



Reconciling Hygiene and Cleanliness: A New Perspective from Human Microbiome

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Abstract The term hygiene is deeply rooted with the concept of maintaining sound health and alertness towards cleanliness, while “hygiene hypothesis” depicts the protective role of microbial community exposure in development of early immunity and initial allergic and aesthetic reactions. The tug-of-war has now been pushed toward the literal term “hygiene” over the “hygiene hypothesis” and has continued with disinfection of all microbial loads from the related environments to avoid infections in humans. With the advancement in the microbiome studies, it became clear that humans possess warm, and significant relationships with diverse microbial community. With this opinion article, we have emphasized on the importance of hygiene hypothesis in immunological responses. We also propose the individual/targeted hygiene instead of application of unanimous hygiene hypothesis. This review also elaborates the common practices that should be employed to maintain hygiene along with the balanced microbiome.

Keywords Hygiene · Old friend hypothesis · Targeted hygiene · Allergies

Introduction

The human microbiome is one of the most studied microecosystems representing the symbiotic association of microbes with the body cells. It has been estimated that the human gut harbors approximately 40,000 bacterial species, 9 million inimitable bacterial genes and 100 trillion microbial cells [1–3]. Not only bacteria, but also archaea, fungi, viruses and protozoa contribute to the human microbiome. In the recent years, the in-depth microbiome analysis have revealed that the microbiome is a crucial determinant of our protective immune responses and helps in proper functioning of digestive system, endocrine system as well as nervous system [4–7]. With the advancements in human microbiome research it is becoming increasingly clear that all bacteria are not dangerous and the concept that we should avoid bacteria at all cost i.e., the conflating hygiene with sterilization is proving to be utterly wrong [8]. On the other hand, the hygiene concept is primarily rooted around cleanliness for maintaining the healthy lifestyle. The effect of several factors such as physical, psychological, social, diet, different ancestral origin and hormonal cycles on the human microbiome are in direct influence of hygiene and it has now become an essential directive for microbiome assessment [9–11]. People often conflate hygiene with sterilization, which means the removal of microbes irrespective of their roles. Thus, promoting the healthy microbial diversity should add into the hygiene hypothesis, rather than conflicting it. In this review, we have focused on the key hygiene factors which directly or indirectly in linkage with our microbiome.

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History of Hygiene and Its Stretched Misleading Tale

The “Hygiene Hypothesis” initially proposed by Starchan [12] stated that the lower chances of early childhood infections lead to an upsurge in allergic diseases such as hay fever and asthma during the later stages of an individual’s life. Its original form proposed that the transmission of infections by “unhygienic contacts” in early life prevents allergies in old ages. Common cases of application of this hypothesis were the endemic infections caused by pathogenic bacteria (*Helicobacter pylori*), viruses (hepatitis viruses) and protozoans like *Toxoplasma gondii*. Soon this concept engulfed both the symbionts and mutualistic microorganism outside pathogens, and it was further extended to explain the incidences of inflammatory diseases [13]. During early-90s hygiene hypothesis started to offer better understanding and awareness towards inflammatory diseases like asthma and allergies [14]. During the mid-’90s, immunological studies generated the paradigm of T-helper cells (T_h -1 and T_h -2) and laid down the first basic theoretical foundation of hygiene hypothesis with scientific evidence [15]. With time the paradigm of T_h -cells started to evolve and the hygiene hypothesis itself suffered many alterations. Two of its popular updates—“Old friend” (diverse bacterial communities) [16] and “Disappearing microbes” (complete absence of microbes from a surface) [17] were proposed by different scientific communities. Both the altered hypothesis highlighted the importance of microbes in human health and homeostasis regardless of their commensal, symbiotic or pathogenic associations. During late-90s or early-2000s “Old Friend Hypothesis” was considered as a better description where the bridge between microbial exposure and inflammatory diseases were highlighted and since then human gut microbiome came into the frame [18]. However, the continued rejections of ‘Old-Friend-Hypothesis’ in last few years by western countries has now resulted in despair and under-modulation of human immune system due to less exposure to diverse kind of bacteria [19]. It has been largely accepted worldwide that it can create immune system dysregulation and ultimately cause allergic reactions like asthma, eczema and food allergies at later stages of life [20].

Hygiene, Microbial Diversity and Asthma

Although protective role of the microbial community over the progression of allergies and asthma has long been confirmed in various studies from western countries [21, 22]. But not much information is available from the developing countries. As evident from the literature, the farming environments provide excessive microbial exposure thus deliver

an atopy-protective effect of endotoxins (LPSs) in the farm children [23, 24]. This can be used as a model to scrutinize the effects of microbial community in development of inflammatory diseases. The endotoxins secreted by Gram-negative bacteria have been considered as the major source of asthmatic effects in humans [25]. Sequencing based results revealed that the *Acinetobacter lwoffii*, (known for its atopy protective potential in mouse models) is the major key player among the Gram-negative bacteria responsible for causing asthma. Effects of endotoxins have been studied in detailed with dust samples from two different agricultural sites (Hutterites and Amish) in the United States [26]. Hutterites run the highly evolved industrialization farming, while Amish farmers stick to their classical old-style type of farming for the last two centuries. In mouse model, it was observed that the crop dust extract from Amish type prohibited the hyper responsiveness whereas in case of Hutterites there was hyper responsiveness in airways sections. Metabolite study revealed that Amish farms extract were found seven-time richer in endotoxins as compared to Hutterites. Endotoxins exposures also induced A20, a ubiquitin modifying enzyme thus suppressing the dendritic and epithelial cell of airway mucosa [27, 28]. Farm toxins also played an important role in Toll-like receptor (TLR) tolerance in gut microbiome inflammation conditions as reported by Yiu et al. [26]. Pregnant women exposed to livestock and farm extracts had significant increase of TLR-4, CD-14 and endotoxin receptors in their offspring at their school-going [29] in comparison to less exposed. This highlights the facts that exposure from farm extract may cause a determined and long-term effect on immunity in human even at early ages.

Indeed, farmhouses offer a diverse exposure of bacterial and fungal species and decrease the chance of asthmatic onsets [30] and thus support “microbial diversity hypothesis”. However, enigmatic questions like, what is the exact mechanism and what specific role diversity has to play in immunity priming remains unanswered. It can be concluded that the exposure to diverse species may increase the probability for advantageous bacterial exposure or at least as a substitute that may keep detrimental pathogens at bay. Summing up all, it can be suggested that sequencing projects targeting a larger population might come up with a detailed resolution of microbial composition with their specific effects on individuals or in clusters of specific formations in the human gut to answer the unreciprocated questions.

Challenges with Hygiene Misnomer and Solution

The label of “hygiene” has caused some serious confusions as it is mostly interpreted as the sense of personal hygiene or cleanliness instead of infection transmissions. Since personal hygiene is maintaining the cleanliness of body

parts, clothes and our environment can be defined as promoting sanitary practices towards self [31]. But it is often considered as literal methods to protect the human body from infections. These methods like excessive sanitation, vaccination, clean food, water and indiscriminate use of antibiotics have put our immune system far away from early priming and ultimately leads to allergic conditions which are difficult to treat [32]. The major trouble with the “hygiene hypothesis” is the word “Hygiene”. In public Hygiene, word comes with prejudices meaning of personal cleanliness. Since mostly term cleanliness is used in place of hygiene as an overlap, but there is clear-cut difference in both these terms. Cleanliness generally is used for washing, removing dirt, etc. using detergent or other methods whereas, hygiene is to prevent or to minimize the frequencies for communicable diseases [33]. The current notion taking place for personal/domestic hygiene is “being too clean our own selves” as an overrated concept of sanitation to prevent microbial infections [34]. Data generated from RNA high throughput sequencing of the western populations suggest that their modern homes are teeming with microbes [35, 36]. Routine and weekly habits of cleaning have no long-lasting effect on indoor hygiene, hence it is compromised in cases of individual cleanliness [37]. Higher standards of personal cleanliness including excessive hand sanitization, bathing, application of beauty products, shampoo, antibiotics, etc. are contributing factor for altered microbial exposure by removing and replacing a large number of bacteria from skin and to the human gut [38]. In a widely accepted study, 399 German families were compared for personal hygiene (lower exposure to endotoxins, muramic acids and dust) with household cleanliness (lower dust but with higher endotoxins and muramic acids). The outcome revealed the less asthma and eczema observations in school-age children in household cleanliness [39]. A similar study in the UK also indicated the higher incidences of wheezing and atopic eczema in 30–42 month-old infants with frequent hand and face washing obsession by their parents [40].

Though there was no direct linkage of hygiene and cleanliness observed in the study cohorts, but it clearly reflected the importance of microbial exposures in early life stages. A key observation arises here that the microbial exposure in modern urban homes is almost different. This is applicable to the different populations all across the world where the standard of living plays an important role in defining personal hygiene and cleanliness. Since gut, oral and skin microbiota constantly changes with internal and external hygiene, thus the direct application of the westernized concept of hygiene is not an appropriate situation in all cases. The common example of the statement is the disruption of internal hygiene (gut microbiota) by altered diet and antibiotic exposure. Not only this but other

factors like house dust, water contamination even hand-shaking are real-time challenges to both individual hygiene and cleanliness. We idealized personal hygiene is a state of individual efforts towards a healthy lifestyle but keeping its environment entangled as limiting factor towards those efforts. Several factors affect the concept of hygiene from science to society that includes media-based TV commercials, governmental policies, education levels, public and medical professionals, etc. Thus, each and every individual seek an open definition of hygiene encompassing microbial exposures and cleaning habits [41, 42]. However, loosening the hygiene standards won't do the needful and it is not even right to adopt poor hygiene to get more exposures. Thus, as a misnomer, hygiene hypothesis fails to include all those immunity factors that have come out in later studies [43]. Various alternate names have been proposed like “microbiome depletion hypothesis” or “microbial diversity hypothesis” or “old friend hypothesis”. Another approach to defining this is “targeted hygiene” which targets the abolition of pathogens and restoration of the diverse microbiome.

Targeted Hygiene as a Means to Prevent Infectious Disease

Maintaining proper hygiene is good for health and preventing infectious diseases, but maintaining too much cleanliness may result in development of allergies, asthma, autoimmune disorders and inflammatory bowel diseases [44, 45]. It has been understood from the past that microbes cause infectious diseases, but the development of microbiome concept highlights the importance of studying microbe: human interactions. The microbes that reside in the natural environment and are associated with human microbiota play a crucial role in human development, digestion and the proper development of the immune system [46]. With an increase in the standard of living of the societies, the individuals are getting very less exposure to microbes which although has reduced the incidence of life-threatening infectious diseases, but making the immune system untrained. Hygiene is becoming synonymous to removing microbes from our environment and body by using sanitizers, alcohols, soaps, and other disinfectants. The gut, skin and oral cavity harbor the maximum microbes which are mostly beneficial and limits the growth of pathogenic bacteria [47]. But on the contrary, the normal public is made to believe that all microbes are “germs” and should be removed for maintaining proper health. There is an urgent need for making the general people aware of microbes, the functions they perform, when they can be harmful to humans and the interactions they play in the environment [11]. Another challenge in maintaining a

proper microbiome is the indiscrete use of antibiotics. Nowadays antibiotics are prescribed very frequently in anticipation of an infection i.e., even before an infection develops. Antibiotic usage during early stages disrupts the gut microbiome and has shown to cause obesity, diabetes, and other cardiovascular disorders [48]. This does not mean that antibiotics are causing these disorders, how microbiome responds to these antibiotics will determine development of disorders. The major problem is antibiotics are given for long durations in mild infections where they are not needed as our immune system can fight of the infection [49]. The paradigm believed is that antibiotics do not harm our body, but the microbiome studies have shown that they can have a negative effect on the normal microbiota and also increases the chance of development of drug resistance. The doctors usually prescribe antibiotics in anticipation of an infection [50]. Here also comes the role of a medical prescription, he/she should look into the probability of a disease getting worse before prescribing an antibiotic dosage and duration. Antibiotics should be correctly used for avoiding resistance and the duration and dosage should be decided after confirmation of the level of infection. Although there is a need for microbial exposure for children, this does not mean the children do not need antibiotics and vaccination. Vaccination and targeted hygiene are required for surviving childhood and the antigenic exposure from the environment is essential for proper training of the immune system. The society should be informed of good and bad microbes and hygiene should only target the bad microbes. The children should be allowed to interact with animals, plants, and environment, which do not possess harmful contaminants and wastes, for building a healthy microbiota and training the immune system. It does not mean we should abandon all hygiene practices but the need of the hour is to adopt targeted hygiene. There is a need to develop lifestyles which relink us with healthy microbial exposures while protecting against infectious disease [44].

Social Attribution and Hygiene in Shaping Human Microbiome

Hygiene hypothesis suggest that the acquiring microbes may essentially help in shaping the immunity, but major goal is to stabilize good and bad bacteria in microbiome. It is highly possible that this evolution has taken place under the influence of social behavior. It has been now proven that infancy is the most critical phase for the growth of an individual's microbiome along with the hygiene practices [51–53]. However, its surrounding individuals can specifically alter the microbiome in childhood stages. Physical interactions like hugging, kissing, skin to skin contact,

hand shaking etc. are directly responsible for microbial drifts among humans and animals. In-fact social relationships like co-housing can stimulate the similarities and dissimilarities in microbiome compositions. A detailed study among the co-housing individuals highlighted the common sharing of skin and inner nose microbiome in a young couple inhabiting together inside a lodge [54] while they were genetically not similar. Microbiome functionality can be varied at different stages of life like pregnancy, attaining puberty, ovarian cycle and menopause [55]. Another major hit taken by our bacterial diversity is the ageing process. Youth is dominated by phylum *Bacteroidetes* which reduces with the increasing age, while the *Firmicutes* follow a reverse trend during the life cycle [56]. This brings attentions of social implications on human microbiome alterations. This also suggest the sharing or transfer of microbiota at adult stages is quite effective. Thus, the transmittance of microbes among individuals is possible at any stage of life. In certain cases, it has been observed that microbiota of oral cavity and semino-vaginal sites has been shared in sport persons of a team [57]. These transmissions can affect moderately constant microbiome profiles in populations. This has created several issues of microbial complexity and variability in recent years. So far, the effects of diet independent lifestyle factors have been highly ignored over microbiome as for example smoking which affects the bowel passage [58]. In Crohn's Diseases (CD), patient smoking can elevate the ratio of *Bacteroides*–*Prevotella* and thus potentially backs the concomitants risks of CD. In fact, industrialization-based environment pollutants and air borne toxic elements have been shown to reach to bowel, increasing the IBD risks [59]. Another societal based factor, the so called “stress” which can randomly accelerate and deaccelerate gut microbiota profile [60]. It is the most profound anxiety factor embedded in our society and affects a large number of populations. It directly impacts the colonic motor activity using the body gut-brain axis which decreases the beneficial *Lactobacillus* count in our body [61]. Since our gut-brain axis has two-way communication between both neuronal and central nervous system, thus stress intervenes the brain and body activities including mood swings [62]. Geography also holds a strong pressure over the microbial compositions for humans and animals [63]. Microbiota in rural African's children was found highly diverse and richer in terms of microbial species as compared to developed countries in Europe [64]. Even the functional gene sets were significantly different in between the individuals of rural Malawi and Venezuela [65]. Travelling to far destinations and overseas wandering surges the potential risk of catching infectious diseases and significant alterations in the gut microbiome, that may lead to the diarrhea [66]. It can also alter the circadian rhythm leading to jet-lag by shifting in

time frames and work hours. It has been directly correlated to affect the gut microbiota in several studies [67, 68].

Do and Don'ts: Hygiene with Microbiome Maintenance

The external hygiene practices followed all around the world affect the skin specifically hand and vaginal microbiome that indirectly affects the gut microbiome. Thus, it is important to follow correct practices in order to maintain hygiene along with maintaining the essential microbiome to avoid diseased conditions. Maintenance of skin hygiene has long been associated with regular bathing and shampooing to eliminate these microbes associated with skin. The use of soaps, antiseptics, cosmetics affect the microbiome makeup of skin [69]. Use of antibiotics as dermatological treatment both orally and topically may instead spur more severe disorders or may cause permanent damage [70]. Therefore, the pathogenesis in context of human skin is not caused merely by the colonization of one species but the balance of the overall skin hygiene is essential for a healthy skin. Mostly hygiene refers to hand hygiene which is indirect example of skin or external hygiene. Hand hygiene plays most important role in infection control activities. Excessive use of commercially available antibacterial toilet soaps removes the beneficial bugs along with the bad microbes. When continued for long duration, this also results into adaptation of pathogens into antibiotic resistance forms. Now a day's health care associated infections (HCAIs) like hospital acquired pneumonia, tuberculosis, MDR infections etc. are prevented by health care practitioners (HCPs) by maintaining hand hygiene. Enough of the scientific evidences backs the reduce risk of cross transmission of infections in various health setups [71–73]. Taking these into account WHO launched global hand and hygiene campaign whereas a part of it “Hand Hygiene in Health Care” in 2006 was drafted. In 2008 first “Global Handwash Day” (<http://www.who.int/gpsc/tools/en/>) was observed and celebrated. Based on its success it was effectively adopted 3863 health care facilities for their commitment of taking effective precautionary measures, next phase of safety program “SAVE LIVES: Clean Your Hands” was launched. Hand hygiene is single most effective and less expensive hygiene practice that can control HAIs and AMRs. Studies has shown the proven result in limiting MSRA infections [74–76]. Even transmission of *Klebsiella* has been observed to reduce by improvement of hand hygiene [71, 74]. Hand hygiene can be maintained by using soap and water in cases of fluid, blood or protein content exposure on hands. Even suspected infections of *Bacillus anthracis* to be taken care by soap not by alcohol-based sanitizers since alcohol and

chlorine bases sanitizers have poor activities against them. In clinical situations like dealing a patient, catheter insertion, invasion procedures, mucus or wound exposures, alcohol-based hand rub should be used according to CDC/HICPAC and other guidelines [71, 77, 78] as they require less time. Further, vaginal microbiome dysbiosis due to over-hygienic conditions also lead to diseased conditions in women. Thus, there is need for early detection of the condition using various screening methods including Gram stain Nugent Score to detect lactobacilli and pathogens. The strategies employed by the physicians to improve the condition without the use of antibiotics include douching, probiotics, steam baths etc. These methods help the women get rid of the toxins and other problems like menstrual cramps, UTI, and fertility issues. Apart from these methods, probiotics have been widely used. This can be administered directly to vagina or taken via oral route. These probiotics containing lactobacilli targets the pathogen in the vaginal area and help maintain healthy microbiome. Thus, maintaining planned hygienic habits can help to avoid diseased conditions.

Conclusions

With passing on age's microbiome has now linked with evolutionary rational. It highlights the coevolution theory of human and its gut microbiome substituting the host–pathogen notion which earlier was a warfare for human rather than a biological evolution. Conclusively bacteria are neither friend or foe nor they have gone vanish from any place and hence we cannot live in a sterile place no matter how much hygiene practices we apply to our routine. We are in an era of dynamic changes which has just started. Applied evolutionary forces may come out with more insights into the relationship between man and its microbiome. In this coming relationship, we might take over more active role adaptation or methods of the desired microbiome in future. Since long hygiene hypothesis has been our guide to elucidate the various phenomenon in adequate responses of immunity. With time our understanding has been increased however its limitation should be acknowledged now. Cases of asthma and their relation with microbial diversity cannot be illustrated by hygiene hypothesis as a generic expiation of immunity dysregulation. Sticking to the only single aspect of hygiene and its application to the worldwide population is a matter of reassessment and rethinking. International Scientific Forum on Home Hygiene (IFH) established in 1997 to addresses the challenges of hygiene more effectively but lacks provisions for individual hygiene concept. But, the involvement of factors indirectly associated with microbiome interactions such as pollen/allergen exposure, pollution,

genetic makeup, etc. can be the additional factors. Studies relating to hygiene and allergies are going very fast, but still there is a long way before any conclusion is reached.

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Compliance with Ethical Standards

Conflict of interest Authors declares no conflict of interest.

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