Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Microbiology

Antibiotics and the resistant microbiome

Morten OA Sommer[1,2] and Gautam Dantas[3,4]

Since the discovery and clinical application of antibiotics, pathogens and the human microbiota have faced a near continuous exposure to these selective agents. A well-established consequence of this exposure is the evolution of multidrug-resistant pathogens, which can become virtually untreatable. Less appreciated are the concomitant changes in the human microbiome in response to these assaults and their contribution to clinical resistance problems. Studies have shown that pervasive changes to the human microbiota result from antibiotic treatment and that resistant strains can persist for years. Additionally, culture-independent functional characterization of the resistance genes from the microbiome has demonstrated a close evolutionary relationship between resistance genes in the microbiome and in pathogens. Application of these techniques and novel cultivation methods are expected to significantly expand our understanding of the interplay between antibiotics and the microbiome.

Addresses
[1] Department of Systems Biology, Technical University of Denmark, DK-2800 Lyngby, Denmark
[2] Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, DK-2800 Lyngby, Denmark
[3] Center for Genome Sciences & Systems Biology, Washington University School of Medicine, St Louis, MO, USA
[4] Department of Pathology & Immunology, Washington University School of Medicine, St Louis, MO, USA

Corresponding authors: Sommer, Morten OA (msom@bio.dtu.dk) and Dantas, Gautam (dantas@wustl.edu)

Current Opinion in Microbiology 2011, **14**:556–563

This review comes from a themed issue on
Antimicrobials
Edited by James Collins and Stefano Donadio

Available online 27th July 2011

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2011.07.005

Introduction

Antibiotic compounds work to either stop bacteria from growing (bacteriostatic agents) or to kill them outright (bacteriocidal agents). The effectiveness of these compounds 'against life' stems from their ability to block critical bacterial cellular processes [1,2]. Since these cellular targets are often highly genetically and structurally conserved across diverse members of the bacterial kingdom, deployment of an antibiotic compound against a specific bacterium (e.g. a pathogen) is quite likely to impart collateral damage to the bacterial community which shares the environment of the intended target organism. The spectrum of unintended effects of antibiotic therapy is likely to be considerably larger than simple growth inhibition of susceptible strains, especially considering the role of these bioactive organic compounds as multi-activity signaling molecules [3]. Almost every surface of the human body after birth is colonized by a rich and diverse community of commensal microbes [4–6], and this microbiota has substantial and continuous effects on human health and physiological development, including dietary and nutritional processing, prevention of pathogen invasion, and immune system maturation [7–9]. Accordingly, antibiotic treatment to prevent or eradicate a pathogenic infection at virtually any body site is likely to produce both short-term and long-term impacts on the commensal microbiota and its encoded microbiome [10]. These effects can be broadly categorized into two interrelated categories (Figure 1): the ones which change the relative proportions of different species in the microbiota, with the introduction of a new species or complete eradication of an existing species as extreme scenarios [11–13,14•,15••,16] and the other ones which alter the antibiotic resistome encoded by members of the microbiota [17••,18]. In the first case, changes in the architecture of the microbiota can dramatically perturb the carefully evolved homeostasis of microbiota–host mutualism, which can lead to both transient and persistent changes in host physiology and health [19••,20••]. In the latter case, enrichment and exchange of resistance genes within the microbiota increase the accessibility of these elements to potentially pathogenic organisms, challenging our ability to treat their subsequent infections [21,22].

Antibiotic-induced changes in microbial community structure

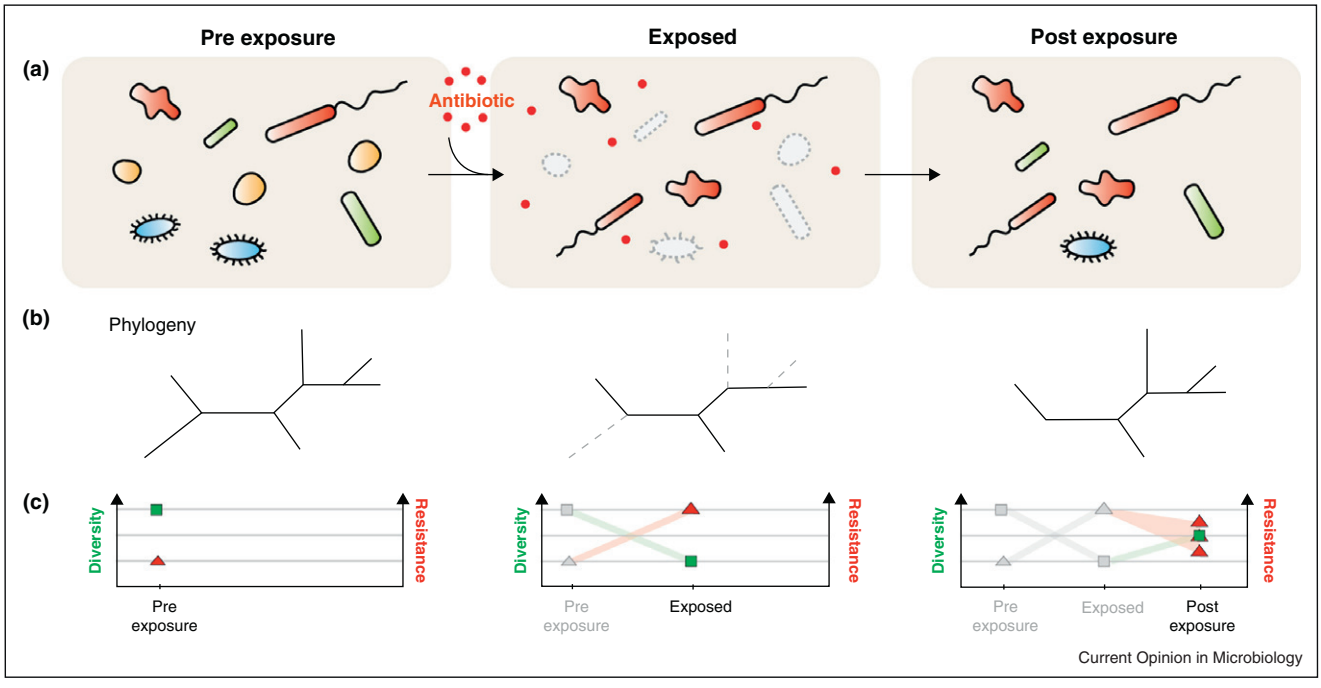
Dramatic advances over the past few years in next-generation DNA sequencing technology have enabled a series of recent high-resolution molecular characterizations of the change in microbiota architecture in humans and model organisms in response to antibiotic treatment. Deep 16S rDNA pyrosequencing was used to investigate the effect of two courses of ciprofloxacin on the gastrointestinal microbiota of three healthy individuals, which revealed dramatic and immediate antibiotic-induced decreases in phylogenetic diversity of the previously stable microbial ecosystem [12,15••]. While the microbiota began to recover to a relatively stable state within a week after antibiotic insult, the pretreatment architecture had not fully recovered within 6–10 months, including the apparent complete loss of low-abundance members of the community. Similarly, a combination of 16S sequencing and

Glossary
Human microbiota: the collection of microbes living in and on the human body
Human microbiome: the collective genomic content of the human microbiota
Resistome: the complement of all antibiotic resistance genes encoded by a microbial community
Metagenomic functional selection: a culture-independent method for capture of selectable functions from microbial metagenomic DNA through heterologous expression in an indicator host strain.

terminal-restriction fragment length polymorphism was used to measure the short-term and long-term effects of clarithromycin and metronidazole, which are standard treatment for *Helicobacter pylori* eradication, on the throat and gastrointestinal microbiota of three treated human subjects compared with three controls [16]. Like the ciprofloxacin study, the combination antibiotic treatment caused transient decreases in microbiota diversity in both the throat and the gut, and the overall community architecture had only partially recovered one and four years after treatment. Interestingly, the throat microbiota was found to be more stable after antibiotic treatment and over long periods than the intestinal microbiota.

One of the challenges in fully understanding the mechanisms of antibiotic-induced microbiota perturbations is the inherent baseline variability of the microbiota of different human individuals, which is confounded by the small sample size employed in human studies to date. One approach to address this variation is to use animal models, where host genotype, diet and other variables, which may impact baseline microbiota composition can be controlled, while still employing modern high-resolution molecular methods to characterize the microbiota [13,14•]. A major finding of such studies is that different antibiotic regimens cause reproducible distinct short-term and long-term impacts on the microbiota in murine models. Mice treated with a combination of amoxicillin, metronidazole, and bismuth had significant changes in abundance and structure of their cecal microbiota following treatment, but there was also significant rapid recovery of the community structure towards baseline after removal of the treatment. In contrast, treatment with the broad-spectrum antibiotic cefoperazone caused persistent depression in overall community diversity up to six weeks after treatment withdrawal, though community composition was 'normalized' when the treated cohort was co-housed with a nontreated animal, presumably via coprophagy [14•]. In another experiment, mice treated with the glycopeptide vancomycin, which exclusively targets gram-positive organisms, showed similar effects to the combination treatment, where the antibiotic-induced community perturbations recovered

Figure 1



The effects of antibiotic treatment on the microbiota. **(a)** Before the antibiotic selection, the constituents of the microbiota range in sensitivities towards antibiotics from sensitive (blue, green and yellow) to resistant (red). During exposure susceptible organisms will decrease in abundance, unless they acquire resistance determinants from viable resistant organisms. Upon cessation of treatment some of the susceptible organisms may increase in abundance to previous levels, whereas others will be lost from the microbiota. **(b)** During antibiotic exposure the microbial community architecture is significantly altered due to the differing sensitivities of the constituent microbes. Following cessation of antibiotic treatment community architecture largely re-establishes yet certain changes persist. **(c)** Schematic depiction of the changes in species diversity and antibiotic resistance levels upon antibiotic treatment followed by partial recovery of microbial community structure and resistance levels upon cessation of treatment.

after removal of treatment but differed from both the previous treatments in that vancomycin did not result in a major decrease in overall bacterial biomass [13]. Of note, all treatments caused transient increases in the normally low-abundance Proteobacteria at the expense of the normally dominant Firmicutes and Bacteroidetes, which is particularly intriguing given the recent rapid global proliferation of extensively drug resistant strains of Protoeobacterial human pathogens, including *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and extended-spectrum beta-lactamase producing *Enterobacteriaceae* [23].

The simplest mechanistic explanation for short-term antibiotic-induced changes in the microbiota is differences in the activity spectrum of the drug; drug resistant or insensitive bacteria proportionally increase at the expense of susceptible ones. For instance, the relative enrichment of the Proteobacteria and Tenericutes in mice cecal microbiota in response to Vancomycin is rationalized by innate resistance to this drug by these groups [13]. Vancomycin targets a peptidoglycan precursor needed for normal cell-wall synthesis, but is ineffective against these bacteria due to its inability to penetrate the Proteobacterial outer membrane, and the lack of a cell wall in Tenericutes comprised peptidoglycan. However, antibiotics can also perturb the complex architecture of the microbiota through indirect or multilayered effects; for example, bacteria which are insensitive to direct action of an antibiotic might nevertheless still be affected if other bacteria or environmental factors which they are dependent on are changed by antibiotic treatment [19••]. For instance, mice treated with the broad-spectrum combination metronidazole, neomycin, and vancomycin significantly downregulated intestinal expression of RegIIIγ, a secreted C-type lectin which is induced by commensal microbes and kills gram-negative bacteria [20••]. This downregulation of the host innate immune defense in turn significantly decreased *in vivo* killing of vancomycin-resistant enterococci, demonstrating the indirect effects of antibiotic treatment on a pathogenic microbe.

Persistent effects on the antibiotic resistome following antibiotic treatment

A major expectation from repeated antibiotic exposure of the microbiota is an increasing abundance of antibiotic resistance determinants, caused by the growth advantage of resistant organisms during antibiotic treatment and further exacerbated through the lateral exchange of resistance determinants between diverse bacteria. Growth of this resistant population and their associated resistance genes is obviously dependent on the existence of a basal level of resistance in the microbiota; however, even in the absence of direct anthropogenic treatment with antibiotics such populations are generally found present in human, animal, and even 'pristine' environmental microbiota [24]. Studies using anaerobic and aerobic cultivation methods have shown that the oral microbiota of children that have not received antibiotics contain a subpopulation of different species that are resistant to tetracycline and other antibiotics [25]. Aerobic cultivation of enterobacteria from the gut of human volunteers free of antibiotics for over one year documented mean levels of resistance in excess of 20% to 13 different antibiotics [17••]. Antibiotic treatment is expected to selectively increase the abundance of these organisms, leading to increased prevalence of their resistance genes and hence increasing the likelihood of their dissemination via lateral gene transfer. Indeed, a cultivation-based study documented significant increases in amoxicillin resistance levels in children exposed to this antibiotic in the three months after exposure, compared with a non-exposed control group [26].

It has long been hypothesized that resistance elements confer a fitness disadvantage to the host, and hence resistant strains should be outcompeted by susceptible counterparts once antibiotic pressure is removed [27]. Unfortunately, there is a growing appreciation that antibiotic resistance can persist for long periods in the absence of treatment, as highlighted by the basal levels of resistance in the microbiota described above. Cultivation-based studies have shown that treatment with clarithromycin leads to high-level resistance within intestinal *Enterococcus* sp. during treatment and that the highly resistant *Enteroccocus* sp. persisted in several patients for one to three years after treatment ended [28]. Similar results have been obtained for *Staphyllococcus epidermis* isolated from the nostrils of antibiotic-treated patients [29] and *Bacteriodes* sp. isolated from the gut [30]. Furthermore, a culture-independent PCR assay showed that the abundance of specific macrolide resistance genes had increased up to 10,000 fold compared with pretreatment levels, and persisted up to two years after the cessation of antibiotic treatment [31].

Mechanisms underlying the persistence of antibiotic resistance are complex and include compensatory adaptations and coselection [32•,33–35]. An elegant demonstration of how compensatory mutations lead to a reduced fitness cost associated with antibiotic resistance gene carriage was shown for two lineages of *Bacteroides thetaiotaomicron* from the human gut microbiota [36••]. In individuals exposed to clindamycin over seven days, a dominant clone of *B. thetaiotaomicron* acquired the *erm*G or F resistance gene, initially leading to a fitness cost in the absence of antibiotic selection. However, resistant clones from this lineage isolated two weeks later had significantly reduced fitness cost associated with the carriage of this resistance gene, highlighting the rapid adaptations that can occur *in vivo* [36••]. Interestingly studies on the persistence of antibiotic resistance after the cessation of antibiotic therapy in livestock microbiota document more drastic reductions in the prevalence of antibiotic resistance compared with those shown in the human microbiota [37]. The larger reductions in

antibiotic resistance levels in livestock microbiota may result from the turnover of the sampled individuals; yet even under these conditions of rapid turnover, antibiotic resistance genes are expected to persist in life stock microbiota for decades after abolishment of antibiotic use [32•].

Transfer of antibiotic resistance determinants

The dissemination of antibiotic resistance genes necessitates the physical transfer or acquisition of the genetic element encoding antibiotic resistance. The mechanisms responsible for this transfer are conjugation, transformation, or transduction, all of which are fully capable of transferring antibiotic resistance determinants *in vitro* from resistant donor organisms to susceptible recipient organisms [21,38]. Such transfer events have been documented for strains isolated from virtually any reservoir *in vitro* under laboratory conditions. More complex *in vitro* systems mimicking the biofilm community structure of the oral microbiota have also been used to demonstrate transfer of antibiotic resistance genes between distant genera through both conjugation and transformation [39]. *In vitro* fermentation systems simulating the human intestinal system have also been used for the investigation of the horizontal transfer of extended-spectrum beta-lactamases. While transfer of resistance genes was accelerated greatly by the administration of cefotaxime to which the donor strain harbored resistance, a baseline of transconjugants was present shortly after the introduction of the resistant donor strain even in the absence of antibiotic selection [40].

To extend these *in vitro* findings, a number of groups have studied the *in vivo* transfer of antibiotic resistance genes in animal models. Germ-free mice colonized with enterococcal donor and recipient strains were used to demonstrate the conjugal transfer of vancomycin resistance with appreciable rates and resulting in a stable population of transconjugants [41]. However, transfer occurred at much lower rates in mice colonized with the complex microbiota from feces recovered from healthy human subjects. Indeed transfer events were only documented between strains of the same species and these transconjugants did not stably colonize the microbiota [41]. The strong preferential transfer of vancomycin resistance to closely related strains was also recently confirmed for a larger array of clinical gram-positive isolates *in vitro* [42]. Recently, transfer of the extended-spectrum beta-lactamase CTX-M-9 was shown in rats colonized with human microbiota between strains of *Salmonella enterica* and *Escherichia coli*; however, the transconjugants were again only transiently present in the rat microbiome and only during treatment with selective antibiotics [43].

Notably, a series of experiments have directly investigated the transfer of antibiotic resistance genes within the gut microbiota of healthy human subjects. In one study healthy human volunteers ingested cultures of *Enterococcus faecium* resistant to either glycopeptide or streptogramin antibiotics. Fecal samples from the individuals were sampled using cultivation-based methods and enterococcal isolates resistant to virginiamycin or vancomycin were enumerated. It was found that the ingested resistant strains were able to multiply and persist in the human gut environment for seven days; however, no transfer events were detected as all resistant isolates were identical to the ingested strains based on the pulsed field gel electrophoresis pattern [22]. These experiments were followed up with a study demonstrating the *in vivo* transfer of the *van*A resistance determinant from a donor *E. faecium* strain of animal origin to a recipient *E. faecium* strain of human origin in the intestine of healthy human subjects [44•]. Similar results were shown for the sulfonamide resistance gene *sul2* within strains of *E. coli* in the human intestine [45].

The *in vivo* studies on antibiotic resistance transfer highlight the importance not only of the transfer event but also the subsequent ability of the strain to establish within the microbiota. While there is a growing body of research documenting persistent effects on the microbiome community structure and the resistance levels within the microbiota, there is limited support yet for this from controlled *in vivo* studies in both animal and human microbiota. This apparent discrepancy may result from the somewhat artificial conditions of controlled *in vivo* studies where donor and recipient strains from a different microbiota are introduced to an already established microbiota. The stability of the established microbiota and the foreign origin of donor and recipient strains may be an important factor explaining the contrasting results. Another consequence of focusing the attention on predefined donor and recipient strains in controlled *in vivo* experiments so far is that the dissemination of antibiotic resistance genes within the total microbial community is not quantified.

Microbiome wide characterization of the antibiotic resistome

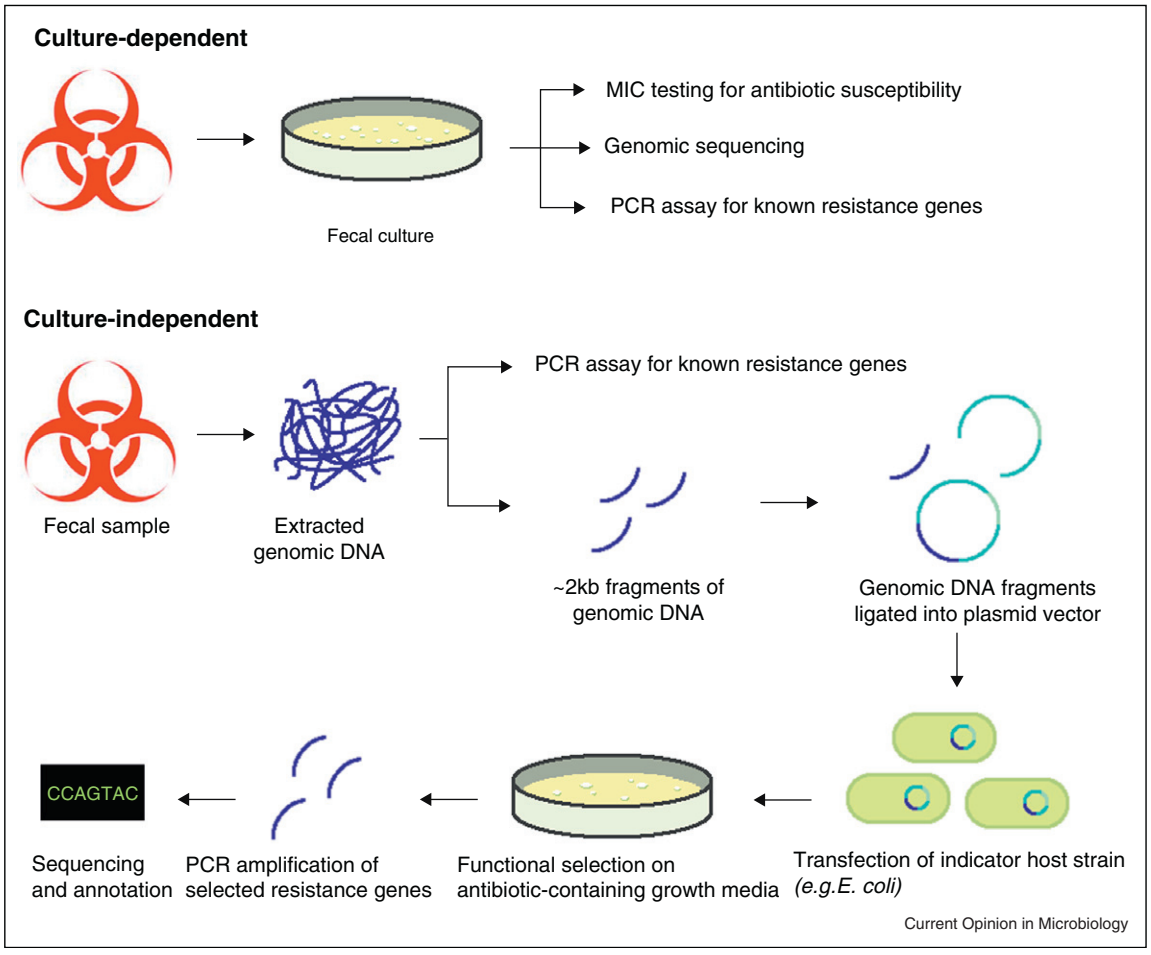
Previous culture-based studies have thus clearly established that the human commensal microbiota harbors many multidrug-resistant isolates, whose load of antibiotic resistance genes have increased steadily over the past decades. However, the majority of constituents in the microbiota have remained inaccessible to traditional culturing techniques, and molecular methods have estimated the gastrointestinal tract is home to nearly 1000 different species and over 7000 different strains [4,10], indicating our picture of the antibiotic resistome of the human microbiota from culturing-studies is incomplete. In collaboration with Gordon and colleagues, we (Dantas) recently demonstrated that use of strict anaerobic conditions and a specially formulated rich gut microbiota medium enabled culturing of over 70% of genus level

Operational Taxonomic Units observed in corresponding uncultured 'complete' microbiota [46••]. While this is undoubtedly a dramatic improvement over past estimates of microbiota 'culturability', the need for culture-independent methods for the complete characterization of the resistome remains acute.

The most commonly used culture-independent method for detection and quantification of antibiotic resistance genes in polymicrobial samples is PCR with primers homologous to known resistance gene sequences [47] (Figure 2b). For example, quantitative PCR screens were recently used to quantify the levels of *tet* and *erm* genes, conferring resistance to tetracycline and macrolide, lincosamide, and streptogramin B antibiotics respectively, in animal and human waste water [48], highlighting potential routes of dissemination of resistance genes among human, animal, and environmental microbiota. However, the extraordinary specificity of PCR-based studies is also an important limitation of the technique: because PCR can only be used to interrogate a sample for known genes, it is an ineffective method for identifying novel resistance genes.

A complementary culture-independent method which enables the characterization of both known and novel resistance genes is termed 'metagenomic functional selection' (Figure 2b), wherein total community

Figure 2



Schematic representation of methods for characterizing antibiotic resistance from human fecal microbiota. **(a)** The traditional method for characterizing antibiotic resistant isolates from the fecal microbiota begins with phenotypic growth assays of individual cultured clones on antibiotic-containing media to determine the minimum inhibitory concentration (MIC). Resistance genes are detected by either PCR amplification based on the sequence of known genes or by whole genome sequencing followed by computational annotation of resistance genes using homology to known genes. **(b)** Culture-independent resistome approaches enable microbiota-wide characterization of resistance genes. Detection and quantification of known resistance genes can be accomplished by PCR. Characterization of both known and novel resistance genes can be accomplished through metagenomic functional selections, wherein metagenomic DNA is directly extracted from the fecal sample and cloned into an expression system in a cultivable, genetically tractable host strain (e.g. *E. coli*). Metagenomic transformants harboring DNA fragments that encode antibiotic resistance genes are selected by subjecting the library of clones to specific antibiotics at concentrations which inhibit the growth of the untransformed indicator strain. Selected DNA fragments can then be sequenced to identify the specific resistance genes.

microbial (metagenomic) DNA is extracted from any arbitrary source (e.g. a fecal microbiota sample) and cloned into an antibiotic susceptible indicator strain (e.g. *E. coli*) [17••,47]. Indicator strains transformed with DNA that encode resistance genes can be functionally selected by plating the recombinant library on antibiotic media. Functionally selected metagenomic DNA inserts are amplified and sequenced to identify the captured resistance genes. We use the phrase *metagenomic functional selections* to specifically refer to the idea of subjecting metagenomic DNA to an experimental functional assay, generally through shot-gun expression in a heterologous host [49], in an attempt to distinguish this approach from broader definitions of '*functional metagenomics*', which include sophisticated computational approaches for the annotation of functions in shot-gun metagenomic sequence data, but without direct experimental validation [50]. As with PCR-based screens, metagenomic functional selections were first developed and applied for resistome characterization of environmental microbial communities [24,47], and have subsequently been successfully applied to identify a variety of enzymatic functions from cultured and uncultured microbes, including genes and pathways for degradation of or resistance towards numerous xenobiotics [51,52].

We have used metagenomic functional selections to demonstrate that the resistome of the human gut microbiota had been profoundly undersampled using traditional culture-based methods [17••,49]. We identified several hundred antibiotic resistance genes within the microbial communities of two healthy, unrelated adults. Over 95% of the resistance genes in cultured Proteobacterial isolates from these microbiota were highly similar to previously characterized genes (>90% nucleotide identity), many of which had been identified in pathogens. In sharp contrast, application of the same method to directly interrogate uncultured microbiota from the same fecal samples yielded predominantly novel resistance genes, with low sequence identity to previously characterized genes (<61% nucleotide identity). Furthermore, while over 65% of the resistance genes derived from cultured isolates were highly similar between the two individuals, <10% of the resistance genes from culture-independent sampling were similar between these two individuals. Our results exposed the substantial under-sampling of resistance genes in prior studies of human microbiota, which stemmed from a reliance on culture-dependent methods. Importantly, our data underscore the utility of metagenomic functional selections for resistance gene discovery and obtaining a culture-unbiased depiction of the diverse and personalized resistomes of the microbiota in humans.

With Gordon and coworkers, we (Dantas) recently showed that inter-individual differences in human microbiota antibiotic resistomes could be detected by subjecting both uncultured 'complete' fecal microbiota as well as pools of phylogenetically representative fecal culture collections to metagenomic functional selections [46••]. Notably, the metagenomic detection of specific resistance genes (e.g. encoding amikacin resistance) in complete samples significantly correlated with the fraction of sample-matched cultured isolates phenotypically resistant to those compounds, and the exact genes were then reconfirmed to be present in those cultured isolates by PCR. Additionally, when transplanted into gnotobiotic mice, complete and cultured communities exhibited similar colonization dynamics, biogeographical distribution, and responses to dietary perturbations, and these personalized culture collections could be enriched *in vivo* for taxa suited to specific perturbations. These methods for careful recapitulation and manipulation of microbiota both *in vitro* and *in vivo* hold great promise for quantifying the phylogenetic specificity of specific resistance genes and interrogating correlations between prevalence and dynamics of antibiotic resistomes and specific genetic and environmental variables.

Conclusion

Antibiotic treatment leads to pervasive changes in the microbial community structure and persistent increases in antibiotic resistance amongst sampled cultivatable species. This results from the rapid proliferation of antibiotic resistant strains present in low basal levels in the microbiota and through horizontal transfer of antibiotic resistance genes. While advances in sequencing technology have driven increases in our knowledge of the effects of antibiotic treatment on total community architecture, we still have a limited understanding of the evolutionary pathways through which the genetic elements encoding antibiotic resistance spreads within a community. However, the application of metagenomic functional selections, novel cultivation methods and high-throughput sequencing on microbiota exposed to antibiotics should enable a more detailed understanding of the processes governing the interplay between antibiotics and the resistant microbiome and its contribution to clinical problems with antibiotic resistance.

Acknowledgements

We thank H.J. Genee and Dr A.M. Moore for assistance in creating figures. Work in M.O.A.S's lab is supported by grants from the Novo Nordisk Foundation, Lundbeckfonden and EU FP7 Health program EvoTAR project. Work in G.D.'s lab is supported by the National Academies Keck Futures Initiative, the Washington University/Pfizer Biomedical Research Program, the Children's Discovery Institute and the International Center for Advanced Renewable Energy and Sustainability.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Walsh C: *Antibiotics: Actions, Origins, Resistance*. Washington, DC: ASM Press; 2003.

2. Kohanski MA, Dwyer DJ, Collins JJ: **How antibiotics kill bacteria: from targets to networks**. *Nat Rev Microbiol* 2010, **8**:423-435.

3. Yim G, Wang HH, Davies J: **Antibiotics as signalling molecules**. *Philos Trans R Soc Lond B Biol Sci* 2007, **362**:1195-1200.

4. Nelson KE, Weinstock GM, Highlander SK, Worley KC, Creasy HH, Wortman JR, Rusch DB, Mitreva M, Sodergren E, Chinwalla AT *et al.*: **A catalog of reference genomes from the human microbiome**. *Science* 2010, **328**:994-999.

5. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R: **Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns**. *Proc Natl Acad Sci U S A* 2010, **107**:11971-11975.

6. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T *et al.*: **A human gut microbial gene catalogue established by metagenomic sequencing**. *Nature* 2010, **464**:59-65.

7. Round JL, Mazmanian SK: **The gut microbiota shapes intestinal immune responses during health and disease**. *Nat Rev Immunol* 2009, **9**:313-323.

8. Khoruts A, Dicksved J, Jansson JK, Sadowsky MJ: **Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent *Clostridium difficile*-associated diarrhea**. *J Clin Gastroenterol* 2010, **44**:354-360.

9. Muegge BD, Kuczynski J, Knights D, Clemente JC, Gonzalez A, Fontana L, Henrissat B, Knight R, Gordon JI: **Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans**. *Science* 2011, **332**:970-974.

10. Jernberg C, Lofmark S, Edlund C, Jansson JK: **Long-term impacts of antibiotic exposure on the human intestinal microbiota**. *Microbiology* 2010, **156**:3216-3223.

11. Blaser MJ, Falkow S: **What are the consequences of the disappearing human microbiota?** *Nat Rev Microbiol* 2009, **7**:887-894.

12. Dethlefsen L, Relman DA: **Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation**. *Proc Natl Acad Sci U S A* 2011, **108(Suppl. 1)**:4554-4561.

13. Robinson CJ, Young VB: **Antibiotic administration alters the community structure of the gastrointestinal micobiota**. *Gut Microbes* 2010, **1**:279-284.

14. • Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML, Young VB: **Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation**. *Infect Immun* 2009, **77**:2367-2375.

The authors use massively parallel 16S rRNA sequencing to demonstrate that that different antibiotic regimens cause reproducible distinct short-term and long-term impacts on the phylogenetic architecture of mouse microbiota.

15. •• Dethlefsen L, Huse S, Sogin ML, Relman DA: **The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing**. *PLoS Biol* 2008, **6**:e280.

Deep 16S rDNA pyrosequencing revealed dramatic ciprofloxacin-induced decreases in phylogenetic diversity of the gastrointestinal microbiota of three healthy humans, and persistent changes were observed up to six months after treatment. First high-resolution culture-independent analysis of antibiotic changes in the human microbiota.

16. Jakobsson HE, Jernberg C, Andersson AF, Sjolund-Karlsson M, Jansson JK, Engstrand L: **Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome**. *PLoS One* 2010, **5**:e9836.

17. •• Sommer MO, Dantas G, Church GM: **Functional characterization of the antibiotic resistance reservoir in the human microflora**. *Science* 2009, **325**:1128-1131.

Metagenomic functional selections reveal the massively undersampled antibiotic resistome of the uncultured human microbiota, while also showing that aerobic cultured microbiota isolates share resistance genes with human pathogens. First culture-independent functional characterization of resistance genes in human samples.

18. Shoemaker NB, Vlamakis H, Hayes K, Salyers AA: **Evidence for extensive resistance gene transfer among Bacteroides spp. and among Bacteroides and other genera in the human colon**. *Appl Environ Microbiol* 2001, **67**:561-568.

19. •• Willing BP, Russell SL, Finlay BB: **Shifting the balance: antibiotic effects on host–microbiota mutualism**. *Nat Rev Microbiol* 2011, **9**:233-243.

Excellent review of how antibiotics perturb the evolved homeostasis of host–microbiota mutualism.

20. •• Brandl K, Plitas G, Mihu CN, Ubeda C, Jia T, Fleisher M, Schnabl B, DeMatteo RP, Pamer EG: **Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits**. *Nature* 2008, **455**:804-807.

Broad-spectrum combination antibiotic treatment in mice induced down-regulation of RegIIIγ, in turn significantly decreasing *in vivo* killing of antibiotic-resistant enterococci. First paper linking reduced innate immune defense due to antibiotic treatment to increased susceptibility to infection.

21. Vignaroli C, Zandri G, Aquilanti L, Pasquaroli S, Biavasco F: **Multidrug-resistant enterococci in animal meat and faeces and co-transfer of resistance from an *Enterococcus durans* to a human *Enterococcus faecium***. *Curr Microbiol* 2011, **62**:1438-1447.

22. Sorensen TL, Blom M, Monnet DL, Frimodt-Moller N, Poulsen RL, Espersen F: **Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork**. *N Engl J Med* 2001, **345**:1161-1166.

23. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J: **Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America**. *Clin Infect Dis* 2009, **48**:1-12.

24. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J: **Call of the wild: antibiotic resistance genes in natural environments**. *Nat Rev Microbiol* 2010, **8**:251-259.

25. Lancaster H, Ready D, Mullany P, Spratt D, Bedi R, Wilson M: **Prevalence and identification of tetracycline-resistant oral bacteria in children not receiving antibiotic therapy**. *FEMS Microbiol Lett* 2003, **228**:99-104.

26. Ready D, Lancaster H, Qureshi F, Bedi R, Mullany P, Wilson M: **Effect of amoxicillin use on oral microbiota in young children**. *Antimicrob Agents Chemother* 2004, **48**:2883-2887.

27. Andersson DI, Hughes D: **Antibiotic resistance and its cost: is it possible to reverse resistance?** *Nat Rev Microbiol* 2009, **8**:260-271.

28. Sjolund M, Wreiber K, Andersson DI, Blaser MJ, Engstrand L: **Long-term persistence of resistant *Enterococcus* species after antibiotics to eradicate *Helicobacter pylori***. *Ann Intern Med* 2003, **139**:483-487.

29. Sjolund M, Tano E, Blaser MJ, Andersson DI, Engstrand L: **Persistence of resistant *Staphylococcus epidermidis* after single course of clarithromycin**. *Emerg Infect Dis* 2005, **11**:1389-1393.

30. Lofmark S, Jernberg C, Jansson JK, Edlund C: **Clindamycin-induced enrichment and long-term persistence of resistant *Bacteroides* spp. and resistance genes**. *J Antimicrob Chemother* 2006, **58**:1160-1167.

31. Jernberg C, Lofmark S, Edlund C, Jansson JK: **Long-term ecological impacts of antibiotic administration on the human intestinal microbiota**. *ISME J* 2007, **1**:56-66.

32. • Johnsen PJ, Townsend JP, Bohn T, Simonsen GS, Sundsfjord A, Nielsen KM: **Retrospective evidence for a biological cost of vancomycin resistance determinants in the absence of glycopeptide selective pressures**. *J Antimicrob Chemother* 2011, **66**:608-610.

Elegant model for predicting the fitness cost of antibiotic resistance gene carriage and time scale for eventual clearance from microbial community.

33. Johnsen PJ, Townsend JP, Bohn T, Simonsen GS, Sundsfjord A, Nielsen KM: **Factors affecting the reversal of antimicrobial-drug resistance**. *Lancet Infect Dis* 2009, **9**:357-364.

34. Andersson DI, Hughes D: **Antibiotic resistance and its cost: is it possible to reverse resistance?** *Nat Rev Microbiol* 2010, **8**:260-271.

35. zur Wiesch PA, Kouyos R, Engelstadter J, Regoes RR, Bonhoeffer S: **Population biological principles of drug-resistance evolution in infectious diseases**. *Lancet Infect Dis* 2011, **11**:236-247.

36. Lofmark S, Jernberg C, Billstrom H, Andersson DI, Edlund C:
•• **Restored fitness leads to long-term persistence of resistant *Bacteroides* strains in the human intestine**. *Anaerobe* 2008, **14**:157-160.
Seminal paper documenting in the human microbiota the acquisition of an antibiotic resistance gene upon antibiotic treatment following by the restoration of the fitness defect associated with resistance gene carriage.

37. Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F: **Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark**. *Antimicrob Agents Chemother* 2001, **45**:2054-2059.

38. Mazaheri Nezhad Fard R, Barton MD, Heuzenroeder MW: **Bacteriophage-mediated transduction of antibiotic resistance in enterococci**. *Lett Appl Microbiol* 2011, **52**:559-564.

39. Hannan S, Ready D, Jasni AS, Rogers M, Pratten J, Roberts AP: **Transfer of antibiotic resistance by transformation with eDNA within oral biofilms**. *FEMS Immunol Med Microbiol* 2010, **59**:345-349.

40. Smet A, Rasschaert G, Martel A, Persoons D, Dewulf J, Butaye P, Catry B, Haesebrouck F, Herman L, Heyndrickx M: ***In situ* ESBL conjugation from avian to human *Escherichia coli* during cefotaxime administration**. *J Appl Microbiol* 2011, **110**:541-549.

41. Bourgeois-Nicolaos N, Moubareck C, Mangeney N, Butel MJ, Doucet-Populaire F: **Comparative study of vanA gene transfer from *Enterococcus faecium* to *Enterococcus faecalis* and to *Enterococcus faecium* in the intestine of mice**. *FEMS Microbiol Lett* 2006, **254**:27-33.

42. Werner G, Freitas AR, Coque TM, Sollid JE, Lester C, Hammerum AM, Garcia-Migura L, Jensen LB, Francia MV, Witte W *et al.*: **Host range of enterococcal vanA plasmids among gram-positive intestinal bacteria**. *J Antimicrob Chemother* 2011, **66**:273-282.

43. Faure S, Perrin-Guyomard A, Delmas JM, Chatre P, Laurentie M: **Transfer of plasmid-mediated CTX-M-9 from *Salmonella enterica* serotype Virchow to *Enterobacteriaceae* in human flora-associated rats treated with cefixime**. *Antimicrob Agents Chemother* 2010, **54**:164-169.

44. Lester CH, Frimodt-Moller N, Sorensen TL, Monnet DL,
• Hammerum AM: ***In vivo* transfer of the vanA resistance gene from an *Enterococcus faecium* isolate of animal origin to an *E. faecium* isolate of human origin in the intestines of human volunteers**. *Antimicrob Agents Chemother* 2006, **50**:596-599.
In vivo demonstration of transfer of antibiotic resistance genes in the intestinal microbiota of healthy human subjects.

45. Trobos M, Lester CH, Olsen JE, Frimodt-Moller N, Hammerum AM: **Natural transfer of sulphonamide and ampicillin resistance between *Escherichia coli* residing in the human intestine**. *J Antimicrob Chemother* 2009, **63**:80-86.

46. Goodman AL, Kallstrom G, Faith JJ, Reyes A, Moore A, Dantas G,
•• Gordon JI: **Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice**. *Proc Natl Acad Sci U S A* 2011, **108**:6252-6257.
High-throughput anaerobic culturing techniques are coupled with gnotobiotic animal husbandry and metagenomics to show that the human fecal microbiota consists largely of taxa and predicted functions that are represented in its readily cultured members. Metagenomic functional selections were used to detect inter-individual differences in the resistomes of both uncultured 'complete' fecal microbiota as well as pools of phylogenetically representative fecal culture collections.

47. Handelsman J, Rondon MR, Brady SF, Clardy J, Goodman RM: **Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products**. *Chem Biol* 1998, **5**:R245-R249.

48. Chen J, Michel FC Jr, Sreevatsan S, Morrison M, Yu Z: **Occurrence and persistence of erythromycin resistance genes (erm) and tetracycline resistance genes (tet) in waste treatment systems on swine farms**. *Microb Ecol* 2010, **60**:479-486.

49. Sommer MO, Church GM, Dantas G: **The human microbiome harbors a diverse reservoir of antibiotic resistance genes**. *Virulence* 2010, **1**:299-303.

50. Dinsdale EA, Edwards RA, Hall D, Angly F, Breitbart M, Brulc JM, Furlan M, Desnues C, Haynes M, Li L *et al.*: **Functional metagenomic profiling of nine biomes**. *Nature* 2008, **452**:629-632.

51. Uchiyama T, Miyazaki K: **Functional metagenomics for enzyme discovery: challenges to efficient screening**. *Curr Opin Biotechnol* 2009, **20**:616-622.

52. Sommer MO, Church GM, Dantas G: **A functional metagenomic approach for expanding the synthetic biology toolbox for biomass conversion**. *Mol Syst Biol* 2010, **6**:360.