REVIEW

Bygiene: The New Paradigm of Bidirectional Hygiene

Gabriel A. Al-Ghalith[a] and Dan Knights[a,b*]

[a]Biomedical Informatics and Computational Biology, University of Minnesota, Minneapolis, Minnesota, and [b]Department of Computer Science and Engineering, University of Minnesota, Minneapolis, Minnesota

The pervasive dogma surrounding the evolution of virulence — namely, that a pathogen's virulence decreases over time to prevent threatening its host — is an archaic assertion that is more appropriately cast as an optimization of virulence cost and benefit. However, the prevailing attitudes underlying practices of medical hygiene and sanitization remain entrenched in these passé ideas. This is true despite the emergence of evidence linking those practices to mounting virulence and antimicrobial resistance in the hospital. It is, therefore, our position that just as the microbe has sought an optimized balance in virulence, so should we seek such an optimized balance in vigilance, complementing warfare with restoration. We call this approach "bygiene," or bidirectional hygiene.

INTRODUCTION

Hospitals throughout the world are increasingly following the lead set by Western biomedicine and are looking to emulate its core tenets and practices, from biochemically engineered pharmaceuticals to hospital hygiene, as closely as possible under what are often challenging economic and geopolitical conditions. Developed Western nations, however, are paradoxically seeing a rise in a particular variety of infection in the hospital: health care-acquired infection (HAI†) [1]. The problem of eliminating nosocomial and other HAIs has proven intractable despite continually emerging sanitization technologies [2] and protocols. This is in large part due to the evolution of highly pathogenic, multidrug-resistant microbes, popularly termed "superbugs." As we will explore, however, these and other contributing factors may have underlying causes rooted in excessive hygienic practices and can be mitigated through a microbial remediation approach based in evolutionary theory.

Virulence, loosely considered here as the degree to which an organism can damage its host, is subject to evolution. Although now beginning to change, a common yet outdated mode of thought regarding the evolution of virulence is that pathogens will seek to maximize their chances of survival over time by minimizing harm to the host and will always, therefore, evolve toward "benign coexistence" [3]. Supplanting this outmoded reasoning is a parameterized model for the evolution of virulence [4]. Under this newer model, each organism has a specific optimal virulence at which it is in a state of balance between cost and benefit. In addition to modifying the degree of virulence, this optimization also occurs in temporal terms, as the case of lysogenic viruses and opportunistic pathogens demonstrates by their varying ability to exist in non-pathogenic states indefinitely. Hence, virulence is a trade-off; in effect, this means that our own actions can contribute to the extent a microbe benefits from pathogenicity and, therefore, the ultimate virulence of the pathogen.

Behaviors or institutions that facilitate microbial transmission to an extent that would not otherwise be expected lower the cost of virulence and collectively can be termed "cultural vectors" of disease transmission [5]. This label applies to the modern hospital, in which some of the most susceptible patients are crowded into the same barracks as our veteran, war-hardened "enemies," much like during the Spanish Flu epidemic of 1912 that
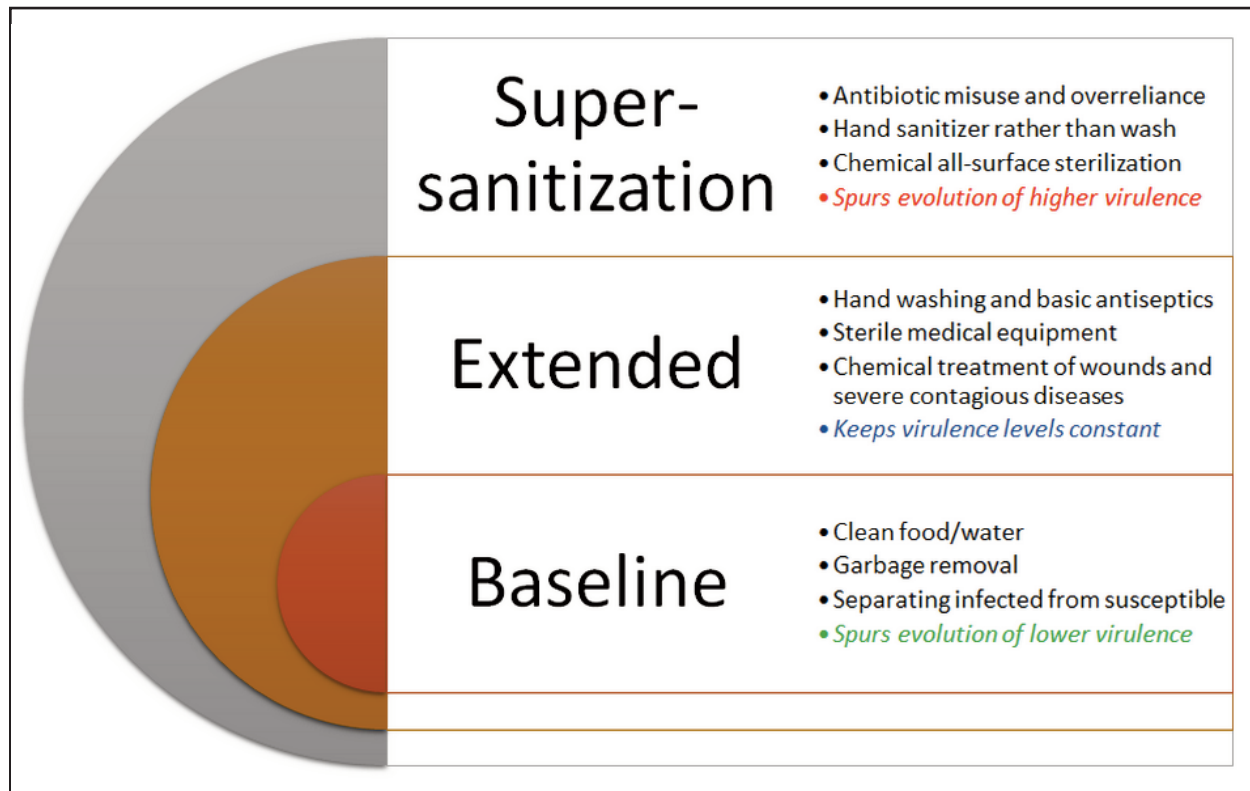
Figure 1. The continuum of modern hygiene. Hygienic practices in the outer circles are typically practiced along with those in the inner circles. An ideal scenario would involve practicing baseline and extended hygienic practices while eliminating super-sanitization.

exhibited a rapid evolution toward high virulence [6]. Our stance, therefore, is that we should turn to our microbial allies instead of considering the entire microbial world to be our "enemies" and complement the sterilization side of hygiene with microbial restoration.

MICROBIAL MELEE

We must accept quickly and decisively that our medical system is unwittingly creating a competitive arms race that we are unlikely to ever truly win. Microbes have proven their ability to quickly develop resistance to antimicrobial compounds in the hospital and, by some estimates, are now poised to flood beyond the hospital gates into the community [7]. The pace of new narrow-spectrum antimicrobial discovery is tapering off, with resistance to each accruing shortly after its introduction [8], while the cost of discovering new drugs is rising exponentially [9]. The cost of treating antibiotic-resistant infections hit an all-time high in 2010, with the trend continuing upward [10].

This isn't to say there is no hope in novel drug discovery. On the contrary, understanding the evolutionary underpinnings of the virulence trade-off hypothesis has recently given rise to an entirely new class of drugs intended to demonstrate ecologically grounded resistance to resistance [11]. Supposedly, by targeting virulence factors themselves at the molecular level, pathogenesis can be specifically selected against without threatening benign communities [12]. However, in practice, such drugs have rapidly seen resistance emerge [13,14]. Recent developments have since modified this premise, suggesting that despite the inevitable development of resistance, nuances in the drug treatment protocol itself, rather than indiscriminate use of the drug, may sway the trade-off [14]. This line of thinking is compatible with the present proposal that the answer to the virulence dilemma lies within us — specifically, in our own community of microbes.

Extensive recent findings from human microbiome research provide evidence that overly hygienic and antimicrobial practices are detrimental to human health [10]. The healthy human body houses hundreds of trillions of microbes inside and out; within the gut alone, there are more than 10 microbial cells to each one human cell and 100 times the number of genes [15]. Theories of the human "superorganism" are even taking shape to account for this striking reality, and yet, the importance of this "forgotten organ" has remained largely unappreciated until recently [16]. It is, therefore, reasonable to assume that as a valuable part of the healthy self, excessive perturbations of our microbial ecology might well be deleterious, and indeed, many health conditions have now been associated with such "dysbiosis" of our microbiome [17].

As distinguished from baseline hygienic practices (Figure 1), "super-sanitization" has become the norm in modern hospitals. In its current form, super-sanitization involves the

extensive use of broad-spectrum antibiotics, antimicrobial hand sanitizer stations in lieu of hand-washing, sophisticated mechanical air filtration systems, and rigorous hospital surface sterilization, in addition to liberal use of antimicrobial agents. Despite prevalent over-prescription of antibiotics [18], these antimicrobial agents have sweeping effects throughout the body in addition to precipitously raising levels of antimicrobial resistance in and out of the hospital. The hygienic ideal in the hospital, therefore, seems to have taken on unrealistic and counterproductive ambitions to "sanitize" the patient environment from the inside out, the effects of which we will now explore.

Beginning with the bowels of the patient, perturbations of the microbiome result in multiple undesirable effects. As the gut microbiome is normally involved in helping with digestion, a reduction in gut microbes following antibiotic treatment renders the gut less effective in harvesting nutrients from food [19]. Antibiotics also have been implicated in intestinal dysbiosis [20], potentially contributing to serious health conditions such as *Clostridium difficile* infection (CDI), antibiotic resistance [21], inflammatory bowel disease, diabetes, obesity [22], metabolic disease, and even conditions as seemingly distant from the gut as autism [23]. Indeed, the microbiome may play a role in depression, mental health, and decision-making [24,25]. From an ecological vantage, the disrupted gut community attempts to shield itself against extinction from antibiotics by engaging in protective measures such as induced horizontal gene transfer of antimicrobial resistance factors, which results in a rapid evolution of antimicrobial resistance [26]. The microbial landscape of the gut in the wake of antibiotic insult is ripe for opportunistic infection in accordance with niche theory after a destructive habitat event, and the ecological succession that follows suit is far from optimal for the host, as with CDI.

Moving up to the next layer of the patient-environment system, the skin is often liberally scoured with alcoholic povidone-iodine or chlorhexidine-based antiseptics to reduce risk of puncture infection secondary to injection, a sanitization effort that may affect much of the region's normal epidermal microbiome. This healthy microbiome of the skin acts in tandem with the immune system as a barrier (or, even in some cases, a standing army) against potential pathogens [27] and even plays a role in modulating the human host's own inflammatory responses to infection or injury [28]. Ironically, "disinfecting" the region in this manner, in addition to clearing it of some pathogens, may, in theory, be removing its sentinels and clearing the way for opportunistic pathogens like methicillin-resistant *Streptococcus aureus* (MRSA).

Proceeding another level outward, the surfaces around the patient, from walls to window sills to the floor, are routinely washed with bleach and other powerful antimicrobial chemicals (disinfectants). Although designed to eliminate a large proportion of microbial species, disinfectants simply do not kill all of them, and as such, "some organisms and bacterial spores" do survive, even if their concentrations are such that they are usually unable to cause disease on their own; common household antibacterial cleaners also suffer from this dilemma [29]. It is not outside the realm of possibility that the microbes that do survive will likely be the community's hardiest members. Today's aseptic protocol, while not selecting specifically for virulence, nonetheless constitutes an evolutionary pressure for the development of antimicrobial resistance, which may potentiate virulence or other disease mechanisms under certain conditions [30].

Finally, the airflow in the hospital is pumped through mechanical filtration systems intended to clear harmful microbes from the air. However, not only do high-efficiency particulate air (HEPA) filters fail to completely filter out potentially pathogenic species [31], a survey of the hospital microbiome pointed to an increase in indicators for opportunistic pathogens in mechanically ventilated rooms as opposed to window-ventilated rooms, and the major determinants for pathogen accumulation were found to be higher humidity and airflow rate [32]. Ultimately, the filter built in the name of sanitization is no cleaner in many cases than pulling in fresh air from outside, although this may not apply to patients with special need of antifungal filters or transplant patients with extremely compromised immune systems.

A common theme underlying these observations is that extensive emphasis on creating a completely sterile environment is not only impractical, but also results in the killing of benign or beneficial microbes while imperfectly controlling the extremely pathogenic ones, furthering resistance mechanisms and selecting for pathogenicity. As a cultural vector, the hospital often weakens the protective normal human microbiome, selects for resistant microbes, and then disseminates them to susceptible patients. It is clear that the modern super-sanitized hospital represents the overly clean end of the hygiene spectrum.

IF YOU CAN'T BEAT 'EM, JOIN 'EM

We suggest that one way to address this dilemma is to restore healthful communities [33] to over-sterilized environments. Instead of treating all microbes as the enemy, it eventually may benefit health care experts to recognize and support our microbial allies by reintroducing them to the appropriate niches in and around patients and the hospital. The culturing process may ultimately run the gamut from gut to filtration system or beyond the hospital entirely, although in most cases, substantial research needs to be done to determine how this remediation would take place.

The practice of fecal microbiota transplantation (FMT) fits this prescription precisely. This procedure, which has been practiced in some form for a variety of ailments for at least half a century [34], has seen a recent resurgence in study and practice since the advent of technology capable of quantifying host microbial configuration [35]. For example, 16S rDNA sequencing technology has enabled investigators to quantitatively determine that

"engraftment" of this microbial organ unequivocally takes place and that it is responsible for patient recovery through ecological means free of significant adverse side effects [36]. The concept of transplanting a donor's fecal microbiota into a patient's gut is the very antithesis of cultural conceptions of hygiene in the developed world, yet the comparatively low-cost FMT has an unprecedented cure rate of nosocomial *C. difficile* infection (approximately 96 percent) in severely colonized individuals with no other recourse [36,37]. Other applications of FMT are currently emerging, perhaps in inflammatory bowel disease [38]. As the field advances, there may be potential to perturb or introduce select members of this complex ecosystem to effect the desired outcome [39]. For FMT currently, the perturbation desired is general: a restoration to healthfulness. Hence, donors are screened using rigorous general health criteria to maximize this directionality. The future of FMT, however, may personalize this process in the form of targeted microbiome interventions, averting the potential for unwittingly introducing opportunistic pathogens or incompatible species in specific recipients.

The skin has received less attention from a restorative perspective, but some evidence seems to indicate a benefit to the use of probiotics for skin conditions, one citing the restorative advantage of applying microbes to compensate for antibiotic treatment, similar to FMT. This restorative intervention has successfully used a commensal bacterium, acne-associated *Proprionobacterium acnes*, to suppress growth of MRSA [40]. There is ongoing research to restore the microbiome to newborn babies delivered via cesarean section, as microbial inoculation by the vaginal tract during childbirth is associated with positive health outcomes later in life [41]. Recent evidence also demonstrates the potential for oral probiotics to improve oral health markers and protect against periodontal pathogenic bacteria [42]. Inoculation of hospital surfaces and ventilation systems with normal or "healthful" species seems a logical next step, with the added potential to outcompete pathogens in environmental niches before they even reach the human body. Some gut bacterial taxa are currently under study for their ability to fulfill these roles in the gut, including *Bifidobacterium*, *Lactobacillus*, *Akkermansia miciniphila*, *Faecalibacterium prausnitzii*, *Roseburia* spp., and *Eubacterium hallii* [43].

Although microbial restoration holds considerable potential, the particularities of the distribution thereof warrant additional layers of consideration such that the inoculation strategy proceeds in accordance with individual patient needs. A severely immunocompromised patient would require a different strategy (or hospital ward) than a patient with an airborne respiratory infection, for instance. There has been some investigation into use of surface inoculation as a biocontrol strategy, with promising early results [44,45]. More research into the ecological and mechanistic actions of the inoculants is required to find the optimal balance of microbes for each set of patient conditions.

THE CASE OF THE MIS-EVOLVED PREMISE

It is important to consider a paradigm shift of this magnitude from various angles, including an examination of some foreseeable counter-arguments against the present ecological hypothesis. One such counter-argument to the proposal of bidirectional hygiene would be to question the universal validity of virulence trade-off optimization — a foundation upon which the bulk of the analysis is based. So-called "coincidental" and "short-sighted" evolution have been proposed as two alternative routes [46] adopted by certain pathogens in the establishment of virulence in humans. This counter-argument would posit that it would be safest to super-sanitize, because despite its incompleteness, sanitization is universal and independent of the ultimate "cause" of virulence.

The theory of coincidental evolution seems to contradict the trade-off hypothesis by explaining the emergence of virulence in a microbe as a coincidental result of some other arbitrary reason unrelated to its pathogenicity in humans [47]. By this logic, coincidental evolution might be an expected evolutionary model for certain generalist pathogens for whom the human is a "dead-end host." Similarly, short-sighted evolution [48] is proposed as a form of "accidental" evolution of virulence due to selection pressures within an individual host that may not confer upon the unwitting microbe any additional advantage for between-host transmission or even may be detrimental to this end. Either case seems to violate the trade-off hypothesis, but the utility of bidirectional hygiene still holds.

First, in regard to coincidental evolution, a restorative plan effectively would select against the pathogen within its non-native host or niche. Second, in the case of short-sighted evolution, should a short-sighted microbe succeed in occupying a particular niche to its own detriment, it still would be impeded by an entrenched and resilient healthy microbiome at every level in and around the patient.

Another counter-argument might suggest that the expanding size of hospitals is the ultimate cause of its being a cultural vector. As previously mentioned, a hospital makes for an ideal mixing vessel in which patients congregate in close quarters to facilitate pathogen spread. However, a study considering the influence of hospital size on rates of nosocomial infection has not seen this assumption borne out in practice [49]. In sum, a policy shift toward bidirectional hygiene, or "bygiene," remains tenable from a variety of evolutionary and financial perspectives.

SIFTING THE CURVE SHIFTING

In the virulence cost-benefit theory, the benefits of virulence from the perspective of a microbe include a higher reproductive rate as well as heavier pathogen load within a host. Since fitness in the short term is quantified as a species' immediate number of progeny, higher virulence would imply more efficient use of the host as a re-

productive vessel. However, this fitness is offset in the longer term by the costs associated with that same virulence. These costs may include the energetic requirements of maintaining and expressing antimicrobial resistance genes, the between-host transmission penalty incurred by shutting down a potential conduit for transmission, and the toll exacted by otherwise restricting transmission efficacy despite higher pathogen load within an individual host [50]. In general, the result of reducing the efficacy of the hospital as a cultural vector would be to force pathogens to pay a higher virulence cost, as transmission would no longer be facilitated between incapacitated hosts. Likewise, the specific harmful impacts of super-sanitization on the evolution of virulence can be understood in terms of their contributions to this tradeoff.

The destruction of the normal microbiome in and around patients can be seen as increasing the benefit of virulence. A landscape devoid of most potential competitors allows reproduction of a pathogen to occur without restraint, resulting in pathogenic blooms, and pathogens are free to compete within the same host to produce even more virulent strains. Less virulent pathogens would be disadvantaged because they would be less expeditious in propagating into the niche, which would instead be quickly filled by their more efficient kin. Additionally, depletion of the normal microbiome can be seen as decreasing the cost of virulence. Without the sentinels, immune-priming agents, and various other regulatory bodies within an intact community, whatever virulence factors normally might have been recognized and systematically excluded by the community no longer elicit a negative reaction from the host. This is especially true of hosts whose immune systems are already in a compromised state, which is frequently the case in susceptible populations of hospital patients.

There is much ongoing research into molecular links between specific resistance mechanisms and their individual implications in virulence [51]. However, antimicrobial resistance resulting from super-sanitization may be seen in general as decreasing the cost of virulence in the long term. When a pathogen reaches a high level of virulence, it may be exposed to antimicrobial drugs, effectively imposing a virulence cost. However, in the case of a pathogen with evolved antimicrobial resistance, rather than being curtailed by the application of antimicrobial agents, a wave of resistant infection can persist and transmit undeterred, thereby maintaining or even increasing virulence at the population level. This may be best exemplified by pathogens for which antimicrobial interventions, rather than modulation of behavior or other host-side factors, serve as both the primary mode of control and hurdle to evolving increased pathogenicity, since the only weapon holding them back would have lost its effect.

As mentioned previously, dysbiosis, whether caused by broad-spectrum antimicrobial agents or other factors in the complex web of host genetics, environment, and microbiome [52], plays a critical role in rendering the host susceptible to pathogenic blooms from within and without. Nosocomial agents, opportunistic pathogens, or imbalances of healthful species may result in health consequences, both known and unknown. It may be possible by extension that our own "evolving" behaviors and microbial community compositions contribute to a growing "Western dysbiosis," as potentially healthful members of the microbiome in industrialized nations disappear from one generation to the next [53].

Microbial remediation may hold significant promise in counterbalancing these deleterious shifts toward increased virulence. Restoring depleted members of a healthy microbiome to a damaged community can reverse the virulence benefits of pathogenicity and re-impose community- and niche-associated virulence costs. Diverse, species-rich habitats are less amenable to perturbation [54], likely through regulatory mechanisms that enforce the system's equilibrium population dynamics, actively excluding high-virulence invaders. Pursuing restorative microbial treatments in addition to antimicrobial warfare as strategies for disease control and prevention may prove useful for controlling the mounting problems of antimicrobial resistance at their source.

CONCLUSIONS

If we were to imagine the entirety of pathogenic microbes as influenza viruses and the sum of our current attempts at super-sanitization as an antiviral drug, we might see some conclusions akin to the surprising findings of Lipsitch et al. in their model of antiviral resistance in pandemic influenza [55]. One such finding is that the benefit of antiviral use (medical hygiene) is highest at "intermediate levels of antiviral use," for the reason that doing nothing in the way of stopping infection will of course not diminish pathogenicity at all, but doing too much will eventually cause complete resistance to our efforts and become exactly like doing nothing.

The personalization of microbial remediation in the hospital is one important future research direction for personalized medicine, as hygienic policy could be tailored on a per-patient basis based on known risk factors and microbiome assays. It also would promote a more holistic form of medical practice in which hygiene becomes about balancing antimicrobial warfare against pathogens with restoration of essential microbial diversity. This balanced approach could include administration of probiotics, natural air supplies with protective microbial infusions, microbiome-sensitive skin care as part of standard practice, and other interventions that require further research. Preliminary evidence indicates that at least some hospital sterilization practices can be safely removed, replaced, or supplemented with microbial restoration [45].

A bidirectional approach to hygiene espousing hygienic best-practices in tandem with restoration of normal microbial communities is preferable to continuing down a road solely focused on antimicrobial warfare. Such a bidi-

rectional technique holds the potential to improve health care on both an individual and global level. Indeed, beyond the hospital and in all walks of life, the notion of bidirectional hygiene, or "bygiene," highlights the growing need for balanced and nuanced approaches to contend with pathogens on an ecological scale in the face of mounting antimicrobial resistance. As such, future work should study restorative techniques in addition to germ warfare.

REFERENCES

1. Dancer SJ. The role of environmental cleaning in the control of hospital-acquired infection. J Hosp Infect. 2009;73(4):378-85.
2. Sciortino CV, Giles RA. Validation and comparison of three adenosine triphosphate luminometers for monitoring hospital surface sanitization: a Rosetta Stone for adenosine triphosphate testing. Am J Infect Control. 2012;40(8):e233-9.
3. Ewald PW. The evolution of virulence. Sci Am. 1993;268(4):86-93.
4. Alizon S, Hurford A, Mideo N, Van Baalen M. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. J Evol Biol. 2009;22(2):245-59.
5. Ewald PW. Transmission modes and the evolution of virulence. Hum Nat. 1991;2(1):1-30.
6. Reid AH, Taubenberger JK, Fanning TG. The 1918 Spanish influenza: integrating history and biology. Microbes Infect. 2001;3(1):81-7.
7. Sefton AM. Mechanisms of Antimicrobial Resistance. Drugs. 2002;62(4):557-66.
8. Clatworthy AE, Pierson E, Hung DT. Targeting virulence: a new paradigm for antimicrobial therapy. Nat Chem Biol. 2007;3(9):541-8.
9. Scannell JW, Blanckley A, Boldon H, Warrington B. Diagnosing the decline in pharmaceutical R&D efficiency. Nat Rev Drug Discov. 2012;11(3):191-200.
10. Vangay P, Ward T, Gerber JS, Knights D. Antibiotics, pediatric dysbiosis, and disease. Cell Host Microbe. 2015;17(5):553-64.
11. Cegelski L, Marshall GR, Eldridge GR, Hultgren SJ. The biology and future prospects of antivirulence therapies. Nat Rev Microbiol. 2008;6(1):17-27.
12. Marra A. Can virulence factors be viable antibacterial targets? Expert Rev Anti Infect Ther. 2004;2(1):61-72.
13. Maeda T, García-Contreras R, Pu M, Sheng L, Garcia LR, Tomás M, et al. Quorum quenching quandary: resistance to antivirulence compounds. ISME J. 2012;6(3):493-501.
14. Allen RC, Popat R, Diggle SP, Brown SP. Targeting virulence: can we make evolution-proof drugs? Nat Rev Microbiol. 2014;12(4):300-8.
15. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, et al. Metagenomic analysis of the human distal gut microbiome. Science. 2006;312(5778):1355-9.
16. Kinross JM, von Roon AC, Holmes E, Darzi A, Nicholson JK. The human gut microbiome: implications for future health care. Curr Gastroenterol Rep. 2008;10(4):396-403.
17. Ianiro G, Bibbò S, Gasbarrini A, Cammarota G. Therapeutic modulation of gut microbiota: current clinical applications and future perspectives. Curr Drug Targets. 2014;15(8):762-70.
18. Linder JA, Stafford RS. Antibiotic Treatment of Adults With Sore Throat by Community Primary Care Physicians: A National Survey, 1989-1999. JAMA. 2001;286(10):1181-6.
19. Cani PD, Delzenne NM. The role of the gut microbiota in energy metabolism and metabolic disease. Curr Pharm Des. 2009;15(13):1546-58.
20. Hawrelak JA, Myers SP. The causes of intestinal dysbiosis: a review. Altern Med Rev. 2004;9(2):180-97.
21. Deshpande A, Pasupuleti V, Thota P, Pant C, Rolston DDK, Sferra TJ, et al. Community-associated Clostridium difficile infection and antibiotics: a meta-analysis. J Antimicrob Chemother. 2013;68(9):1951-61.
22. Al-Ghalith GA, Vangay P, Knights D. The guts of obesity: progress and challenges in linking gut microbes to obesity. Discov Med. 2015;19(103):81-8.
23. Wakefield AJ. The Gut–Brain Axis in Childhood Developmental Disorders. J Pediatr Gastroenterol Nutr. 2002;34:S14-7.
24. Dash S, Clarke G, Berk M, Jacka FN. The gut microbiome and diet in psychiatry: focus on depression. Curr Opin Psychiatry. 2015;28(1):1-6.
25. Alcock J, Maley CC, Aktipis CA. Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. Bioessays. 2014;36(10):940-9.
26. Stecher B, Maier L, Hardt W-D. "Blooming" in the gut: how dysbiosis might contribute to pathogen evolution. Nat Rev Microbiol. 2013;11(4):277-84.
27. Liu CM, Price LB, Hungate BA, Abraham AG, Larsen LA, Christensen K, et al. Staphylococcus aureus and the ecology of the nasal microbiome. Science Advances. 2015;1(5): e1400216.
28. Lai Y, Di Nardo A, Nakatsuji T, Leichtle A, Yang Y, Cogen AL, et al. Commensal bacteria regulate Toll-like receptor 3–dependent inflammation after skin injury. Nat Med. 2009;15(12):1377-82.
29. Levy SB. Antibacterial household products: cause for concern. Emerg Infect Dis. 2001;7(3 Suppl):512-5.
30. Cameron DR, Howden BP, Peleg AY. The interface between antibiotic resistance and virulence in Staphylococcus aureus and its impact upon clinical outcomes. Clin Infect Dis. 2011;53(6):576-82.
31. Araujo R, Cabral JP, Rodrigues AG. Air filtration systems and restrictive access conditions improve indoor air quality in clinical units: Penicillium as a general indicator of hospital indoor fungal levels. Am J Infect Control. 2008;36(2):129-34.
32. Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, Womack AM, et al. Architectural design influences the diversity and structure of the built environment microbiome. ISME J. 2012;6(8):1469-79.
33. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature. 2007;449(7164):804-10.
34. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery. 1958;44(5):854-9.
35. Furrie E. A molecular revolution in the study of intestinal microflora. Gut. 2006;55(2):141-3.
36. Bakken JS, Borody T, Brandt LJ, Brill JV, Demarco DC, Franzos MA, et al. Treating Clostridium difficile infection with fecal microbiota transplantation. Clin Gastroenterol Hepatol. 2011;9(12):1044-9.
37. Khoruts A, Sadowsky MJ. Therapeutic transplantation of the distal gut microbiota. Mucosal Immunol. 2011;4(1):4-7.
38. Cui B, Feng Q, Wang H, Wang M, Peng Z, Li P, et al. Fecal microbiota transplantation through mid-gut for refractory Crohn's disease: safety, feasibility, and efficacy trial results. J Gastroenterol Hepatol. 2015;30(1):51-8.
39. Ubeda C, Bucci V, Caballero S, Djukovic A, Toussaint NC, Equinda M, et al. Intestinal Microbiota Containing Barnesiella Species Cures Vancomycin-Resistant Enterococcus faecium Colonization. Infect Immun. 2013;81(3):965-73.
40. Shu M, Wang Y, Yu J, Kuo S, Coda A, Jiang Y, et al. Fermentation of Propionibacterium acnes, a commensal bacterium in the human skin microbiome, as skin probiotics against methicillin-resistant Staphylococcus aureus. PLoS One. 2013;8(2):e55380.
41. Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG. The infant microbiome development: mom matters. Trends Mol Med. 2015;21(2):109-17.

42. Flichy-Fernández AJ, Ata-Ali J, Alegre-Domingo T, Candel-Martí E, Ata-Ali F, Palacio JR, et al. The effect of orally administered probiotic Lactobacillus reuteri-containing tablets in peri-implant mucositis: a double-blind randomized controlled trial. J Periodontal Res. 2015 Feb 25. [Epub ahead of print].

43. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11(8):506-14.

44. Falagas ME, Makris GC. Probiotic bacteria and biosurfactants for nosocomial infection control: a hypothesis. J Hosp Infect. 2009;71(4):301-6.

45. Vandini A, Temmerman R, Frabetti A, Caselli E, Antonioli P, Balboni PG, et al. Hard surface biocontrol in hospitals using microbial-based cleaning products. PLoS One. 2014;9(9):e108598.

46. Levin BR. The evolution and maintenance of virulence in microparasites. Emerg Infect Dis. 1996;2(2):93-102.

47. Adiba S, Nizak C, van Baalen M, Denamur E, Depaulis F. From grazing resistance to pathogenesis: the coincidental evolution of virulence factors. PLoS One. 2010;5(8):e11882.

48. Levin BR, Bull JJ. Short-sighted evolution and the virulence of pathogenic microorganisms. Trends Microbiol. 1994;2(3):76-81.

49. Sax H, Pittet D. Interhospital Differences in Nosocomial Infection Rates: Importance of Case-Mix Adjustment. Arch Intern Med. 2002;162(21):2437-42.

50. Dieckmann U. Adaptive Dynamics of Pathogen–Host Interactions. In: Dieckmann U, Metz JAJ, Sabelis MW, Sigmund K, editors. Adaptive Dynamics of Infectious Diseases. Cambridge: Cambridge University Press; 2002. p. 39-59.

51. Beceiro A, Tomás M, Bou G. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev. 2013;26(2):185-230.

52. Knights D, Silverberg MS, Weersma RK, Gevers D, Dijkstra G, Huang H, et al. Complex host genetics influence the microbiome in inflammatory bowel disease. Genome Med. 2014;6(12):107.

53. Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? Nat Rev Microbiol. 2009;7(12):887-94.

54. McCann KS. The diversity-stability debate. Nature. 2000;405(6783):228-33.

55. Lipsitch M, Cohen T, Murray M, Levin BR. Antiviral resistance and the control of pandemic influenza. PLoS Med. 2007;4(1):e15.