microorganisms (van der Waa et al., 1971). In the study by Donkey et al. (2008), 15 vancomycin-resistant enterococci (VRE)-carriers received antibiotics active against anaerobes, while 10 VRE-carriers received antibiotics poorly active against anaerobes. Strikingly, the average density of VRE (expressed in log CFU/g of feces) significantly increased (by 2.2 logs) in patients who received antibiotics that were active against anaerobes, whereas the average density decreased by 0.6 log in those patients receiving antibiotics with minimal activity against anaerobes. In a subsequent study, the same group has reported that the density of resistant Gram-negative bacilli also increased under exposure to antibiotics that were active against anaerobes (Bhalla et al., 2003). In the latter case, the resistant bacteria occupied niches that appeared to be left by anaerobes (Figure 1).

The barrier effect can also be studied by considering one species, such as E. coli. In healthy volunteers, the E. coli population is composed of a variable number of clones of different abundances: dominant and subdominant clones (Lidin-Janson et al., 1978). When these E. coli have different susceptibilities, antibiotic exposure will change their respective proportions and promote the growth of the resistant strains over that of the susceptible ones (Figure 1). This phenomenon has recently been reported for fluoroquinolones, which cause a sharp decrease in total counts of intestinal enterobacteria during treatments. The available niches can then be occupied by resistant enterobacteria that were initially present in low fecal concentrations (Fantin et al., 2009) or from a new acquisition (de Lastours et al., 2012). If the number of total enterobacteria remains unchanged, then the relative abundance of resistant enterobacteria increases (Figure 1).

CONSEQUENCES OF INCREASED DENSITY OF COLONIZATION ON INFECTIONS

ANTIBIOTICS INCREASE THE RISK OF INFECTIONS CAUSED BY RESISTANT ENTEROBACTERIA

Several studies have shown that patients with infections caused by resistant bacteria were more likely to have taken antibiotics recently (Ben-Amiti et al., 2009). Indeed, the link between antibiotic exposure and antibiotic-resistant infection could lie in the intestinal microbiota, as antibiotics would allow the overgrowth of resistant bacteria (i.e., increase the density of resistant bacteria). Interestingly, the association between antibiotic use and infections caused by resistant bacteria is also found for antibiotics with little effect on anaerobes, e.g., co-trimoxazole or quinolones, suggesting that it is not only the overgrowth of resistant bacteria within niches left empty by anaerobes that increases the risk of infection by resistant bacteria, but more likely the augmentation of the relative abundance of resistant bacteria (i.e., the augmentation of the proportion of resistant bacteria) within specific niches (Figure 1). MDRE are extensively antibiotic-resistant and not only to beta-lactams, but also to many other antibiotics including among others fluoroquinolones, aminoglycosides, or co-trimoxazole (Pitout, 2009). Thus, MDRE can overgrow and increase the risk of their involvement in further infection under almost any antibiotic exposure.

URINARY-TRACT INFECTIONS

Urinary-tract infections (UTIs) are the most common bacterial infections (Hooton, 2012) and are most often caused by enterobacteria, and especially E. coli. There is some evidence that in most cases, the infecting clone originates from the intestinal microbiota (Yamamoto et al., 1997), even if it cannot always be retrieved in the stool at the onset of the symptoms (Moreno et al., 2008). Specific strains of E. coli appear to have the ability (through virulence factors) to colonize the urethra and bladder, causing cystitis; others are able to further colonize the ureter to cause pyelonephritis (Pfo et al., 1995). According to this “virulence theory,” some
strains of *E. coli* could cause UTIs, even if they are subdominant in the intestines (low relative abundance). Alternatively, the relative abundance of the various clones of *E. coli* present in the feces may also play a key role in the pathogenesis of UTIs, in that the dominant clone of *E. coli* would have the maximum likelihood to colonize the urinary tract (Moreno et al., 2008). In 42 women with cystitis caused by *E. coli*, Moreno et al. (2008) compared the UTI-causing strain to 30 randomly picked colonies from concomitant stool samples. In 90% of the women, the urinary clones were found in the feces. In 71% of the women, the urinary clone that was present in the feces was also the dominant fecal clone. Urine clones mostly belonged to B2 and D phylotypes and had an increased content of urovirulence factors. For one given fecal clone, predictors of infection were that the urinary clone belonged to the B2 phylotype and was dominant. Thus, the findings of this study reconciled the dominance and the virulence theories.

### Bacteremia

Translocation is defined as the passage of viable indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes (Berg and Garlington, 1979). This passage is the first step for bacteria to settle in other locations, such as the bloodstream, or cause secondary forms of infections, such as in the cardiac valves. Bacterial translocation can be part of normal physiological processes in healthy subjects, but to a limited extent and without deleterious consequences. By contrast, sustained translocation is observed in subjects with specific deficiencies, such as neutropenia, starvation, or hemorrhagic shock and then leads to severe septic consequences (Tancrède and Andremont, 1985; Youssef et al., 1998). A key point determining bacterial translocation is the intestinal density: the translocating bacteria are mostly dominant within the intestinal microbiota (Youssef et al., 1998; Taur et al., 2012). Furthermore, translocation of enterobacteria has been reported in immunocompetent mice exposed to penicillin, clindamycin, and metronidazole (Berg, 1981). In countries with a high prevalence of bacteria, this passage may also play a key role in the pathogenesis of UTIs, in that the dominant clone of *E. coli* would have the maximum likelihood to colonize the urinary tract (Moreno et al., 2008). In 42 women with cystitis caused by *E. coli*, Moreno et al. (2008) compared the UTI-causing strain to 30 randomly picked colonies from concomitant stool samples. In 90% of the women, the urinary clones were found in the feces. In 71% of the women, the urinary clone that was present in the feces was also the dominant fecal clone. Urine clones mostly belonged to B2 and D phylotypes and had an increased content of urovirulence factors. For one given fecal clone, predictors of infection were that the urinary clone belonged to the B2 phylotype and was dominant. Thus, the findings of this study reconciled the dominance and the virulence theories.

Patients to Patient Cross-Transmission

In the above-mentioned study from Donskey et al. (2000), the surrounding environment of 10 VRE-carrying patients was investigated for the presence of VRE. Environmental samples from 21 patients were analyzed and compared according to the intestinal density of VRE. Strikingly, when the density was ≤ 4 log CFU/g of stool, VRE were found in the patient’s environment only in one out of nine sample sets (11%). Conversely, when density was ≥ 4 log CFU/g of stool, VRE were found in 10 of 12 sets (83%). Other studies have shown that patients can acquire resistant bacteria from a former occupant of the room through the persistence of the bacteria on environmental surfaces (Datta et al., 2011). Although no study has shown that cross-transmission occurs less often when the density of resistant bacteria is low, the results from Donskey et al. (2000) support this notion. To date, no data are available for MDRE.
| Name                                                   | Rationale                                                                 | Effect on the IM                                                                 | Advantages                                                                 | Limitations                                                                 | Phase     | Reference                                      |
|--------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|-----------|------------------------------------------------|
| Selective digestive decontamination (SDD)              | Killing resistant bacteria                                               | Loss of bacteria susceptible to the SDD regimen                                 | Simple                                                                     | Emergence of resistance to SDD agents; Causes damage to intestinal microbiota | In use    | de Smidt et al. (2011), Overdevest et al. (2011), Saidel-Odes et al. (2012) |
| Antibiotic colonic inactivation (ACI)                  | Inactivating residues of antibiotics in the colon                        | None                                                                            | No effect on the intestinal microbiota                                     | Incomplete inactivation in the colon                                        | Proof of concept | Tarkkanen et al. (2009), Khoder et al. (2010) |
| Fecal microbiota transplantation (FMT)                | Restoration of a new intestinal microbiota by allo- or autotransplantation | Restoration after antibiotics                                                   | Restore a full, healthy intestinal microbiota with barrier effect           | Acceptance; Transmission of pathogens                                       | Used occasionally | Gough et al. (2011), Ubeda et al. (2013), van Noot et al. (2013) |
| Antibiotic stewardship programs (ASPs)                 | Prioritizing antibiotics with minimal effects on the intestinal microbiota| The least effect as possible                                                    | Optimized infection management for patients                                | Necessity of close collaboration between trained clinicians and biologists; In some cases, the use of wide-spectrum antibiotics cannot be avoided | In use    | MacDougall and Polk (2009), Davy et al. (2009) |
FIGURE 2 | Schematic representation of solutions employed to maintain a low density of colonization of multidrug-resistant enterobacteria in the intestinal microbiota. Blue, green, and red, respectively, represent anaerobes, antibiotic-susceptible enterobacteria, and MDRE. (A) Selective digestive decontamination (SDD) eradicates all enterobacteria, including MDRE. (B) Fecal microbiota transplantation (FMT) reinstalls the original intestinal microbiota or an intestinal microbiota from a healthy donor after antibiotic-induced perturbations. (C) Antibiotic colonic inactivation (ACI) inactivates antibiotics and residues when they reach the colon. (D) Antibiotic stewardship program (ASP) favors antibiotics with minimal impact on the intestinal microbiota.

concentrations of these bacteria were below the limit of culture detection while SDD was being applied. Nevertheless, keeping MDRE at low intestinal concentrations in the absence of total eradication may be sufficient to prevent further infections or cross-transmission. Interestingly, no colistin or gentamicin resistance emerged among the recovered KPC-producing bacteria.

INACTIVATION OF ANTIBIOTICS IN THE INTESTINE

Instead of killing resistant bacteria, another approach would be to prevent their overgrowth by inactivating the antibiotics in the intestine during treatments. Orally administered antibiotics are primarily absorbed in the proximal jejunum, yet a fraction reaches the colon, where the density of bacteria is maximal. Parenterally administered antibiotics are filtered by the liver, and a fraction is excreted through the gall bladder into the jejunum and then reaches the colon. Thus, the concept of designing drugs or beta-lactamases capable of inactivating antibiotics in the colon, but not earlier, has arisen.

The protective effect of a recombinant beta-lactamase, P1A, has been evaluated in 34 human volunteers taking ampicillin (Taskinen et al., 2009). In the ampicillin group without P1A, a decrease of *Bifidobacterium*, *Streptococcus*, *Lactobacillus*, and an increase of *E. coli* and yeasts, were noted in the intestinal microbiota. Conversely, in the ampicillin + P1A group, no significant changes in the composition of the intestinal microbiota could be observed. Furthermore, the relative abundance of ampicillin-resistant *E. coli* increased from 2.1% at baseline to >72.7% at day 5 under ampicillin only, while it remained under 10% in the ampicillin + P1A group. In addition to target-specific inactivation strategies, adsorbents, such as colonic delivery of activated
charcoal, were shown to be efficient in trapping ciprofloxacin in a rat model (Khoder et al., 2010). Further clinical studies are expected to assess the efficacy of such a strategy.

**PROBIOTICS AND FECAL MICROBIOTA TRANSPLANTATION**

Probiotics are defined as live microorganisms that may confer a health benefit to their host. The most used probiotics are lactic acid bacteria, *Bifidobacteria*, *E. coli* Nissle 1917 or *Saccharomyces boulardii*, a yeast. It is unknown if these probiotics could exert a barrier effect for resistant bacteria. A study from New Zealand competed *E. coli* Nissle with a fluoroquinolone-resistant *E. coli* in elderly residents, and the results showed that there was eventually no difference in terms of carriage of the fluoroquinolone-resistant *E. coli* between the Nissle group and the placebo group (Tannock et al., 2011). Another growing concern about probiotics is that there remains little evidence that the massive ingestion of one single species can restore all the intestinal microbiota at a significant extent. Another potential way to restore microbiota is a fecal microbiota transplantation (FMT), which refers to the process of instilling a liquid suspension of stool from a healthy donor into the gastrointestinal tract of a patient to restore the intestinal microbiota immediately after any perturbation, such as that caused by antibiotics.

When mice with intestinal microbiota affected by antibiotics, were caged with mice without previous antibiotic exposure, a faster restoration of the intestinal microbiota was observed. It was suggested that this was due to the transfer of a normal microbiota to the antibiotic treated mice, likely through coprophagy. It was suggested that this was due to the transfer of a normal microbiota to the antibiotic treated mice, likely through coprophagy (Gough et al., 2011; van Nood et al., 2013). The main limitation of FMT is its obvious repellence, which could be overcome by rectal instillations instead of oral routes (Bakken, 2009).

Another caveat is that the fecal samples administered could include undesected pathogens. This caveat could be overcome by autotransplantation (Bush and McDonald, 2012) or by using preparation containing a cocktail of defined strains (Hamilton et al., 2012). This has recently been done to lower the density of VRE in mice (Ubeda et al., 2013) and of MDRE in chickens (Niruiari et al., 2013). However, these approaches have not been used in humans so far. If data support the efficacy of FMT in resolving *C. difficile* infections, studies assessing its efficacy in the context of outbreaks of MDRE to lower the risk of their transmissions and infections are warranted.

**ANTIBIOTIC STEWARDSHIP PROGRAMS**

Another strategy for combating antibiotic-induced perturbations and to keep MDRE at low densities is to improve the use of antibiotics through antibiotic stewardship programs (ASPs; MacDougall and Polk, 2015). Indeed, these programs can be beneficial to the intestinal microbiota at three levels: (i) avoid prescriptions when antibiotics are not justified (Willemsen et al., 2010), (ii) scale down from the use of empirical wide-spectrum antibiotics to the narrowest spectrum possible, guided by antibiotic-susceptibility tests (Cosgrove et al., 2007), and (iii) choose the antibiotic with the lowest impact on the intestinal microbiota whenever possible (Lesprut and Bruin-Buisson, 2008). In a Cochrane-based review, ASPs have been showed to decrease the overall antibiotic resistance, as well as *C. difficile* infection, suggesting their role in intestinal microbiota preservation (Davey et al., 2005). To reduce the use of wide-spectrum antibiotics further, new rapid diagnostic tests that identify resistance traits in strains in clinical samples or feces are being developed and have attracted interest from clinicians (Cuazon et al., 2012).

**CONCLUSION**

The intestinal microbiota has to be considered an organ that is mistreated with every antibiotic administration. The intestinal microbiota provides several benefits to its hosts, including colonization resistance. When it is disrupted, resistant bacteria overgrow in the empty niches. Although few data are available to date, it appears as though high densities of MDRE may increase the risk of further infections and transmissions between patients. Indeed, controlling levels of MDRE may be a key point in terms of care in the next few years; further studies in this regard are expected. In this perspective, simple methods to measure these quantitative parameters, such as qPCR instead of serial dilutions, are promising (Lerner et al., 2013).

**ACKNOWLEDGMENT**

This work was supported by the Oseo-Nosobio program (www.nosobio.fr).

**REFERENCES**

Antonopoulos, D. A., Hsu, S. M., Morrison, H. G., Schmidt, T. M., Sogin, M. L. and Young, V. B. (2009). Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. *Infect. Immun.* 77, 2376–2383.

Bakken, J. S. (2009). Fecal bacteriotherapy for recurrent *Clostridium difficile* infection. *Annu. Rev. Microbiol.* 63, 285–299.

Bagnoli, F., Zollan, A. P. and Garrel, Y. M. (2013). Antibiotic resistance shaping multi-level population biology of bacteria. *FEMS Microbiol. Rev.* 37, 1–16. doi: 10.1093/femsre/fut034

Ben Ami, R., Rodrigues-Ramos, J., Arslan, H., Pinton, J. D., Quentin, C., Cabio, E. S. et al. (2009). A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in nosocomial patients. *Clin. Infect. Dis.* 49, 602–609.

Borg, R. D. (1981). Promotion of the translocation of enteric bacteria from the gastrointestinal tracts of mice by oral treatment with penicillin, clindamycin, or neomycin. *Infect. Immun.* 35, 874–891.

Borg, R. D. and Gardell, A. W. (1979). Transmission of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in a gastrectomized mouse model. *Infect. Immun.* 29, 403–411.

Bhalla, A., Palus, N. I., Ray, A. J., Heen, C. K., Eksteen, E. C., and Demmke, C. J. (2005). Antimicrobial antibiotic therapy promotes emergence of antimicrobial-resistant, gram-negative bacilli and vancomycin-resistant enterococci in the stool of colonized patients. *Infect. Control Hosp. Epidemiol.* 26, 444–448.

Bestmann, H. J., Kohli, H., and Rangoninla, M. (2008). Impact of colistin and ciprofloxacin on the brood and vaginal flora after single-dose prophylaxis in vaginal hysterectomy. *Dis. Rdge Suppl.* 2, 163–168.

Bruin-Buisson, C., Legrand, F., Renault, A., Richard, C., Montieres, F., Bacle, M., et al. (1989). Intestinal decontamination for control of nosocomial multiresistant gram-negative bacilli. Study of an outbreak in an intensive care unit. *Ann. Infect. Med. Heb.* 110, 875–885.

Buch, A. C., and Cooke, E. M. (1999). The fate of imputed *Pseudomonas aeruginosa* on normal human skin. *J. Med. Microbiol.* 2, 521–529.

Chawner, A. E., Pierson, E., and Hung, D. T. (2007). Targeting virulence: a new paradigm for antimicrobial therapy. Nat. Clin. Pract. *Microbiol.* 3, 541–548.

Cooke, E. M., Hermsen, I. G., and Buck, A. C. (1972). Fate of ingested...
Pitout, J. D., and Laupland, K. B. (2008). Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect. Dis. 8, 1356–1368.

Pitout, J. D., and Laupland, K. B. (2009). IPSA T P1A, a randomized, double-blind, placebo-controlled trial of selective digestive decontamination using oral gentamicin and oral polymyxin E for eradication of carbapenem-resistant Enterobacteriaceae pulmonary carriage. Infect. Control Hosp. Epidemiol. 30, 4–9.

Skurnik, D., Nicola, A., Bussol, R., Lazzarini, S., Muller-Ser Skip.jet, D., and Church, G. M. (2009). Functional characterization of the antibiotic resistance reservoir in the human microbiota. Science 325, 1128–1131.

Stapleton, T. S., Hooper, L. V., and Gordon, J. J. (2005). Developmental regulation of intestinal angiogenesis by indigenous microbes via panell cells. Proc. Natl. Acad. Sci. U.S.A. 102, 17545–17549.

Summers, M. C., Lynch, M. D., Sanddal, D. B., Tenenbaum, H. C., Goldfinger, M. B., Christensen, D. G., et al. (2011). Bacterial Biogeography of the human digestive tract. ISME J. 1, 170.

Terrorle, C. H., and Andremont, A. O. (1995). Bacterial translocation and gram-negative bacteria in patients with hematological malignancies. J. Infect. Dis. 171, 625–631.

Terrorle, C., Opi, C., Grifet, E., and Lambelle, C. (2010). Emergence of ampicillin resistance in intestinal Clostridium difficile in hospitalized children with severe acute malnutrition in Niger. Clin. Infect. Dis. 50, 657–665.

Van Nood, E., Vrieze, A., Nieuwdorp, M., van Der Kooij, T., Vandeboom, H. C., De Vries, J. M., and Lekkerkerk, L. V. (1971). Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. J. Hyg. (Lond.) 69, 405–411.

Van Nood, E., Vries, A., Nieuwdorp, M., Fuentes, S., Zuidema, E. G., De Vos, W. M., et al. (2013). Duodenal colonization of donor feces for recipients. Chronic Disease 6, 313–315.

Vann, S., Carbone, A., Tho- let, J., Jarlier, V., and Coignard, B. (2011). Emergence of carbapenemase-producing Enterococcus faecium in France, 2004 to 2011. Euro Surveill. 16, pii:19088.

Villard, E. J., and Blaser, M. H. (1994). Colonization resistance. Antimicrob. Agents Chemother. 38, 409–414.

Walsh, T. R., Wood, J., Livermore, D. M., and Teunissen, N. C. (2011). Carbanidemases: the versatile beta-lactamases. Nat. Rev. Microbiol. 8, 55–60.

Westh, P. L., Angland, J., Jepson, H., Hegelt, H. C., Jansen, A., Suvalk, S., et al. (2011). Increase in carriage, spread, and exchange of extended spectrum beta-lactamase-producing genes among intestinal Enterobacteriaciae in hospitalized children with severe acute malnutrition in Niger. Clin. Infect. Dis. 53, 677–685.

Whitman, W. B., Coleman, D. C., and Wiebe, W. J. (1998). Prokaryotes: the unseen majority. Proc. Natl. Acad. Sci. U.S.A. 95, 6578–6583.

Williamson, L., Van Der Kooy, T., Van Benthem, B., Wilkins, J., and Kluyw- mans, J. (2010). Appropriations of antimicrobial therapy: a multicentre prevalence survey in the Netherlands, 2006–2009. Euro Surveill. 15, pii:19755.

Woertker, P. L., Angland, J., Jepson, H., Hegelt, H. C., Jansen, A., Suvalk, S., et al. (2011). Increase in carriage, spread, and exchange of extended spectrum beta-lactamase-producing genes among intestinal Enterobacteriaciae in hospitalized children with severe acute malnutrition in Niger. Clin. Infect. Dis. 53, 677–685.

Yamamoto, S., Tsukamoto, T., Terai, A., Kazunori, H., Takaoka, Y., and Yoshida, O. (1997). Genetic evidence supporting the local perinatal-urinary hypothesis in cystitis caused by Escherichia coli. J. Infect. Dis. 173, 1127–1129.

Yoosef, M., Al Sharman, C., Chachaty, E., Boul, A. R., and Andremont, A. (1998). Use of molecular typing to investigate bacterial translocation from the intestinal tract in malnourished children with Gram-negative bacteria. Clin. Microbiol. J. 4, 70–74.

Conflict of Interest Statement: Etienne Ruppé has no conflict. Antoine Andremont is scientific advisor for the DaV olterra Company (www.davolterra.com) with- in the frame of the French law for innovation and research.

Received: 11 February 2013; accepted: 08 May 2013. Published online: 28 May 2013.

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