GUT MICROBES
2016, VOL. 7, NO. 1, 68–74
http://dx.doi.org/10.1080/19490976.2015.1127463

Taylor & Francis
Taylor & Francis Group

ADDENDUM

OPEN ACCESS

Antibiotic-treated versus germ-free rodents for microbiota transplantation studies

Randi Lundberg[a,b], Martin F. Toft[b,†], Benjamin August[b,‡], Axel K. Hansen[a], and Camilla H. F. Hansen[a]

[a]Section of Experimental Animal Models, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark; [b]Internal Research and Development, Taconic Biosciences, Lille Skensved, Denmark

ABSTRACT

We recently investigated the applicability of antibiotic-treated recipient mice for transfer of different gut microbiota profiles. With this addendum we elaborate on perspectives and limitations of using antibiotics as an alternative to germ-free (GF) technology in microbial transplantation studies, and we speculate on the housing effect. It is possible to transfer host phenotypes via fecal transplantation to antibiotic-treated animals, but problems with reproducibility, baseline values, and antibiotic resistance genes should be considered. GF animals maintained in isolators still seem to be the best controlled models for long-term microbial transplantation, but antibiotic-treated recipients are also commonly utilized. We identify a need for systematic experiments investigating the stability of microbial transplantations by addressing 1) the recipient status as either GF, antibiotic-treated or specific pathogen free and 2) different levels of protected housing systems. In addition, the developmental effect of microbes on host physiological functions should be evaluated in the different scenarios.

ARTICLE HISTORY
Received 29 June 2015
Revised 7 November 2015
Accepted 30 November 2015

KEYWORDS
animal models; antibiotics; fecal transplantation; germ-free; gut microbiota; mouse; reproducibility

Breeding of germ-free (GF) rodents started as long ago as in the mid-1930′s,[1] and since the 1960′s the GF state has been the standard starting point for the production of barrier-bred laboratory rodents with a specific pathogen free (SPF) health status.[2] In addition, GF rodents have also proved important for studying the effects of microbial mono- and polycolonizations on host phenotype [3-5] and in the search for a mechanistic understanding of microbe mediated changes in several disease models.[6-15] With the massive interest in host-microbiome interactions and the implications of dysbiosis for human health,[16,17] the use of GF animal models for transplanting microbiotas of interest to investigate causality sees a renaissance in these years. However, the generation of and working with GF or gnotobiotic animals, in which the presence of identified microbes is strictly controlled for, is laborious and costly due to housing in isolators and rigid gnotobiotic working procedures. In two recent studies, we therefore investigated the applicability of antibiotic-treated mice as recipients of microbial transplants and housed them in less protected systems than isolators.[18] With this addendum, we elaborate on the perspectives and limitations of using antibiotics as an alternative to GF technology in microbial transplantation studies, and we speculate on the effect of the housing system on the outcome.

We colonized broad-spectrum antibiotic-treated weaned and adult mice with microbiotas from obese or lean mice with the purpose of addressing the optimal recipient age during microbiota transfer of phenotype to antibiotic-treated recipients, as it has been shown that the timing of transplantation is important for optimal colonization conditions.[7] In pups colonized at weaning, we found that recipients with a lean or obese microbiota clustered separately in principal component analysis plots throughout the study period of 6 weeks post-colonization. The mice were housed in

CONTACT Randi Lundberg randi.lundberg@taconic.com Tornbjergvej 40, Ejby, DK-4623 Lille Skensved, Denmark.
[†] Current address: Adlast, Aabenraa, Denmark
[‡] Current address: LEO Pharma; Ballerup, Denmark

Addendum to: Ellekilde M, Selfjord E, Larsen CS, Jakesevic M, Rune I, Tranberg B, Vogensen FK, Nielsen DS, Bahl MI, Licht TR, Hansen AK, Hansen CHF. Transfer of gut microbiota from lean and obese mice to antibiotic-treated mice. Scientific Reports 2014; 4:5922; doi:10.1038/srep05922; http://www.nature.com/srep/2014/140801/srep05922/full/srep05922.html

open-top cages in an SPF facility using non-aseptic husbandry procedures. Also for adult mice colonized when eight weeks old, the recipients clustered separately, though less related to the donors compared to the situation in the weanlings. In the adult study, a more protected housing approach was attempted by housing in a ventilated cabinet and with autoclaving of all materials. The cabinet was placed in the same SPF facility as for the weaning study. Colonization successfully changed the diminished gut microbiota of antibiotic-treated mice in both studies, and interestingly this was unaffected by recipient age and the level of protection from the surroundings. However, the microbial composition changed in both studies over the study period of 6 weeks, indicating that it was not possible to maintain a stable microbiota resembling the donor in the open cage systems. Perhaps, several rounds of re-colonizations e.g. by transferring bedding from donor animals, continuous colonization via the drinking water or repeated oral gavages would improve the stability. A more stable microbiota over time after transplantation may also be obtained by housing in individually ventilated cages (IVCs) instead,[19] though a side-by-side comparison of stability after transplantation in IVCs or isolators still remains to be done. Apart from considerations of re-colonization and increased protection from the surroundings, it cannot be excluded that an age-matched group of donors housed in parallel under the same conditions as the mice in our study would have displayed a similar change in the gut microbiota over time as we saw. Maybe it is utopian thinking to expect a complex microbiota to be completely stable on a long-term basis even under protected housing conditions as host age and seasonal fluctuations may affect the mutual relationship of the members of the gut microbiota, but this would need further investigations.

GF animals, and in particular GF mice, are usually the first choice for microbial transplantation studies, as they constitute a clean sheet in terms of immunological naivety and the absence of competition between the resident and newly transplanted microbes. However, only a few commercially available GF mouse strains exist and though possible, it is not trivial to generate new GF mouse strains of interest, not to mention the practical challenges in maintaining GF animal colonies. For some research areas, it is extremely difficult to maintain a GF or gnotobiotic health status throughout a study, e.g., in behavioral and neurobiological research, where the animals are often subjected to various test conditions outside the home cage. Capacity constraints are another common problem with microbiome research where the aim often is to evaluate different communities from different donor sources, requiring several gnotobiotic isolators. In this context, the perspective of using IVCs for gnotobiotic studies is also intriguing and has been attempted by different research groups. Most impressive, a GF state was secured in Isocages, a type of IVCs with positive pressure, for 12 weeks and also proved useful for fecal transplantations with high stability.[19] Depletion of the gut microbiota by antibiotic use has similarly been attempted as an alternative to GF animals. This method comes with several caveats, especially when used for microbiota transplantation studies. 1) Though a broad-spectrum antibiotic approach reduces the majority of the bacterial species considerably, there will still be bacteria left in the gut as shown by denaturing gradient gel electrophoresis [18] and by cultivation.[20] It is impossible to control for the exact effect of an antibiotic treatment in terms of which species are fully eradicated and which are only reduced, and the remaining microbiota of the antibiotic-treated mice may thus also have had an impact on colonization and stability over time in our study. 2) As the immune system is known to be primed by the gut microbiota in early life,[5,7,21-23] the exposure to microbes before depletion with antibiotics can have long-lasting effects on host physiology and should also be controlled or taken into consideration. On the contrary, the complete lack of bacteria in GF animals before microbiota transplantation probably has an even larger impact due to permanent alterations of the immune system even after colonization.[7] The difference in immune response to a microbiota transplant in GF mice, which have never previously encountered bacteria, in contrast to antibiotic-treated recipients with prior microbial stimulation of the immune system, must be expected to be major at least for certain disease models, and therefore yield different outcomes though colonization is equally successful. 3) Antibiotic treatment can lead to an overgrowth of a few species, such as *Klebsiella spp*., which may have a substantial dominating role in the microbial profile also after recolonization,[20] or may be detrimental to the health of the animal. 4) Oral administration of antibiotics disrupts the gut microbiota, but other microbial communities, e.g. the skin and lung microbiota, are not always directly affected. This is

dependent on the pharmacokinetics of the antibiotic substance and may have developmental impact on the immune system as well.[24,25] 5) Increasing evidence points to the fact that fungi, bacteriophages, and eukaryotic viruses which are not directly targeted by bactericidal antibiotics cannot be ignored in gut microbiota homeostasis and immune priming.[26,27] 6) Direct effects of antibiotics on host physiology should be taken into consideration. The effects of antibiotic treatment on disease expression in animal models are usually hypothesized to be mediated by the gut microbiota. This can be tested by transplanting antibiotic-altered microbiotas into GF recipients.[28] Interestingly, this causal relationship is not always the case, e.g., it was recently reported that antibiotics had an ameliorating effect on the intestinal inflammation of SPF as well as GF TRAF6ΔDC mice, in which dendritic cell-intrinsic expression of the signaling mediator TRAF6 is ablated, thus implying a microbiota-independent and direct immunomodulatory effect of the antibiotic treatment.[29] The direct effects of antibiotics on the host was also recently systematically investigated, with repression of mitochondrial and ribosomal function as remarkable findings in GF mice treated with antibiotics.[30] 7) Last but not least, in a discussion of the use of antibiotics for non-therapeutic purposes, the risk of favoring bacteria with antibiotic resistance genes cannot be ignored.[31] Exchange of resistant bacteria between staff and animals in a laboratory animal facility may be limited via the use of personal protective equipment, but the risk is present. Spillage of antibiotics to the environment, e.g. from residual antibiotic-treated drinking water and waste from antibiotic-treated animals, should also be considered and is a legitimate concern.

The impact of the gut microbial composition on animal models is increasingly being revealed, as has recently been reviewed.[32-34] Thus, antibiotic treatment has the potential to alter expression in disease models and this has been useful in studying effects of different microbial compositions on immunology and metabolism in animal models.[22,28,35,36] Antibiotics, as opposed to the GF state, have the advantage of enabling investigations of the consequence of gut microbial depletion in different life stages.[37] Also, by targeting different groups of bacteria via different classes of antibiotics, it is possible to generate hypotheses as to which bacteria are responsible for disease manifestation, e.g., treatment with vancomycin in non-obese diabetic (NOD) mice resulted in several immunological alterations and a reduced degree of insulitis,[22] whereas GF NOD mice have accentuated insulitis.[38,39] Nonetheless, treating with antibiotics in order to create a pseudo-GF state represents an uncontrolled situation which may have a negative effect on the reproducibility of the study.

One can turn the kaleidoscope from seeing the GF animal as a controlled, clean sheet to the perspective where the GF animal is viewed upon with altered baseline values compared to SPF mice colonized from birth. It is well-known that there are anatomical, immunological, and metabolic features of GF mice that are well adapted to the specific physiologic state they exist in and that are different from the SPF mice until colonized.[40-42] The researcher may therefore consider SPF mice with a complex microbiota that later in life are treated with antibiotics before transplantation with a microbiota of interest, as a better model due to the fact that the early GF state can have permanent effects on important host parameters that are not possible to reverse by introduction of bacteria.[7] However, the GF state constitutes a controlled situation, where different baseline microbial profiles of mice within or between replicate studies do not have a substantial effect on the result of a gnotobiotic experiment. Laboratory animals are only model systems and each model comes with its own limitations, but reproducibility should always be emphasized as an inarguable necessary feature of the system. A way to bypass both the uncontrolled situation of antibiotic-treated animals and the effect of an early GF life is to use a GF parent generation for the microbial transplantation, and using the subsequent generations as study subjects.

Another approach than using GF or antibiotic-treated recipients is simply to use recipients with a complex microbiota. This was attempted in rats, and surprisingly did antibiotic-treatment prior to transplantation not enhance establishment of the transplant.[43] In contrast, it was nicely demonstrated that transfer of microbiota from protected NOD mice had a more substantial effect on newborn pups when their endogenous microbiota was limited by treating the recipient mothers with an antibiotic cocktail before the transfer.[44] The same authors have previously shown that donor microbiota from wild-type and MyD88 deficient NOD mice could be resuspended in the drinking water of non-treated SPF recipient NOD mice. The gut microbiota transfer had long-term effect

on the composition of the recipient mice but did not fully resemble the donor microbiota.[45] Transfer of certain species seemed to have been sufficient to partly transfer the host phenotype as also evident in our own study. Co-housing of lean and obese mice with different microbiotas also led to at least partial transfer of the microbiota and transfer of phenotype.[46] However, this appeared to only happen in a unidirectional way where the healthy microbiota dominates over the dysbiotic, as only the lean microbiota was transmissible and prevented co-housed mice with obese microbiotas from becoming obese. In a model of acute liver injury, where the same strains of mice originating from different vendors were shown to have different susceptibility in the model, co-housing of mice with high and low susceptibility resulted in intermediate disease susceptibility in all co-housed mice.[47] This was probably due to a resulting mixture of protective and inducing bacterial taxa. It is poorly understood why some taxa are more capable of transmission between cage mates than others, though diet has been shown to be an important factor.[46] The mentioned alternatives to using antibiotic-treated or GF recipients can be useful for investigating ecological dynamics and effects on phenotype, but these methods are probably also associated with a low reproducibility due to lack of control of the microbial starting point and how it affects the stability of the new microbiota. Partial or limited transmission of only certain species can, however, be beneficial in understanding the properties of only these specific bacteria if they alone are sufficient to transfer a host phenotype; especially considering that mono-colonization studies of suspected candidates have repeatedly disappointed as a tool for transferring e.g. disease or protection thereof.[48] Yet another way to transfer microbiotas between individuals is by cross-fostering recipient pups born vaginally or by Caesarian section with foster mothers harboring the microbiota of interest.[49,50] Cross-fostering after vaginal birth was shown to be an effective way to lastingly change the gut microbiota of the pups,[50] but the early-life immune priming by the vaginal microbiota would have to be addressed as well.

Table 1. Applicability of antibiotic-treated or germ-free rodents. Different research aims within translational microbiome research and the recommended use of either antibiotic-treated or germ-free rodent hosts for the purpose.

	Status of Host	
Research Aim	Antibiotic-treated	Germ-free
Investigate microbial phenotype transfer of manifestations known to be microbiota dependent	X	x
Investigate microbial phenotype transfer of manifestations not known to be microbiota dependent		x
Investigate effect of disrupting the microbiome in certain life stages of the host	x	
Investigate effect of targeting certain groups of bacteria	x	
Investigate effect of monocolonization		x
Investigate effect of colonization with a few, defined organisms		x

Concluding remarks

We and others have shown that it is possible to transfer phenotypes via microbial transplantation in antibiotic-treated animals, superseding the need for GF recipients in these types of studies, though potential problems with reproducibility and concerns of spreading antibiotic resistance genes should be acknowledged. Nevertheless, GF animals still seem to be the best controlled model systems for microbial transplantation and thus, given the lack of reliable phenotype transfers, the antibiotic-treated models cannot be regarded to serve as good models for discovering novel roles of gut microbiota in disease states where gut microbiota has not previously been implicated. The antibiotic-treated recipients should rather be considered when studying phenotype transfers in conditions already known to be associated with alterations in gut microbiota, such as obesity (Table 1). When it is necessary to use GF animals, an approach where GF parents receive the microbial transplant and the subsequent offspring generations are used as study subjects is advisable to overcome the problems associated with an early GF life. When antibiotic treatment is used as an alternative to the GF state, e.g., because of limited access to certain GF mouse and rat strains, it should thoroughly be evaluated if the approach is truly applicable in the given situation. With this addendum, we identify a need for systematic experiments investigating the stability of microbial transplantations by addressing 1) the recipient status as either GF, antibiotic-treated or SPF, and 2) different levels of protected housing systems. In addition, the developmental effect on host functions, in particular the immune system should be evaluated in the different recipient types.

Abbreviations

GF germ-free
NOD non-obese diabetic
IVC individually ventilated cage
SPF specific pathogen free.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

Randi Lundberg is partly funded by Innovation Fund Denmark and collaborates with the strategic research center 3G (Gut, Grain & Greens).

References

1. Kirk RG. "Life in a germ-free world:" isolating life from the laboratory animal to the bubble boy. Bull Hist Med 2012; 86:237-75; PMID:23000838; http://dx.doi.org/10.1353/bhm.2012.0028
2. Hansen AK, Nielsen DS. Handbook of Laboratory Animal Bacteriology, Second Edition - CRC Press Book. 2nd ed. CRC Press; 2014
3. 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
4. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, Deng Y, Blennerhassett P, Macri J, McCoy KD, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 2011; 141:599-609, 609.e1-3; http://dx.doi.org/10.1053/j.gastro.2011.04.052
5. Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 2008; 455:1109-13; PMID:18806780; http://dx.doi.org/10.1038/nature07336
6. Bäckhed F, Ding H, Wang T, Hooper L V, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 2004; 101:15718-23; http://dx.doi.org/10.1073/pnas.0407076101
7. Hansen CHF, Nielsen DS, Kverka M, Zakostelska Z, Klimesova K, Hudcovic T, Tlaskalova-Hogenova H, Hansen AK. Patterns of early gut colonization shape future immune responses of the host. PLoS One 2012; 7:e34043; PMID:22479515; http://dx.doi.org/10.1371/journal.pone.0034043
8. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005; 122:107-18; PMID:16009137; http://dx.doi.org/10.1016/j.cell.2005.05.007
9. Neufeld K-AM, Kang N, Bienenstock J, Foster JA. Effects of intestinal microbiota on anxiety-like behavior. Commun Integr Biol 2011; 4:492-4; PMID:21966581; http://dx.doi.org/10.4161/cib.15702
10. Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, Glickman JN, Siebert R, Baron RM, Kasper DL, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. Science 2012; 336:489-93; PMID:22442383; http://dx.doi.org/10.1126/science.1219328
11. Schéle E, Grahnemo L, Anesten F, Hallén A, Bäckhed F, Jansson JO. The gut microbiota reduces leptin sensitivity and the expression of the obesity-suppressing neuropeptides proglucagon (Gcg) and brain-derived neurotrophic factor (Bdnf) in the central nervous system. Endocrinology 2013; 154:3643-51; http://dx.doi.org/10.1210/en.2012-2151
12. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, Kubo C, Koga Y. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol 2004; 558:263-75; PMID:15133062; http://dx.doi.org/10.1113/jphysiol.2004.063388
13. Bleich A, Mahler M. Environment as a critical factor for the pathogenesis and outcome of gastrointestinal disease: experimental and human inflammatory bowel disease and helicobacter-induced gastritis. Pathobiology 2005; 72:293-307; PMID:16582581; http://dx.doi.org/10.1159/000091327
14. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun 1998; 66:5224-31; PMID:9784526
15. Song F, Ito K, Denning TL, Kuninger D, Papaconstantinou J, Gourley W, Klimpel G, Balish E, Hokanson J, Ernst PB. Expression of the neutrophil chemokine KC in the colon of mice with enterocolitis and by intestinal epithelial cell lines: effects of flora and proinflammatory cytokines. J Immunol 1999; 162:2275-80; PMID:9973504
16. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012; 490:55-60; PMID:23023125; http://dx.doi.org/10.1038/nature11450
17. Salonen A, de Vos WM, Palva A. Gastrointestinal microbiota in irritable bowel syndrome: present state and perspectives. Microbiology 2010; 156:3205-15; PMID:20705664; http://dx.doi.org/10.1099/mic.0.043257-0
18. Ellekilde M, Selfjord E, Larsen CS, Jakesevic M, Rune I, Tranberg B, Vogensen FK, Nielsen DS, Bahl MI, Licht TR, et al. Transfer of gut microbiota from lean and obese mice to antibiotic-treated mice. Sci Rep 2014; 4:5922; PMID:25082483; http://dx.doi.org/10.1038/srep05922
19. Paik J, Pershutkina O, Meeker S, Yi JJ, Dowling S, Hsu C, Hajjar AM, Maggio-Price L, Beck DAC. Potential for using a hermetically-sealed, positive-pressured isocage system for studies involving germ-free mice outside a flexible-film isolator. Gut Microbes 2015; 6:255-65; PMID:26177210; http://dx.doi.org/10.1080/19490976.2015.1064576
20. Hansen AK. Antibiotic treatment of nude rats and its impact on the aerobic bacterial flora. Lab Anim 1995;

29:37-44; PMID:7707676; http://dx.doi.org/10.1258/002367795780740410

21. Candon S, Perez-Arroyo A, Marquet C, Valette F, Foray AP, Pelletier B, Milani C, Ventura M, Bach JF, Chatenoud L. Antibiotics in early life alter the gut microbiome and increase disease incidence in a spontaneous mouse model of autoimmune insulin-dependent diabetes. PLoS One 2015; 10:e0125448; PMID:25970503; http://dx.doi.org/10.1371/journal.pone.0125448
22. Hansen CHF, Krych L, Nielsen DS, Vogensen FK, Hansen LH, Sørensen SJ, Buschard K, Hansen AK. Early life treatment with vancomycin propagates Akkermansia muciniphila and reduces diabetes incidence in the NOD mouse. Diabetologia 2012; 55:2285-94; PMID:22572803; http://dx.doi.org/10.1007/s00125-012-2564-7
23. Weng M, Walker WA. The role of gut microbiota in programming the immune phenotype. J Dev Orig Health Dis 2013; 4:203-14; PMID:24353893; http://dx.doi.org/10.1017/S2040174412000712
24. Barfod KK, Roggenbuck M, Hansen LH, Schjørring S, Larsen ST, Sørensen SJ, Krogfelt KA. The murine lung microbiome in relation to the intestinal and vaginal bacterial communities. BMC Microbiol 2013; 13:303; PMID:24373613; http://dx.doi.org/10.1186/1471-2180-13-303
25. Srinivas G, Möller S, Wang J, Künzel S, Zillikens D, Baines JF, Ibrahim SM. Genome-wide mapping of gene-microbiota interactions in susceptibility to autoimmune skin blistering. Nat Commun 2013; 4:2462; PMID:24042968; http://dx.doi.org/10.1038/ncomms3462
26. Underhill DM, Iliev ID. The mycobiota: interactions between commensal fungi and the host immune system. Nat Rev Immunol 2014; 14:405-16; PMID:24854590; http://dx.doi.org/10.1038/nri3684
27. Virgin HW. The virome in mammalian physiology and disease. Cell 2014; 157:142-50; PMID:24679532; http://dx.doi.org/10.1016/j.cell.2014.02.032
28. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, Kim SG, Li H, Gao Z, Mahana D, et al. Altering the Intestinal Microbiota during a Critical Developmental Window Has Lasting Metabolic Consequences. Cell 2014; 158:705-21; PMID:25126780; http://dx.doi.org/10.1016/j.cell.2014.05.052
29. Han D, Walsh MC, Kim KS, Hong SW, Lee J, Yi J, Rivas G, Surh CD, Choi Y. Microbiota-independent ameliorative effects of antibiotics on spontaneous th2-associated pathology of the small intestine. PLoS One 2015; 10:e0118795; PMID:25689829; http://dx.doi.org/10.1371/journal.pone.0118795
30. Morgun A, Dzutsev A, Dong X, Greer RL, Sexton DJ, Ravel J, Schuster M, Hsiao W, Matzinger P, Shulzhenko N. Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks. Gut 2015; 64:1732-43; PMID:25614621; http://dx.doi.org/10.1136/gutjnl-2014-308820
31. Modi SR, Lee HH, Spina CS, Collins JJ. Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. Nature 2013; 499:219-22; PMID:23748443; http://dx.doi.org/10.1038/nature12212
32. Bleich A, Hansen AK. Time to include the gut microbiota in the hygienic standardisation of laboratory rodents. Comp Immunol Microbiol Infect Dis 2012; 35:81-92; PMID:22257867; http://dx.doi.org/10.1016/j.cimid.2011.12.006
33. Hansen AK, Friis Hansen CH, Krych L, Nielsen DS. Impact of the gut microbiota on rodent models of human disease. World J Gastroenterol 2014; 20:17727-36; PMID:25548471
34. Macpherson AJ, McCoy KD. Standardised animal models of host microbial mutualism. Mucosal Immunol 2015; 8:476-86; PMID:25492472; http://dx.doi.org/10.1038/mi.2014.113
35. Cho I, Yamanishi S, Cox L, Methé BA, Zavadil J, Li K, Gao Z, Mahana D, Raju K, Teitler I, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 2012; 488:621-6; PMID:22914093; http://dx.doi.org/10.1038/nature11400
36. Desbonnet L, Clarke G, Traplin A, O'sullivan O, Crispie F, Moloney RD, Cotter PD, Dinan TG, Cryan JF. Gut Microbiota Depletion from Early Adolescence in Mice: Implications for Brain and Behaviour. Brain Behav Immun 2015; 50:335-6
37. Rune I, Hansen CHF, Ellekilde M, Nielsen DS, Skovgaard K, Rolin BC, Lykkesfeldt J, Josefsen K, Tranberg B, Kihl P, et al. Ampicillin-improved glucose tolerance in diet-induced obese C57BL/6NTac mice is age dependent. J Diabetes Res 2013; 2013:319321; PMID:24369539; http://dx.doi.org/10.1155/2013/319321
38. Alam C, Bittoun E, Bhagwat D, Valkonen S, Saari A, Jaakkola U, Eerola E, Huovinen P, Hänninen A. Effects of a germ-free environment on gut immune regulation and diabetes progression in non-obese diabetic (NOD) mice. Diabetologia 2011; 54:1398-406; PMID:21380595; http://dx.doi.org/10.1007/s00125-011-2097-5
39. Greiner TU, Hyötyläinen T, Knip M, Bäckhed F, Orešič M. The gut microbiota modulates glycaemic control and serum metabolite profiles in non-obese diabetic mice. PLoS One 2014; 9:e110359; PMID:25390735; http://dx.doi.org/10.1371/journal.pone.0110359
40. Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Toth M, Korecka A, Bakocevic N, Guan NL, Kundu P, et al. The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med 2014; 6:263ra158-263ra158; PMID:25411471; http://dx.doi.org/10.1126/scitranslmed.3009759
41. Heijtz RD, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci 2011; 108:3047-52; PMID:21282636; http://dx.doi.org/10.1073/pnas.1010529108
42. Smith K, McCoy KD, Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin Immunol 2007;

19:59-69; PMID:17118672; http://dx.doi.org/10.1016/j.smim.2006.10.002

43. Manichanh C, Reeder J, Gibert P, Varela E, Llopis M, Antolin M, Guigo R, Knight R, Guarner F. 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
44. Hu Y, Peng J, Tai N, Hu C, Zhang X, Wong FS, Wen L. Maternal Antibiotic Treatment Protects Offspring from Diabetes Development in Nonobese Diabetic Mice by Generation of Tolerogenic APCs. J Immunol 2015; 195:4176-84; PMID:26401004; http://dx.doi.org/10.4049/jimmunol.1500884
45. Peng J, Narasimhan S, Marchesi JR, Benson A, Wong FS, Wen L. Long term effect of gut microbiota transfer on diabetes development. J Autoimmun 2014; 53:85-94; PMID:24767831; http://dx.doi.org/10.1016/j.jaut.2014.03.005
46. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013; 341:1241214; PMID:24009397; http://dx.doi.org/10.1126/science.1241214
47. Celaj S, Gleeson MW, Deng J, O'Toole GA, Hampton TH, Toft MF, Morrison HG, Sogin ML, Putra J, Suriawinata AA, et al. The microbiota regulates susceptibility to Fas-mediated acute hepatic injury. Lab Invest 2014; 94:938-49; PMID:25068658; http://dx.doi.org/10.1038/labinvest.2014.93
48. Yurkovetskiy L, Burrows M, Khan AA, Graham L, Volchkov P, Becker L, Antonopoulos D, Umesaki Y, Chervonsky AV. Gender bias in autoimmunity is influenced by microbiota. Immunity 2013; 39:400-12; PMID:23973225; http://dx.doi.org/10.1016/j.immuni.2013.08.013
49. Hansen CHF, Andersen LSF, Krych L, Metzdorff SB, Hasselby JP, Skov S, Nielsen DS, Buschard K, Hansen LH, Hansen AK. Mode of delivery shapes gut colonization pattern and modulates regulatory immunity in mice. J Immunol 2014; 193:1213-22; PMID:24951818; http://dx.doi.org/10.4049/jimmunol.1400085
50. Daft JG, Ptacek T, Kumar R, Morrow C, Lorenz RG. Cross-fostering immediately after birth induces a permanent microbiota shift that is shaped by the nursing mother. Microbiome 2015; 3:17; PMID:25969735; http://dx.doi.org/10.1186/s40168-015-0080-y