Review Article

Indian J Med Res 142, November 2015, pp 515-524
DOI:10.4103/0971-5916.171275

Microbiome: Paediatricians' perspective

Shilpa Khanna Arora, Pooja Dewan* & Piyush Gupta*

*Department of Pediatrics, Postgraduate Institute of Medical Education & Research & Dr Ram Manohar Lohia Hospital, New Delhi & *Department of Pediatrics, University College of Medical Sciences & GTB Hospital, Delhi, India*

Received October 29, 2013

Millions of microorganisms inhabit the human body and affect its homeostasis in multiple ways. Alterations in this microbial community have implications for the health and survival of the human hosts. It is believed that these microorganisms should be included as part of the human genome because of their influence on human physiology hence the term "microbiome" is commonly used to refer to these microbes along with their genetic make-up and their environmental interactions. In this article we attempt to provide an insight into this recently discovered vital organ of the human body which is yet to be fully explored. We herein discuss the composition and role of microbiome in human health and disease with a special emphasis in children and culture-independent techniques employed in mapping of the microbiome. Alteration in the gut microbiome has been associated with causation of several paediatric diseases like infantile colic, necrotizing enterocolitis, asthma, atopy, obesity, type -1 diabetes, and autism. Atopic dermatitis and psoriasis have also been associated with changes in the cutaneous microbiome. Respiratory microbial imbalances during infancy have been linked with wheezing and bronchial asthma. Dysbiosis in the regional microbiome has been linked with caries, periodontitis, and chronic rhinosinusitis. The future therapeutic implications of this rapidly evolving area of research are also highlighted.

Key words Children - metagenomics - microbiome - microbiota - 16S RNA - virome

Introduction

Humans inhabit a whole community of microorganisms in and on their body[1]. Almost 100 trillion symbiotic microbes inhabit a single human body which is almost 10 times the number of cells present in an adult human. It amounts to almost 1-3 per cent of the body weight[2]. The term "microbiome" was first used by Joshua Lederberg[3] for "the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share the human body". He suggested that these microorganisms should be included as part of the human genome because of their influence on human physiology. Research in the field of metagenomics suggests that the brigade of microorganisms living in close proximity, both in and on, human beings is indispensible for human survival. This has led to the hypothesis that the human being must be regarded as a super-organism in whom symbiotic microorganisms perform multiple tasks ranging from digestion of food to angiogenesis[4,5]. The disruption in this flora is associated with many disease conditions ranging from diarrhoea to neoplasia.

Basic terminology

The more commonly used term 'microflora' or microbial 'flora' is actually a misnomer as it technically means the plant or vegetable microbial community of a region. Recently, the term 'micobiota' has become prevalent which literally means all the living organisms of a region. Considering the fact that these organisms are an integral part of the human genetic landscape, the term 'microbiome' seems to be best suited as it symbolises all the microorganisms along with their genetic make-up as well as their interactions in a particular environment[3]. Conventionally, the major part of the research relevant to human microbiome has focussed on identification and study of the inhabiting bacteria, however, there also exists a 'virome' *i.e.* the viruses inhabiting the body, that are probably as important[6]. 'Phylotypes' or 'operational taxonomic units' (OTUs) are a group of microbes generally defined by the level of sequence similarity (percentage) between the 16S rRNA genes (*e.g.* ≥ 98% for a 'species'-level phylotype)[7].

The recent advent in the knowledge of human microbiome is undoubtedly attributed to the preceding advances in the field of metagenomics. 'Metagenomics' is the science of directly analysing the genome (the complete set of DNA present in a single cell) contained in an environmental sample[8]. Metagenomics has opened up the avenues for obtaining the genetic information on potential biocatalysts, genomic links between function and phylogeny along with the evolutionary profile of the microbial community[8]. Three closely related fields that are coming up are 'metatranscriptomics' *i.e.* the study of the transcriptomes (reverse-transcribed RNA transcripts) of a group of interacting microbes[9], 'metaproteomics' *i.e.* the study of the entire protein complement of the microbial community[10] and 'metabolomics' *i.e.* the study of small-molecule metabolites of the microorganisms[11].

Studies carried out in 'germ-free animals' *i.e.,* the ones born and reared in sterile environments free of any microbiota have provided useful insight into the complex interactions taking place between the microbiome and its host[12]. Likewise, 'gnotobiotic systems' have been developed to get animals with desired microbiome by transferring or synthetically implanting the desired microbes from another host into the germ-free animals[13]. Researchers have developed gnotobiotic systems with humanized microbiota to evaluate the effects of controlled interventions[14].

Mapping the microbiome

The foundation of present day microbiology was laid down in the nineteenth century after Robert Koch developed the technique of selectively growing and isolating bacteria in culture medium. Until recently we were dependent on the conventional methods of 'cultivating' bacterial 'pure cultures' for isolating the microorganisms in a sample and then depending on growth on a particular type of medium, colony morphology and consumption or production of a particular metabolite to finally identify it. But the observation that only a few of the microbes out of the many visualised microscopically could be isolated by culture prompted scientists to develop the newer 'culture-independent' techniques to recognise these yet unidentified microbes[15]. *Escherichia coli* that was considered as the major component of gut microbiota actually constitutes much less than 1 per cent of gut bacteria, but as it grows easily in culture, it can be detected even at low abundance[16]. It is estimated that almost 20-60 per cent of our microbiome, depending on the region of the body, cannot be cultured by the conventional methods[17].

The prokaryotic cell contains 16S RNA gene in its ribosome that has about 1500 nucleotide; it is highly conserved between different species of bacteria but has several hypervariable regions that can allow identification at genus or species level[13]. Sequence analysis of 16S rRNA gene is thus utilised in many phylogenetic studies to simultaneously amplify the genetic regions of clusters of diverse bacteria by PCR. Initial DNA sequencing techniques were based on tedious fingerprinting methods for the separation of 16S RNA like denaturing gradient gel electrophoresis (DGGE) and restriction fragment length polymorphism (RFLP)[12]. Sanger technique which was developed in 1977 was used for more than two decades for sequencing of the amplified and cloned 16S RNA. It was based on the classical chain termination methods which could be used only on short strands (100-1000 base pairs). Cloning longer DNA strands became possible with the advent of Shot-gun sequencing by which longer sequences could be subdivided into smaller fragments, and subsequently re-assembled to give the overall sequence[18]. With the recent technological explosion, many low-cost, high-throughput sequencing technologies have been developed that parallelize the sequencing process and can produce millions of sequences concurrently. These 'highly-parallelized' or 'next-generation'

sequencing techniques like 454 Pyrosequencing, Sequencing by Synthesis (Illumina) And Sequencing by Ligation (SOLiD) are much more accurate and less time consuming[19]. Whole-Genome Shotgun (WGS) metagenomic sequencing has emerged as an important strategy enabling scientists to analyze the DNA extracted directly from a sample and evaluate not only its composition (taxonomic diversity), but also the metabolic tasks (functional metagenomics) performed by the microbial community[12,13]. The sequenced clones are utilised for the purpose of identification by finding the closest match in the existing gene database bank if available; and the novel sequences are added to the database to facilitate future research.

Virus identification is a relatively tougher task as the viruses lack this 16S RNA gene. Probably that is the reason why the researchers believe that virome research has lagged much behind than that has occurred for their bacterial counterparts[6]. But with the advent of shotgun sequencing that enables deciphering each and every sequence of DNA in the sample, viral metagenomics is being utilised to discover the role of human virome in health and disease[20,21].

The task of mapping the human microbiome has been taken up by scientists around the world through large scale projects like Human Microbiome Project (HMP) and Metagenomics of the Human Intestinal Tract (MetaHIT). The United States National Institutes of Health (NIH) launched the HMP in 2008 with the goal of characterizing healthy human microbiome using the culture-independent methods, to determine whether perturbations in the microbiome affect health/disease status, to provide a standardized data resource and new technological approaches to enable further research, and to evaluate the ethical, legal, and social implications of the same[17]. The European Commission initiated the MetaHIT in 2008 to decipher the intestinal metagenome and analyze their association with human phenotypes[22]. These projects have helped scientists to discover an enormous database comprising thousands of taxonomic profiles that constitute more than trillion bytes of data of metagenomic sequences[22,23].

Composition and biodiversity of healthy human microbiome

The human body is home for taxonomically diverse classes of friendly microbes ranging from eukaryotes, archaea, bacteria and even viruses[23]. The gastrointestinal tract is the largest reservoir of commensals in the body and hence has been studied most extensively[12,14,24-27]. Other sites which have been sampled and analysed include skin, oral cavity, nasal cavity, lower respiratory tract, and vagina. Costello *et al*[26] in their study of multiple body sites were able to detect members of 22 bacterial phyla, of which > 90 per cent was contributed by four predominant phyla, *viz.*, Actinobacteria (36.6%), Firmicutes (34.3%), Proteobacteria (11.9%), and Bacteroidetes (9.5%)[26,28-30]. Though the number of phyla is limited, the biodiversity increases at the level of class, family, genus and becomes enormous at the level of species[24]. Each body habitat is dominated by certain signature microbes like *Propionibacterium* on the skin and *Lactobacillus* in the vagina[17,31,32].

Bacteroidetes and Firmicutes are the predominant phyla inhabiting the gut amounting to more than 95 per cent of the adult gut microbiome[25-27,30]. Family members share more similar gut microbiome as compared to unrelated individuals[7,33]. Each adult individual has a unique microbiome whose composition tends to remain stable over a period of time[33]. Neonates are born with an almost sterile gastrointestinal tract. Environmental exposures during infancy lead to dense colonization of the gut that is highly variable over time but by the end of infancy the microbiome converges to resemble almost like an adult[33]. The amount and variety of microorganisms inhabiting the gut during this time have a significant impact on the development of a person's immune system with life-long consequences[34]. Multiple factors affect the timing and composition of this ecosystem in the neonatal gut. Penders *et al*[35] observed that infants delivered by caesarean section had lower colonization rates and counts of bifidobacteria and *Bacteroides fragilis* but higher prevalence and counts of *Clostridium difficile* and *E. coli.* Breastfed infants at the age of one month were predominantly colonized with bifidobacteria whereas formula-fed infants were colonized with *E. coli*, *C. difficile*, *B. fragilis*, and lactobacilli. Hospitalization and prematurity were associated with higher rates of colonization with *C. difficile*. Use of oral antibiotic decreased the levels of obligate anaerobes like bifidobacteria and *Bacteroides*. Infants having an older sibling demonstrated higher bifidobacterial counts. They concluded that term infants, delivered vaginally at home and exclusively breastfed have the most beneficial gut microbial composition, *i.e*. having large number of bifidobacteria and less of *C. difficile* and *E. coli*[35]. Antibiotics are known to inhibit the healthy microbiota allowing pathogenic microbes like *C. difficile* to proliferate. Antibiotic exposure, direct as

well as indirectly through breast milk in neonates has been shown to result in significant alterations in the gut microbiome that may last for days to months[35,36].

The predominant phyla present on the skin are Actinobacteria, Firmicutes, and Proteobacteria whereas Bacteroidetes which is predominant in the gut is a minor component of the skin[37,38]. In comparison to adults, infant skin shows Firmicutes predominance[39]. Akin to gut microbiome, the cutaneous microbiome also evolves during infancy with staphylococci dominance during the initial period giving way to a more diverse ecosystem by the end of the first year[39]. Skin is an indispensible physical and immune barrier for humans hence its early microbial colonization is an important determinant of body's defence against pathogens. The microbial composition of infants as well as adults is highly variable but site specific depending upon local anatomy, lipid content, *p*H, sweat, and sebum secretion at the site[40]. This could be the underlying reason as to why certain diseases of the skin that have been linked with microbial causation have predilection for specific skin sites like acne, atopic dermatitis, psoriasis and seborrhoeic dermatitis[40].

Majority of the bacteria sampled at the nostrils belong to Firmicutes and Actinobacteria; but Proteobacteria are very few unlike the skin elsewhere[41]. Oropharynx is known to inhabit microbes belonging to Firmicutes, Proteobacteria, and Bacteroidetes[41]. Till date, very few researchers have been able to characterize the healthy lower respiratory tract microbiome as obtaining pure lung derived samples not contaminated by upper airway microbes is a relatively difficult task. Charlson *et al*[42] have observed that the lung microbiome is similar in composition to upper respiratory tract but has a lesser biomass and hypothesised that it probably originates by micro-aspiration from upper airways. Pyrosequencing techniques have established that the female genital microbiome is dominated by lactobacilli that belong to phylum Firmicutes[32].

It is not only the taxonomic composition, but also the taxonomic diversity that has been implicated in causation of several diseases. Two parameters that are routinely employed for this purpose are alpha diversity, *i.e*. how many kinds of taxa or lineages are within a sample and beta diversity, *i.e*. how many kinds of taxa or lineages are shared among samples from same habitat among different subjects[43]. Data from NIH-HMP demonstrated that saliva had the maximum variety of bacteria (highest alpha diversity) but different individuals had similar microorganisms (lowest beta diversity) in their saliva[43]. On the other hand, skin microbiome showed intermediate alpha diversity but highest beta diversity, whereas vaginal samples demonstrated the lowest alpha as well as beta diversity at the genus level. Temporal analysis has revealed that each adult harbours a unique microbiome that stays more or less stable over time as compared to the population as a whole both in terms of microbial composition as well as metabolic functions[43].

Studies evaluating the metagenomics have proved that in spite of variations in the taxonomic profile, the metabolic functions carried out by the microbiome of a particular body site were similar among different individuals. This has prompted scientists to hypothesise about the possible existence of a 'Core Microbiome' that might be sharing a set of genes and/or metabolic capabilities[7]. Tap and colleagues[30] observed that inspite of presence of a large number of species in the gut a limited number of OTUs were shared amongst many individuals and hence these might represent the phylogenetic core of human gut microbiome. Recently, Li and colleagues[44] attempted to characterize the core microbiome of different body habitats using two parameters *viz*. ubiquity and abundance. These have been represented in the Figure.

Microbiome and diseases of childhood

Gut microbiome plays a vital role in several body functions like nutrient processing and assimilation; defence against pathogenic microbes; and even stimulation of angiogenesis[4,5,45,46]. Alteration in this ecosystem has been associated with causation of several diseases of the gut in children, like infantile colic and necrotizing enterocolitis (NEC)[47-51]. Imbalances in intestinal microbiome have been implicated in causation of many non-gastrointestinal disorders as well, like asthma, atopy, obesity, type -1 diabetes, and autism as depicted in the Table[7,27,47-64].

Several skin disorders like atopic dermatitis and psoriasis have been associated with changes in the local cutaneous microbiome[57-59]. Similarly alterations in respiratory microbiome during infancy have been linked with wheezing as well as future development of bronchial asthma[60,61]. Researchers have demonstrated the role of altered microbiome in many oronasal diseases like caries, periodontitis, and chronic rhinosinusitis[62-64].

Virome

Majority of the viruses that inhabit humans are the bacteriophages, *i.e*. viruses that infect bacteria. Hence

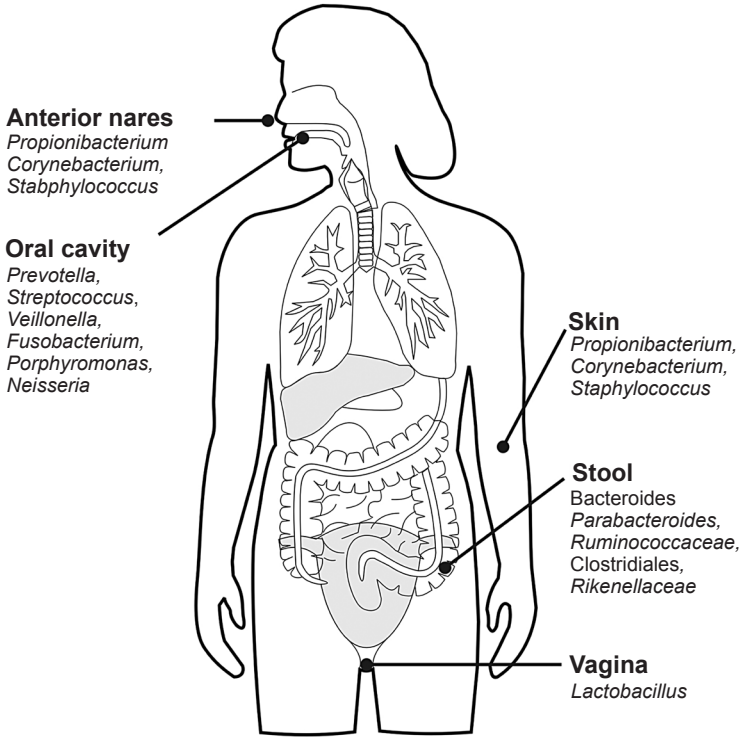

Figure. Representation of the core taxa at different human microbial habitats**.**

the human virome apart from influencing the cellular processes directly could be acting indirectly by altering the symbiotic bacterial functioning, composition, or abundance[6]. Lysholm *et al*[21] analysed the respiratory secretions of children with severe lower respiratory tract infection by metagenomic sequencing and observed that three of the RNA virus families were responsible for more than 90 per cent of these infections. These were *Paramyxoviridae* - human respiratory syncytial virus (hRSV), human metapneumovirus (hMPV) and human parainfluenza virus (hPIV); *Orthomyxoviridae* - influenza virus; and *Picornaviridae* - human rhinovirus (HRV)[21]. It has been observed that viruses that may be non-pathogenic otherwise, interact with various susceptibility genes in predisposed individuals and result in diseases like Crohn's disease, type-I diabetes and bronchial asthma which would not have manifested if either the susceptibility allele or the virus was absent[65,66]. On the other hand, certain pathogenic viruses like herpes viruses tend to adapt to the host body causing lifelong infection, are at times considered a part of the human virome[67]. EBV and other herpes viruses have been implicated in causation of allergic and atopic diseases like asthma and eczema by immunomodulatory mechanisms[67]. Wylie *et al*[68] carried out sequence analysis of the human virome in nasal swabs from febrile and afebrile children and observed that children with unexplained fevers had more viruses than healthy kids hinting towards a viral aetiology. This knowledge could be utilized in developing tests that would help in rapid identification of the virus and thus avoid unnecessary antibiotic usage.

Microbiome research in children in the Indian context

Very few paediatric studies have been carried out in India to evaluate the role of microbiome in health and disease. A study from Pune observed that the intestinal flora of infants born by caesarean section was more diverse as compared to infants delivered vaginally[69]. The most abundant bacterial species present in vaginally delivered infants were *Acinetobacter* spp., *Bifidobacterium* spp. and *Staphylococcus* spp. whereas caesarean delivered infants' faecal microbiota was dominated by *Citrobacter* spp., *E. coli* and *C. difficile* but lacked in *Bifidobacterium* spp. In another study published from Vellore, it was observed that one species of *Bifidobacterium i.e. B. longum* subspecies *infantis* colonized and predominated the neonatal gut[70]. They also observed that asymptomatic rotavirus

Table. Paediatric diseases implicated to result from dysbiosis in human microbiome

Regional microbiome	Disease	Authors	Observations
Gastrointestinal system	Infantile colic	de Weerth *et al*[47]	Microbial flora of infants with colic had significantly lower diversity with predominance of Proteobacteria and significantly reduced number of Bifidobacteria and lactobacilli as compared to controls.
	Necrotizing enterocolitis (NEC)	Fell[48] de la Cochetiere *et al*[49] Mai *et al*[50]	Abnormal gut microbial patterns have been observed in neonates with NEC in all these studies.
		Azcarate-Peril *et al*[51]	Study in preterm piglets has demonstrated an important role of *Clostridium* spp., and members of the Actinobacteria and Cyanobacteria in the pathogenesis of NEC.
	Bronchial asthma	Azad and Kozyrskyij[52]	Studies have linked factors like caesarean delivery, breastfeeding, perinatal stress, probiotics, and antibiotics, which influence intestinal microbial evolution during infancy, with the development of bronchial asthma in childhood suggesting an important role of gut microbiome in perinatal programming of asthma.
	Atopy	Candela *et al*[53]	Gut microbiome of atopic children was found to have a decreased number of immunomodulatory bacteria of *Clostridium* cluster IV, *Faecalibacterium prausnitzii*, and *Akkermansia muciniphila* along with a relative increase in the members of *Enterobacteriaceae*.
	Obesity	Turnbaugh and colleagues[7,27,54]	The relative proportion of Bacteroidetes to Firmicutes was seen to be decreased in obese people in comparison to lean subjects and it has been hypothesized that this obese microbiome has an increased capacity to harvest energy from the diet.
	Diabetes	Giongo *et al*[55]	Studied the role of gut microbiome in development of autoimmunity underlying type-1 diabetes mellitus (TIDM) in genetically predisposed young children and observed that their bacterial biodiversity decreased with time as compared to controls. They also observed the predominance of a single species, *Bacteroides ovatus* that might serve as a tool for early diagnosis of T1DM in future.
	Autism	Louis[56]	Several researchers have linked alterations in gut microbiome with autistic spectrum disorders but there is disparity in the various results with a lack of a particular trend in microbial composition.
Skin	Atopic dermatitis	Kong *et al*[57]	Increase in *Staphylococcus aureus* and *S. epidermidis* is observed during disease flares; whereas following therapy there occurs increase in *Streptococcus*, *Propionibacterium*, and *Corynebacterium* species.
		Dekio *et al*[58]	Observed the presence of several microbial species on skin of atopic subjects that were not previously linked with atopic dermatitis. *Stenotrophomonas maltophilia* was the predominant species in this study.
	Psoriasis	Gao *et al*[59]	A significant increase in Firmicutes along with a reduction in Proteobacteria and Actinobacteria was observed.

Contd...

Regional microbiome	Disease	Authors	Observations
Respiratory System	Wheezing, bronchial asthma	Bisgaard *et al*[60]	This longitudinal study observed that asymptomatic neonates whose upper airways were found to be colonized with *S. pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis* showed higher susceptibility to develop recurrent wheezing and asthma during childhood.
		Hilty *et al*[61]	Molecular analysis of 16S RNA derived from respiratory secretions of asthmatic subjects demonstrated the predominance of members of the phylum Proteobacteria which include pathogens *Haemophilus*, *Moraxella* and *Neisseria* spp. with relatively lesser predominance of *Bacteroidetes* (particularly *Prevotella* spp.) as compared to controls.
Oral cavity	Dental caries	Luo *et al*[62]	Alteration in salivary flora is associated with dental caries in children with a higher biodiversity observed in patients.
	Periodontitis	Hajishengallis *et al*[63]	*Porphyromonas gingivalis* which is a low-abundance oral anaerobic bacterium has been observed to cause periodontitis by disturbing the local microbial homeostasis.
Nasal cavity	Rhinosinusitis	Abreu *et al*[64]	Patients with chronic rhinosinusitis were seen to have a significantly reduced bacterial diversity with a relative reduction in lactobacilli and predominance of a single species *Corynebacterium tuberculostearicum*, in comparison to healthy controls. The pathogenic role of *C. tuberculostearicum* and the protective role of *Lactobacillus sakei* was also confirmed in a murine model.

infection in neonates did not alter the development of the intestinal microbiota in terms of bifidobacterial diversity or colonization.

Future implications

The observed gut microbiome dysbiosis in diseases like gastroenteritis, necrotizing enterocolitis, inflammatory bowel disease, malabsorption, obesity and atopy can open up the avenues for prevention and management of these diseases by several ways. Animal studies have demonstrated that many gut bacteria produce immunomodulatory, anti-inflammatory, and growth promoting molecules[71-73]. These microorganisms also produce antibacterial substances like bacteriocin and lacticin that inhibit the growth of pathogens like *C. difficile*[74,75]. Further characterization of human microbiome might help to utilize these bacterial derivatives as therapeutic agents to control various disease states. The term "pharmabiotic" has thus been used to denote any material that has been obtained from the intestinal microbiome and can be utilized for health promotion, be it a molecular by-product or a microorganism itself.

Microbiological modification of gut microbiome in a desired manner can be attempted by means of administering various prebiotics and probiotics, separately or in combination as synbiotics. These "functional food ingredients" are being utilized to enrich the gut microbiome with bacteria like *Bifidobacterium* and *Lactobacillus* that are believed to be health promoting[76]. The role of prebiotics and probiotics has been well established in acute gastroenteritis as well as antibiotic associated diarrhoea. In a meta-analysis by Deshpande *et al*[77], it was observed that probiotic supplementation in preterm neonates not only reduced the risk of necrotizing enterocolitis but also the risk of mortality. Similarly in a meta-analysis by Brenner *et al*[78], probiotic usage in patients of irritable bowel syndrome has been found to be efficacious.

Faecal microbiota transplantation (FMT) has been tried by occasional researchers in the last century also as a therapeutic intervention for antibiotic associated diarrhoea caused by *C. difficile*. A systematic review found out its efficacy to the tune of 92 per cent but further research is required to standardize the procedure; evaluate its safety and suitability before it is approved as a treatment modality by the regulatory agencies[79,80].

de Weerth *et al*[47] in their study on infants with colic, observed the consistent presence of a few

Proteobacteria linked to *Escherichia*, *Klebsiella*, *Serratia*, *Vibrio*, *Yersinia*, and *Pseudomonas* with the colic phenotype. Saulnier *et al*[81] have been able to link specific "signature" microbes with several diseases like irritable bowel syndrome. These disease specific signature phylotypes might serve to devise diagnostic as well as therapeutic strategies in the future.

Recent advances in pharmacogenomics that focus on the role of genetics in an individual's response to a drug have prompted researchers to explore other environmental influences that affect drug metabolism. Gut microbiome is one such important determinant. Personalized drug therapy to improve efficacy and reduce adverse effects might become feasible with an approach utilizing pre-dose metabolite profiling to predict an individual's response to a drug. This novel approach has been termed as pharmaco-metabonomic approach[82].

The ultimate objective of the microbiome research is to utilize this knowledge to predict the risk of disease development, develop newer techniques to diagnose related diseases and evolve therapeutic approaches for manipulation of the human microbiome for betterment of mankind.

References

1. Trivedi B. Microbiome: The surface brigade. *Nature* 2012; *492* : S60-1.
2. Fritz JV, Desai MS, Shah P, Schneider JG, Wilmes P. From meta-omics to causality: experimental models for human microbiome research. *Microbiome* 2013; *1* : 14.
3. Lederberg J, McCray AT. 'Ome sweet 'omics - a genealogical treasury of words. *Scientist* 2001; *15* : 8.
4. Flint HJ, Scott KP, Louis P, Duncan SH. The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol* 2012; *9* : 577-89.
5. Stappenbeck TS, Hooper LV, Gordon JI. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. *Proc Natl Acad Sci USA* 2002; *99* : 15451-5.
6. Williams SC. The other microbiome. *Proc Natl Acad Sci USA* 2013; *110* : 2682-4.
7. Turnbaugh PJ, Gordon JI. The core gut microbiome, energy balance and obesity. *J Physiol* 2009; *587* : 4153-8.
8. Thomas T, Gilbert J, Meyer F. Metagenomics - a guide from sampling to data analysis. *Microb Inform Exp* 2012; *2* : 3.
9. Li X, LeBlanc J, Truong A, Vuthoori R, Chen SS, Lustgarten JL, *et al*. A metaproteomic approach to study human-microbial ecosystems at the mucosal luminal interface. *PLoS One* 2011; *6* : e26542.
10. Wilmes P, Bond PL. Metaproteomics: studying functional gene expression in microbial ecosystems. *Trends Microbiol* 2006; *14* : 92-7.
11. Turnbaugh PJ, Gordon JI. An invitation to the marriage of metagenomics and metabolomics. *Cell* 2006; *134* : 708-13.
12. Grice EA, Segre JA. The human microbiome: Our second genome. *Annu Rev Genom Hum Genet* 2012; *13* : 151-70.
13. Morgan XC, Huttenhower C. Human Microbiome Analysis. *PLoS Comput Biol* 2012; *8* : e1002808.
14. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* 2009; *1* : 6ra14.
15. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol Rev* 1995; *59* : 143-69.
16. Hamady M, Knight R. Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. *Genome Res* 2009; *19* : 1141-52.
17. Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, *et al*. The NIH human microbiome project. *Genome Res* 2009; *19* : 2317-23.
18. Shotgun sequencing. 2013. Available from: *http://en.wikipedia.org/wiki/Shotgun_sequencing*, accessed on October 4, 2013.
19. Liu L, Li Y, Li S, Hu N, He Y, Pong R, *et al*. Comparison of next-generation sequencing systems. *J Biomed Biotechnol* 2012; *2012* : 251364.
20. Victoria JG, Kapoor A, Li L, Blinkova O, Slikas B, Wang C, *et al*. Metagenomic analyses of viruses in stool samples from children with acute flaccid paralysis. *J Virol* 2009; *83* : 4642-51.
21. Lysholm F, Wetterbom A, Lindau C, Darban H, Bjerkner A, Fahlander K, *et al*. Characterization of the viral microbiome in patients with severe lower respiratory tract infections, using metagenomic sequencing. *PLoS One* 2012; *7* : e30875.
22. Dusko Ehrlich S, MetaHIT consortium. Metagenomics of the intestinal microbiota: potential applications. *Gastroenterol Clin Biol* 2010; *34* : S23-8.
23. Methé BA, Nelson KE, Pop M, Creasy HH, Giglio MG, Huttenhower C, *et al*. A framework for human microbiome research. *Nature* 2012; *486* : 215-21.
24. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 2006; *124* : 837-48.
25. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichahn C, *et al*. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; *464* : 59-65.
26. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science* 2009; *326* : 1694-97.
27. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, *et al*. A core gut microbiome in obese and lean twins. *Nature* 2009; *457* : 480-4.
28. Johnson CL, Versalovic J. The human microbiome and its potential importance to pediatrics. *Pediatr* 2012; *129* : 950-60.
29. Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat Rev Microbiol* 2011; *9* : 279-90.

30. Tap J, Mondot S, Levenez F, Pelletier E, Caron C, Furet J, *et al*. Towards the human intestinal microbiota phylogenetic core. *Environ Microbiol* 2009; *11* : 2574-84.
31. Grice EA, Kong HH, Renaud G, Young AC, NISC Comparative Sequencing Program, Bouffard GG, et al. A diversity profile of the human skin microbiota. *Genome Res* 2008; *18* : 1043-50.
32. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, *et al*. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA* 2011; *108* : 4680-7.
33. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol* 2007; *5* : e177.
34. Vael C, Desager K. The importance of the development of the intestinal microbiota in infancy. *Curr Opin Pediatr* 2009; *21* : 794-800.
35. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, *et al*. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 2006; *118* : 511-21.
36. Torrazza RM, Neu J. The developing intestinal microbiome and its relationship to health and disease in the neonate. *J Perinatol* 2011; *31* : S29-34.
37. Dekio I, Hayashi H, Sakamoto M, Kitahara M, Nishikawa T, Suematsu M, *et al*. Detection of potentially novel bacterial components of the human skin microbiota using culture-independent molecular profiling. *J Med Microbiol* 2005; *54* : 1231-8.
38. Gao Z, Tseng C, Pei Z, Blaser MJ. Molecular analysis of human forearm superficial skin bacterial biota. *Proc Natl Acad Sci USA* 2007; *104* : 2927-32.
39. Capone KA, Dowd SE, Stamatas GN, Nikolovski J. Diversity of the human skin microbiome early in life. *J Invest Dermatol* 2011; *131* : 2026-32.
40. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, *et al*. Topographical and temporal diversity of the human skin microbiome. *Science* 2009; *324* : 1190-2.
41. Lemon KP, Klepac-Ceraj V, Schiffer HK, Brodie EL, Lynch SV, Kolter R. Comparative analyses of the bacterial microbiota of the human nostril and oropharynx. *MBio* 2010; *1* : e00129-10.
42. Charlson ES, Bittinger K, Haas AR, Fitzgerald AS, Frank I, Yadav A, *et al*. Topographical continuity of bacterial populations in the healthy human respiratory tract. *Am J Respir Crit Care Med* 2011; *184* : 957-63.
43. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* 2013; *486* : 207-14.
44. Li K, Bihan M, Methe´ BA. Analyses of the stability and core taxonomic memberships of the human microbiome. *PLoS One* 2013; *8* : e63139.
45. Hooper LV, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu Rev Nutr* 2002; *22* : 283-307.
46. MacDonald TT, Gordon JN. Bacterial regulation of intestinal immune responses. *Gastroenterol Clin North Am* 2005; *34* : 401-12.
47. de Weerth C, Fuentes S, Puylaert P, de Vos WM. Intestinal microbiota of infants with colic: development and specific signatures. *Pediatrics* 2013; *131* : e550-8.
48. Fell JM. Neonatal inflammatory intestinal diseases: Necrotising enterocolitis and allergic colitis. *Early Hum Dev* 2005; *81* : 117-22.
49. de la Cochetiere MF, Piloquet H, des Robert C, Darmaun D, Galmiche JP, Roze JC. Early intestinal bacterial colonization and necrotizing enterocolitis in premature infants: the putative role of Clostridium. *Pediatr Res* 2004; *56* : 366-70.
50. Mai V, Young CM, Ukhanova M, Wang X, Sun Y, Casella G, *et al*. Fecal microbiota in premature infants prior to necrotizing enterocolitis. *PLoS One* 2011; *6* : e20647.
51. Azcarate-Peril MA, Foster DM, Cadenas MB, Stone MR, Jacobi SK, Stauffer SH, *et al*. Acute necrotizing enterocolitis of preterm piglets is characterized by dysbiosis of ileal mucosa-associated bacteria. *Gut Microbes* 2011; *2* : 234-43.
52. Azad MB, Kozyrskyj AL. Perinatal programming of asthma: the role of gut microbiota. *Clin Dev Immunol* 2012; *2012* : 932072.
53. Candela M, Rampelli S, Turroni S, Severgnini M, Consolandi C, De Bellis G, *et al*. Unbalance of intestinal microbiota in atopic children. *BMC Microbiol* 2012; *12* : 95.
54. 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.
55. Giongo A, Gano KA, Crabb DB, Mukherjee N, Novelo LL, Casella G, *et al*. Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J* 2011; *5* : 82-91.
56. Louis P. Does the human gut microbiota contribute to the etiology of autism spectrum disorders? *Dig Dis Sci* 2012; *57* : 1987-9.
57. Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, *et al*. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* 2012; *22* : 850-9.
58. Dekio I, Sakamoto M, Hayashi H, Amagai M, Suematsu M, Benno Y. Characterization of skin microbiota in patients with atopic dermatitis and in normal subjects using 16S rRNA gene-based comprehensive analysis. *J Med Microbiol* 2007; *56* : 1675-83.
59. Gao Z, Tseng CH, Strober BE, Pei Z, Blaser MJ. Substantial alterations of the cutaneous bacterial biota in psoriatic lesions. *PLoS One* 2008; *3* : e2719.
60. Bisgaard H, Hermansen MN, Buchvald F, Loland L, Halkjaer LB, Bønnelykke K, *et al*. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med* 2007; *357* : 1487-95.
61. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, *et al*. Disordered microbial communities in asthmatic airways. *PLoS One* 2010; *5* : e8578.
62. Luo AH, Yang DQ, Xin BC, Paster BJ, Qin J. Microbial profiles in saliva from children with and without caries in mixed dentition. *Oral Dis* 2012; *18* : 595-601.
63. Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA, *et al*. Low-abundance biofilm species orchestrates

inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe* 2011; *10* : 497-506.

64. Abreu NA, Nagalingam NA, Song Y, Roediger FC, Pletcher SD, Goldberg AN, *et al*. Sinus microbiome diversity depletion and *Corynebacterium tuberculostearicum* enrichment mediates rhinosinusitis. *Sci Transl Med* 2012; *4* : 151ra124.
65. Cadwell K, Patel KK, Maloney NS, Liu TC, Ng AC, Storer CE, *et al*. Virus-plus-susceptibility gene interaction determines Crohn's disease gene *Atg16L1* phenotypes in intestine. *Cell* 2010; *141* : 1135-45.
66. Foxman EF, Iwasaki A. Genome-virome interactions: examining the role of common viral infections in complex disease. *Nat Rev Microbiol* 2011; *9* : 254-64.
67. Dreyfus DH. Herpesviruses and the microbiome. *J Allergy Clin Immunol* 2013; *132* : 1278-86.
68. Wylie KM, Mihindukulasuriya KA, Sodergren E, Weinstock GM, Storch GA. Sequence analysis of the human virome in febrile and afebrile children. *PLoS One* 2012; *7* : e27735.
69. Pandey PK, Verma P, Kumar H, Bavdekar A, Patole MS, Shouche YS. Comparative analysis of fecal microflora of healthy full-term Indian infants born with different methods of delivery (vaginal vs caesarean): *Acinetobacter* sp. prevalence in vaginally born infants. *J Biosci* 2012; *37* : 989-98.
70. Balamurugan R, Magne F, Balakrishnan D, Suau A, Ramani S, Kang G, *et al*. Faecal bifidobacteria in Indian neonates & the effect of asymptomatic rotavirus infection during the first month of life. *Indian J Med Res* 2010; *132* : 721-7.
71. Rachmilewitz D, Katakura K, Karmeli F, Hayashi T, Reinus C, Rudensky B, *et al*. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology* 2004; *126* : 520-8.
72. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 2008; *453* : 620-5.
73. Yan F, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology* 2007; *132* : 562-75.
74. Rea MC, Sit CS, Clayton E, O'Connor PM, Whittal RM, Zheng J, *et al*. Thuricin CD, a post-translationally modified bacteriocin with a narrow spectrum of activity against *Clostridium difficile*. *Proc Natl Acad Sci USA* 2010; *107* : 9352-7.
75. Rea MC, Clayton E, O'Connor PM, Shanahan F, Kiely B, Ross RP, *et al*. Antimicrobial activity of lacticin 3,147 against clinical *Clostridium difficile* strains. *J Med Microbiol* 2007; *56* : 940-6.
76. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J Nutr* 1995; *125* : 1401-12.
77. Deshpande G, Rao S, Patole S. Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: A systematic review of randomised controlled trials. *Lancet* 2007; *369* : 1614-20.
78. Brenner DM, Moeller MJ, Chey WD, Schoenfeld PS. The utility of probiotics in the treatment of irritable bowel syndrome: A systematic review. *Am J Gastroenterol* 2009; *104* : 1033-49.
79. Gough E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent *Clostridium difficile* infection. *Clin Infect Dis* 2011; *53* : 994-1002.
80. Mole B. FDA gets to grips with faeces. *Nature* 2013; *498* : 147-8.
81. Saulnier DM, Riehle K, Mistretta TA, Diaz MA, Mandal D, Raza S, *et al*. Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. *Gastroenterology* 2011; *141* : 1782-91.
82. Clayton TA, Lindon JC, Cloarec O, Antti H, Charuel C, Hanton G, *et al*. Pharmaco-metabonomic phenotyping and personalized drug treatment. *Nature* 2006; *440* : 1073-7.

Reprint requests: Dr Shilpa Khanna Arora, Department of Pediatrics, Postgraduate Institute of Medical Education & Research & Dr Ram Manohar Lohia Hospital, New Delhi 110 001, India
e-mail: drshilpakhanna@yahoo.co.in