Gut Microbes 3:5, 463-467; September/October 2012; © 2012 Landes Bioscience

Collateral effects of antibiotics on mammalian gut microbiomes

Torey Looft and Heather K. Allen*
Food Safety and Enteric Pathogens Research Unit; National Animal Disease Center; Ames, IA USA

Antibiotics are an essential component of the modern lifestyle. They improve our lives by treating disease, preventing disease, and in the case of agricultural animals by improving feed efficiency. However, antibiotic usage is not without collateral effects. The development and spread of antibiotic resistance is the most notorious concern associated with antibiotic use. New technologies have enabled the study of how the microbiota responds to the antibiotic disturbance, including how the community recovers after the antibiotic is removed. One common theme in studies of antibiotic effects is a rapid increase in *Escherichia coli* followed by a gradual decline. Increases in *E. coli* are also associated with systemic host stresses, and may be an indicator of ecosystem disturbances of the intestinal microbiota. Moreover, recent studies have shown additional effects mediated by antibiotics on the gut microbiota, such as the stimulation of gene transfer among gut bacteria and the reduction of immune responses in peripheral organs. Querying the microbiota after antibiotic treatment has led to intriguing hypotheses regarding predicting or mitigating unfavorable treatment outcomes. Here we explore the varied effects of antibiotics on human and animal microbiotas.

Keywords: antibiotics, microbiota, *E. coli*, disturbance, humans, agriculture

Submitted: 05/11/12

Revised: 06/24/12

Accepted: 06/27/12

http://dx.doi.org/10.4161/gmic.21288

*Correspondence to: Heather Allen;
Email: heather.allen@ars.usda.gov

Addendum to: Looft T, Johnson TA, Allen HK, Bayles DO, Alt DP, Stedtfeld RD, et al. In-feed antibiotic effects on the swine intestinal microbiome. Proc Natl Acad Sci U S A 2012; 109:1691-6; PMID:22307632; http://dx.doi.org/10.1073/pnas.1120238109.

In the US, agricultural antibiotics are used for disease treatment, control, and prevention, but decades of use has led some to question the long-term safety of antibiotic usage in livestock and poultry production (i.e., performance-enhancing uses), particularly regarding those antibiotics that are important for human health.[1-3] Primary concerns surrounding antibiotic use in agriculture are the development, dissemination, and persistence of antibiotic resistance genes in the bacterial communities associated both with the host and the environment. The discussion usually centers on antibiotic resistance genes in or transferred to pathogens because of their obvious implications for disease treatment, but the so-called collateral effect of antibiotics on a host and its associated microbial community (microbiota) should be an important part of the debate. Collateral effects are the undefined, unintended, or previously unknown effects that are secondary to the intended objectives of antibiotic use. In this review, we will leave the topic of resistance genes and pathogenic bacteria and instead discuss the breadth of additional collateral effects of antibiotics on host microbiotas, focusing on mammalian systems.

The mammalian gastrointestinal (GI) microbiota is comprised of a diverse collection of bacteria in a dynamic environment. As least 500 species are found in the human gut,[4] with up to 1,000 species found in the swine gut.[5] These diverse GI microbes are important for the maintenance of host health. They have important protective and metabolic functions, such as assisting the host in nutrient extraction, immune system and epithelium development, and are a natural defense against pathogens.[6] This vast array of GI bacteria responds to environmental conditions inside the host,[7] including antibiotic-induced disturbances.

Except for performance-enhancing antibiotics in livestock and poultry production, both veterinary and medical antibiotics are typically administered to target particular bacterial pathogen(s). However, most antibiotics are either

injected or administered orally, thereby circulating throughout the host system and potentially affecting the entire microbiota. Next-generation sequencing technologies have been particularly powerful to view the comprehensive effects of antibiotics on mammalian microbiotas, in particular by using conserved phylogenetic markers (e.g., the 16S rRNA gene sequence) to make taxonomic assignments.[8] For example, 16S rRNA gene sequence analysis of the entire community revealed that the administration of ciprofloxacin to humans affected the majority of bacterial taxa in the gut, resulting in decreased richness (membership), diversity (membership and abundance), and evenness (numerical distribution of the members).[9] However, considering the breadth of antibiotics and their various targets, doses, and durations, it is not surprising that gut microbial communities respond to different antibiotics in different ways. In contrast to the ciprofloxacin example, oral administration of vancomycin to mice did not decrease the abundance of mucosally associated gut bacteria.[10] Discovering common patterns of antibiotic-induced changes in bacterial membership could be important indicators of general community disturbances.

Our recent study in swine used 454 technology to query the 16S rRNA gene sequence diversity and fecal metagenomes of six pigs over 14 d, before and after administration of ASP250 (chlortetracycline, sulfamethazine, and penicillin) in the feed.[11] We showed profound changes in the bacterial membership and functions with antibiotic treatment, including increases in *Escherichia coli* populations, certain antibiotic resistance genes, and clusters of functional genes related to energy production and conversion. We confirmed the *E. coli* increase in a separate study.[5]

Investigation of the literature on antibiotic effects suggested that a bloom in commensal *E. coli* populations could be a response to a non-specific stress, including antibiotic treatment. Increased *E. coli* abundance was also detected in another swine study evaluating parenterally (non-GI) administered amoxicillin in piglets,[12] suggesting that the *E. coli* response is not specific to the route of antibiotic administration. Increases of enteric *E. coli* have also been associated with exposure to many other antibiotics such as amoxicillin and metronidazole,[13] metronidazole alone,[14] and vancomycin and imipenem.[15] Additionally, *E. coli* bloomed in parallel with *Salmonella* in mice that were treated with streptomycin.[16] Throughout these examples, it is unclear if *E. coli* is increasing in abundance due to antibiotic resistance or other factors associated with the community disturbance (such as inflammation), but it is nonetheless intriguing that a wide array of antibiotic classes and administration methods elicits a common response.

Antibiotics are not the only perturbation that leads to an increased abundance of gastrointestinal *E. coli*. Additional stresses, including those directed by or at the host, have been shown to cause this phenomenon. Enteric *E. coli* are more abundant in pregnant women with excessive weight gain,[17] but a seemingly disparate example is elevated *E. coli* populations observed in starving Bangladeshi children[18] who obviously have no weight gain. A more invasive disturbance is surgery, which in mice increased both the abundance and the adherence of gut *E. coli* during the post-surgery recovery.[19] Additionally, models of inflammatory bowel disease have shown an association between *E. coli* blooms and disease onset.[20,21] In livestock, *E. coli* is known to increase in abundance during weaning, which is a very stressful event for young animals. Indeed, one of the leading causes of post-weaning diarrhea and death in piglets is caused by enterotoxigenic *E. coli* (ETEC) infections.[22] Even a simple disturbance such as fasting can induce a population burst of commensal *E. coli* in cattle.[23] The various drivers of *E. coli* blooms might have a common theme in that they all impact the total intestinal bacterial community; *E. coli* may be capitalizing on a general disruption of the microbiota, temporarily expanding its niche in part due to its short doubling time.

Disturbances within the host ecosystem may disrupt gut microbial community homeostasis, resulting in reduction or loss of some members of the intestinal community. It has been suggested that reducing microbial diversity and richness in the intestinal tract increases the susceptibility for enteric infections.[24] Specifically, certain members of the intestinal microbiota have been shown to inhibit enteric Enterobacteriaceae such as *E. coli* and Salmonella.[21,25] Additionally, commensal Lactobacilli spp can induce secretion of intestinal mucins, inhibiting adhesion of enteropathogenic *E. coli* in vitro.[26] Cecal transplants from conventional to germfree mice initially result in reduced diversity of the transplanted community in the recipient, followed by an explosion on *E. coli* growth. In time the diversity recovered and *E. coli* returned to lower levels.[27] Disturbance resulting in dysbiosis and loss of members of the commensal community may contribute to unforeseen collateral damage, such as blooms of *E. coli* within the intestinal microbiota.

With a disturbance such as antibiotic administration, recovery depends on the antibiotic used, its dose, and its duration of administration. With one-time streptomycin administration in mice, the number of bacteria in the feces dropped 90% in the first 12 h, but rebounded to pre-treatment amounts by six days post-treatment.[28] In a microbial community analysis of humans over a longer time period that ended four weeks after the withdrawl of ciprofloxacin, the microbiota of the treated individuals merely resembled the pretreated state, and several taxa did not recover.[9] This is potentially concerning because loss of specific commensal bacteria may impact host health. For example, the bacterium *Oxalobacter formigenes* is lost after antibiotic treatment in humans and can be difficult to re-establish.[29] *O. formigenes* degrades oxalate, the accumulation of which results in the formation of calcium oxalate kidney stones.[30] The re-colonization of *O. formigenes* in hyperoxaluric (high blood oxalate) mice resulted in decreased urinary and blood oxalate levels,[31] implicating this commensal gut microbe in treating or even preventing hyperoxaluria. Indeed, it has been hypothesized that the gradual loss of specific members of our ancestral gut microbiota due to numerous modern practices, including antibiotic consumption, has caused the recent sharp increases in allergic and metabolic diseases in human medicine.[32] The long-term impacts of a

failed microbial recovery on host health are unclear but merit further study.

Alterations in bacterial membership have important implications on the functional capacity of the microbiota. Function-based studies are an important continuation of membership studies because they begin to address questions about what the bacteria in the community are doing, including benefits they may be providing the host. For example, particular changes in the bacterial communities may be associated with improved energy harvesting capacity of the gut microbiota after exposure to antibiotics.[33-36] Changes in the relative abundance of Bacteroidetes and Firmicutes in obese individuals also reflect functional shifts toward increased energy harvesting within the microbial communities.[34,36] Although performance-enhancing antibiotics do not promote obesity in animals, perhaps changes in metabolic activity due to microbial population shifts contribute to host metabolism.[33,36]

Building on studies of the functional capacity of the microbiota, transcriptomics and metabolomics are important tools for querying activity by asking what genes are transcribed and what metabolites are produced, respectively. In a landmark study of the effect of a one-time dose of the antibiotic streptomycin on the gut metabolome of mice, Antunes et al.[37] showed that over 80% of the detectable metabolites in feces showed altered abundance compared with those in the non-treated communities. Most of the affected metabolites discussed were critical for host physiology, including steroid hormone synthesis. This result suggests that streptomycin impacts the microbiota in such a way that certain hormone synthesis is affected, particularly that of steroids and eicosanoids, which might be important regarding their involvement in the inflammatory response in the gut. More studies such as these are needed to define the functional capacity of a microbiota and how it responds to and recovers from an antibiotic perturbation.

In addition to bacteria, bacteriophages are important members of the gut microbiota and are largely unstudied. Globally, phage predation on bacterial populations can impact biogeochemical processes, driving nutrient turnover and shaping microbial communities.[37] Phages are also important causes of food production losses as the leading causes of fermentation failure.[38] An impact of antibiotics on phages has been demonstrated in agricultural systems. The in-feed antibiotic carbadox is fed to swine for disease treatment and prevention against the causative agent of swine dysentery (*Brachyspira hyodysenteriae*). Sub-inhibitory concentrations of carbadox were shown to induce the phage-like gene transfer agent VSH-1 from *B. hyodysenteriae*, and indeed this phage-like element transduced chloramphenicol and tylosin resistance genes between *B. hyodysenteriae* strains.[39] In a later study, fecal phages were isolated from swine that were fed either carbadox or ASP250, and the phage metagenomes were sequenced. The phage metagenomes from medicated animals showed more integrase-encoding genes than those from the non-medicated animals, suggesting that in-feed antibiotics induced prophages from the bacterial community.[5] These studies demonstrate that antibiotics stimulate prophages or phage-like elements, which might transfer DNA; gene transfer is one mechanism of evolution. A further collateral effect of antibiotic treatment could therefore be the promotion of evolution among gut bacteria.

Although the purpose of most antibiotics is to prevent or treat a specific disease, some antibiotic use can actually increase susceptibility to certain bacterial pathogens. For example, reducing the numbers of commensal gut bacteria with broad-spectrum antibiotics can result in a reduction of host-produced antimicrobial molecules in the intestinal mucosa, increasing susceptibility to antibiotic-resistant bacteria and pathogens.[40,41] One suggestion for how antibiotics such as metronidazole lead to increased gut infections is that they alter the mucus layer, thinning it and thereby weakening its barrier function.[42] The microbiota also benefits the host by displacing potential pathogens,[43] and community reductions due to antibiotics may reduce that capacity. A classic example of this is infection by *Clostridium difficile*, which is an opportunistic pathogen that frequently infects patients following antibiotic treatment for other conditions. Although the infection is difficult to treat with antibiotics and can result in the surgical removal of the colon, transplanting the fecal microbiota of a healthy person to the colon of a person infected with *C. difficile* is 90% effective at curing the disease.[45] The microbiota is therefore important both in preventing and treating certain bacterial pathogens. The mechanisms of how the microbiota functions in these capacities are unknown and should be a priority for further research.

Antibiotic alterations of the gut microbiota have also been linked to viral and fungal infections, and even have an impact on disease susceptibility in distal organs. Administering neomycin increased the susceptibility of mice to influenza infection in the lungs, indicating that inflammasome activation by neomycin-sensitive bacteria in the gut prevents influenza infections in the lungs.[45] This is an example of the commensal bacteria protecting against a viral infection, but the commensal bacteria can also directly or indirectly promote viral infection.[46] Infection by the mouse mammary tumor virus is only possible in the presence of the gut microbiota, even if the microbiota is of a random yet defined composition such as altered Schaedler's flora.[47] Additional opportunistic infectious agents that benefit from antibiotic treatment are fungi, which are notorious for causing infections in antibiotic-treated humans, particularly when the anaerobic gut bacteria are disturbed.[48] Administering tetracyline to rats caused the fungal pathogen *Candida albicans* to form significantly larger tongue lesions, although there was no significant difference in the number of lesions compared with infections in non-medicated rats.[49] Clearly there are situations in which the use of antibiotics is essential to treat disease, but the potential for secondary infections is a reminder that antibiotics should be used prudently.

Conclusions

The benefits of medical and agricultural antibiotic usage are straight-forward. However, the collateral consequences are complex and numerous. Negative side effects like the promotion of antibiotic resistance should be expected, but other

changes in the host and its microbiota are harder to predict. It is important to remember that antibiotics aren't simply impacting a potential pathogen; they are also changing the ecology within the gut. One change detected following various disturbances is an increase in gut *E. coli* populations. Culturing fecal coliforms may be an easy and inexpensive way to monitor for dysbiosis in animals. Interestingly, the severity and duration of pathogenic *E. coli* infections in pigs at weaning can be reduced by altering the composition of the feed, including the addition of acidifying agents.[50] This suggests that intestinal changes that result in increased *E. coli* populations could be mitigated with host diet management, including the use of alternatives to in-feed antibiotics such as organic acids. Antibiotic alternatives are an active area of research, both for validating existing alternatives and for developing novel approaches. New technologies have enabled the complexities of the intestinal ecosystem to be explored and provide insights into the extended effects of antibiotics on this ecosystem. Understanding the interplay among the host, the gut microbiota, and antibiotics will inform decisions on antibiotic usage and ultimately improve human and animal health.

Acknowledgments

The authors thank Toni Poole, Tom Casey, Tracy Nicholson, and Thad Stanton for comments on the manuscript. USDA is an equal opportunity provider and employer.

References

1. Collignon P, Powers JH, Chiller TM, Aidara-Kane A, Aarestrup FM. World Health Organization ranking of antimicrobials according to their importance in human medicine: A critical step for developing risk management strategies for the use of antimicrobials in food production animals. Clin Infect Dis 2009; 49:132-41; PMID:19489713; http://dx.doi.org/10.1086/599374.
2. Barbosa TM, Levy SB. The impact of antibiotic use on resistance development and persistence. Drug Resist Updat 2000; 3:303-11; PMID:11498398; http://dx.doi.org/10.1054/drup.2000.0167.
3. Food and Drug Administration, Center for Veterinary Medicine. The judicious use of medically important antimicrobial drugs in food-producing animals. Guidance for Industry 2012; 209:1-26.
4. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science 2005; 308:1635-8; PMID:15831718; http://dx.doi.org/10.1126/science.1110591.
5. Allen HK, Looft T, Bayles DO, Humphrey S, Levine UY, Alt D, et al. Antibiotics in feed induce prophages in swine fecal microbiomes. MBio 2011; 2:10.1128; PMID:22128350; http://dx.doi.org/10.1128/mBio.00260-11.
6. Zoetendal EG, Cheng B, Koike S, Mackie RI. Molecular microbial ecology of the gastrointestinal tract: from phylogeny to function. Curr Issues Intest Microbiol 2004; 5:31-47; PMID:15460065.
7. Leser TD, Amenuvor JZ, Jensen TK, Lindecrona RH, Boye M, Møller K. Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. Appl Environ Microbiol 2002; 68:673-90; PMID:11823207; http://dx.doi.org/10.1128/AEM.68.2.673-690.2002.
8. Cotter PD, Stanton C, Ross RP, Hill C. The impact of antibiotics on the gut microbiota as revealed by high throughput DNA sequencing. Discov Med 2012; 13:193-9; PMID:22463795.
9. 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; PMID:19018661; http://dx.doi.org/10.1371/journal.pbio.0060280.
10. Robinson CJ, Young VB. Antibiotic administration alters the community structure of the gastrointestinal micobiota. Gut Microbes 2010; 1:279-84; PMID:20953272; http://dx.doi.org/10.4161/gmic.1.4.12614.
11. Looft T, Johnson TA, Allen HK, Bayles DO, Alt DP, Stedtfeld RD, et al. In-feed antibiotic effects on the swine intestinal microbiome. Proc Natl Acad Sci U S A 2012; 109:1691-6; PMID:22307632; http://dx.doi.org/10.1073/pnas.1120238109.
12. Janczyk P, Pieper R, Souffrant WB, Bimczok D, Rothkötter HJ, Smidt H. Parenteral long-acting amoxicillin reduces intestinal bacterial community diversity in piglets even 5 weeks after the administration. ISME J 2007; 1:180-3; PMID:18043627; http://dx.doi.org/10.1038/ismej.2007.29.
13. 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; 77:2367-75; PMID:19307217; http://dx.doi.org/10.1128/IAI.01520-08.
14. Pélissier MA, Vasquez N, Balamurugan R, Pereira E, Dossou-Yovo F, Suau A, et al. Metronidazole effects on microbiota and mucus layer thickness in the rat gut. FEMS Microbiol Ecol 2010; 73:601-10; PMID:20579100.
15. Manichanh C, Reeder J, Gibert P, Varela E, Llopis M, Antolin M, et al. Reshaping the gut microbiome with bacterial transplantation and antibiotic intake. Genome Res 2010; 20:1411-9; PMID:20736229; http://dx.doi.org/10.1101/gr.107987.110.
16. Stecher B, Denzler R, Maier L, Bernet F, Sanders MJ, Pickard DJ, et al. Gut inflammation can boost horizontal gene transfer between pathogenic and commensal Enterobacteriaceae. Proc Natl Acad Sci U S A 2012; 109:1269-74; PMID:22232693; http://dx.doi.org/10.1073/pnas.1113246109.
17. Santacruz A, Collado MC, García-Valdés L, Segura MT, Martín-Lagos JA, Anjos T, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr 2010; 104:83-92; PMID:20205964; http://dx.doi.org/10.1017/S0007114510000176.
18. Monira S, Nakamura S, Gotoh K, Izutsu K, Watanabe H, Alam NH, et al. Gut microbiota of healthy and malnourished children in Bangladesh. Front Microbiol 2011; 2:228; PMID:22125551; http://dx.doi.org/10.3389/fmicb.2011.00228.
19. Rocha F, Laughlin R, Musch MW, Hendrickson BA, Chang EB, Alverdy J. Surgical stress shifts the intestinal *Escherichia coli* population to that of a more adherent phenotype: role in barrier regulation. Surgery 2001; 130:65-73; PMID:11436014; http://dx.doi.org/10.1067/msy.2001.115360.
20. Wohlgemuth S, Haller D, Blaut M, Loh G. Reduced microbial diversity and high numbers of one single *Escherichia coli* strain in the intestine of colitic mice. Environ Microbiol 2009; 11:1562-71; PMID:19245530; http://dx.doi.org/10.1111/j.1462-2920.2009.01883.x.
21. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. Cell Host Microbe 2007; 2:204; PMID:18030708; http://dx.doi.org/10.1016/j.chom.2007.08.002.
22. Wu XY, Chapman T, Trott DJ, Bettelheim K, Do TN, Driesen S, et al. Comparative analysis of virulence genes, genetic diversity, and phylogeny of commensal and enterotoxigenic *Escherichia coli* isolates from weaned pigs. Appl Environ Microbiol 2007; 73:83-91; PMID:17056683; http://dx.doi.org/10.1128/AEM.00990-06.
23. Cray WC, Casey TA, Bosworth BT, Rasmussen MA. Effect of dietary stress on fecal shedding of Escherichia coli O157:H7 in calves. Appl Environ Microbiol 1998; 64:1975-9; PMID:9572985.
24. Bailey MT, Dowd SE, Parry NM, Galley JD, Schauer DB, Lyte M. Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by *Citrobacter rodentium*. Infect Immun 2010; 78:1509-19; PMID:20145094; http://dx.doi.org/10.1128/IAI.00862-09.
25. Levine UY, Bearson SM, Stanton TB. Mitsuokella jalaludinii inhibits growth of Salmonella enterica serovar Typhimurium. Vet Microbiol 2012; 159:115-22; PMID:22503601; http://dx.doi.org/10.1016/j.vetmic.2012.03.027.
26. Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA. Probiotics inhibit enteropathogenic *E. coli* adherence *in vitro* by inducing intestinal mucin gene expression. Am J Physiol 1999; 276:G941-50; PMID:10198338.
27. Gillilland MG 3rd, Erb-Downward JR, Bassis CM, Shen MC, Toews GB, Young VB, et al. Ecological succession of bacterial communities during conventionalization of germ-free mice. Appl Environ Microbiol 2012; 78:2359-66; PMID:22286988; http://dx.doi.org/10.1128/AEM.05239-11.
28. Antunes LC, Han J, Ferreira RB, Lolic P, Borchers CH, Finlay BB. Effect of antibiotic treatment on the intestinal metabolome. Antimicrob Agents Chemother 2011; 55:1494-503; PMID:21282433; http://dx.doi.org/10.1128/AAC.01664-10.
29. Duncan SH, Richardson AJ, Kaul P, Holmes RP, Allison MJ, Stewart CS. *Oxalobacter formigenes* and its potential role in human health. Appl Environ Microbiol 2002; 68:3841-7; PMID:12147479; http://dx.doi.org/10.1128/AEM.68.8.3841-3847.2002.
30. Allison MJ, Dawson KA, Mayberry WR, Foss JG. *Oxalobacter formigenes* gen. nov., sp. nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract. Arch Microbiol 1985; 141:1-7; PMID:3994481; http://dx.doi.org/10.1007/BF00446731.
31. Hatch M, Gjymishka A, Salido EC, Allison MJ, Freel RW. Enteric oxalate elimination is induced and oxalate is normalized in a mouse model of primary hyperoxaluria following intestinal colonization with *Oxalobacter*. Am J Physiol Gastrointest Liver Physiol 2011; 300:G461-9; PMID:21163900; http://dx.doi.org/10.1152/ajpgi.00434.2010.
32. Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? Nat Rev Microbiol 2009; 7:887-94; PMID:19898491; http://dx.doi.org/10.1038/nrmicro2245.

33. Looft T, Johnson TA, Allen HK, Bayles DO, Alt DP, Stedtfeld RD, et al. In-feed antibiotic effects on the swine intestinal microbiome. Proc Natl Acad Sci U S A 2012; 109:1691-6; PMID:22307632; http://dx.doi.org/10.1073/pnas.1120238109.
34. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature 2006; 444:1022-3; PMID:17183309; http://dx.doi.org/10.1038/4441022a.
35. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 2005; 102:11070-5; PMID:16033867; http://dx.doi.org/10.1073/pnas.0504978102.
36. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006; 444:1027-31; PMID:17183312; http://dx.doi.org/10.1038/nature05414.
37. Rohwer F, Prangishvili D, Lindell D. Roles of viruses in the environment. Environ Microbiol 2009; 11:2771-4; PMID:19878268; http://dx.doi.org/10.1111/j.1462-2920.2009.02101.x.
38. McGrath S, Fitzgerald GF, van Sinderen D. Bacteriophages in dairy products: pros and cons. Biotechnol J 2007; 2:450-5; PMID:17330220; http://dx.doi.org/10.1002/biot.200600227.
39. Stanton TB, Humphrey SB, Sharma VK, Zuerner RL. Collateral effects of antibiotics: carbadox and metronidazole induce VSH-1 and facilitate gene transfer among *Brachyspira hyodysenteriae* strains. Appl Environ Microbiol 2008; 74:2950-6; PMID:18359835; http://dx.doi.org/10.1128/AEM.00189-08.
40. Brandl K, Plitas G, Mihu CN, Ubeda C, Jia T, Fleisher M, et al. Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature 2008; 455:804-7; PMID:18724361; http://dx.doi.org/10.1038/nature07250.
41. Hill DA, Hoffmann C, Abt MC, Du Y, Kobuley D, Kirn TJ, et al. Metagenomic analyses reveal antibiotic-induced temporal and spatial changes in intestinal microbiota with associated alterations in immune cell homeostasis. Mucosal Immunol 2010; 3:148-58; PMID:19940845; http://dx.doi.org/10.1038/mi.2009.132.
42. Wlodarska M, Willing B, Keeney KM, Menendez A, Bergstrom KS, Gill N, et al. Antibiotic treatment alters the colonic mucus layer and predisposes the host to exacerbated *Citrobacter rodentium*-induced colitis. Infect Immun 2011; 79:1536-45; PMID:21321077; http://dx.doi.org/10.1128/IAI.01104-10.
43. Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol 2004; 4:478-85; PMID:15173836; http://dx.doi.org/10.1038/nri1373.
44. Khoruts A, Sadowsky MJ. Therapeutic transplantation of the distal gut microbiota. Mucosal Immunol 2011; 4:4-7; PMID:21150894; http://dx.doi.org/10.1038/mi.2010.79.
45. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. Proc Natl Acad Sci U S A 2011; 108:5354-9; PMID:21402903; http://dx.doi.org/10.1073/pnas.1019378108.
46. Wilks J, Golovkina T. Influence of microbiota on viral infections. PLoS Pathog 2012; 8:e1002681; PMID:22615558; http://dx.doi.org/10.1371/journal.ppat.1002681.
47. Kane M, Case LK, Kopaskie K, Kozlova A, MacDearmid C, Chervonsky AV, et al. Successful transmission of a retrovirus depends on the commensal microbiota. Science 2011; 334:245-9; PMID:21998394; http://dx.doi.org/10.1126/science.1210718.
48. Krcméry V Jr., Matejicka F, Pichnová E, Jurga L, Sulcova M, Kunová A, et al. Documented fungal infections after prophylaxis or therapy with wide spectrum antibiotics: relationship between certain fungal pathogens and particular antimicrobials? J Chemother 1999; 11:385-90; PMID:10632385.
49. Allen CM, Beck FM, Lurie FA, Pinsky HM. Role of tetracycline in pathogenesis of chronic candidiasis of rat tongues. Infect Immun 1985; 47:480-3; PMID:3967925.
50. Fairbrother JM, Nadeau E, Gyles CL. *Escherichia coli* in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim Health Res Rev 2005; 6:17-39; PMID:16164007; http://dx.doi.org/10.1079/AHR2005105.