Probiotics or antibiotics: future challenges in medicine

Yousef Nami,1,† Babak Haghshenas,1 Norhafizah Abdullah,2 Abolfazl Barzegari,3 Dayang Radiah,2 Rozita Rosli1 and Ahmad Yari Khosroushahi4,5†

1Institute of Biosciences, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
2Chemical and Environmental Engineering Department, Faculty of Engineering, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
3Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
4Drug Applied Research Center, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
5Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

Genetic and environmental factors can affect the intestinal microbiome and microbial metabolome. Among these environmental factors, the consumption of antibiotics can significantly change the intestinal microbiome of individuals and consequently affect the corresponding metagenome. The term ‘probiotics’ is related to preventive medicine rather than therapeutic procedures and is, thus, considered the opposite of antibiotics. This review discusses the challenges between these opposing treatments in terms of the following points: (i) antibiotic resistance, the relationship between antibiotic consumption and microbiome diversity reduction, antibiotic effect on the metagenome, and disease associated with antibiotics; and (ii) probiotics as living drugs, probiotic effect on epigenetic alterations, and gut microbiome relevance to hygiene indulgence. The intestinal microbiome is more specific for individuals and may be affected by environmental alterations and the occurrence of diseases.

Introduction

Antibiotics, as substances that either prevent the growth of or kill a living organism, are considered miracle drugs. The history of antibiotics started in 1932, when the first sulfonamide was prepared by Selman Waksman. In the 1940s to the 1960s, the term ‘antibiotic’ was distinguished from the term ‘chemotherapeutic drug’ in that the former are natural drugs made by using fungi or bacteria (Castanon, 2007; Cheng et al., 2003; Prantera & Scribano, 2009). Antibiotics can enhance human lives by treating or preventing diseases. However, a major public-health threat is the resistance to antibiotics or the increased capability of bacteria to stay alive in the presence of antibiotics. Moreover, antibiotics only treat bacterial infections and cannot be effectively used to treat virus-related infections, such as colds (Cars et al., 2008; Costelloe et al., 2010). Antibiotics have the greatest effect on human health among all medical developments achieved since the beginning of the 20th century. Antibiotics are selective and specific in their target; thus, these drugs can eradicate invading bacteria without inducing toxicity to the infected host (Guarner et al., 2006). Antibiotics are frequently prescribed in most countries (Quigley, 2011). Antibiotics are extensively used in farming, veterinary medicine and medicine in Australia. Almost 400 000 kg of the total amount went into stock feed to stop diseases in intensively raised animals or for use as a growth promoter. In other words, over 20 g of antibiotics are used for agricultural purposes per year for every man, woman and child in Australia. However, other countries consume a significantly higher amount. For example, the mean for the USA is over 27 g per person each year (Ellison, 2006; Friedman, 2012).

Meanwhile, probiotics are considered as living drugs that can reduce antibiotic consumption and increase human health development. The application of probiotics in preventing and managing gastrointestinal (GI) disorders has gained considerable interest over the past two decades. The consumption of these products is increasing worldwide because probiotics are generally regarded as safe. Anti-carcinogenic, anti-diabetic, anti-allergic and anti-inflammatory
genetically modified probiotics, as well as oral vaccine development, are counted as new trends of probiotic usage (Bouton et al., 2002). This review discusses the major future challenges for antibiotics and probiotics, and the advantages and disadvantages of their use during medical consumption.

Challenges faced by antibiotics (present and future)

Antibiotic resistance

Antimicrobial resistance, which has been reported against almost every antibiotic discovered, is one of the most urgent public-health concerns that weaken in a global way the usefulness of the antibiotic treatment of infectious illness. The world of science, since penicillin led to the antibiotic age in the mid-20th century, has been involved in a conflict between bacterial resistance and the expansion of antibacterial agents. Critical concern, throughout the first decade of the 21st century, has been devoted to the development of resistant enterococci, multi-drug resistant staphylococci, and resistant mycobacteria, placing serious public-health and clinical challenges for humans (Preidis & Versalovic, 2009).

Our ability to treat contagious disease has been compromised by the growing levels of multi-drug resistance in human pathogenic bacteria. The unchecked spread of multiple-antibiotic resistance in clinically relevant pathogenic microbes has seemingly started to dull the hue of the golden era of antibiotics considerably. Most clinically used antibiotics are structural products of compounds isolated from natural sources. The common consumption of antibiotics in human medicine and the broad-spectrum activities of antibiotics has probably caused the human microbiome to undertake considerable responsive changes to this therapy. This phenomenon encourages the investigation of the amount to which the human-associated microbiome in the antibiotic era has developed to become a basin of antibiotic-resistance genes (Carlet, 2012; Kazimierczak & Scott, 2007).

Resistance is closely related to antibiotic consumption in human, agricultural and veterinary practice. The use of antibiotics for growth promotion and protection of crops causes resistance elements to appear in saprophytic bacteria. Resistance may be conveyed to human bacteria that are sometimes pathogenic when these crops are eaten. The possibility of finding resistant organisms in the gut is linked to the tonnage of antibiotic consumption in the country where the person lives. This possibility is also related to the ease with which a resistance mechanism can increase. Resistant organisms transferred from one person to another remain resistant. Novel colonizing and infecting flora tend to return to the sensitive phenotype when antibiotic pressure is removed (Porsby et al., 2011; Prescott, 2008).

Resistance might be acquired by the means of genetic elements resulting in three fundamental outcomes: (i) inactivation of enzyme production, (ii) a change of the target site, and (iii) antibiotic exclusion from the natural target site (e.g. vancomycin against Gram-negative organisms) (Scherer et al., 2013). However, the concern remains significant because of the existence of many multiple-resistant organisms, which are expensive to treat or cannot be treated at all. Vancomycin-resistant enterococcal infections emerged as a new and potentially untreatable scourge during the 1990s (Climo et al., 2009; Sievert et al., 2008; Smith et al., 2009).

The appearance of vancomycin resistance among strains of Staphylococcus aureus has been a main academic issue. However, fully resistant clinical isolates have been identified. Although eight confirmed infections with S. aureus with relatively reduced susceptibility to vancomycin have been identified in the United States since 1997, the first fully vancomycin-resistant S. aureus was not discovered until June 2002. S. aureus is one of the hazardous bacterial pathogens for which vancomycin has been the drug of choice for several years; thus, the prospect of vancomycin-resistant S. aureus development has become a great concern (Lodise et al., 2008). Susceptibility reports on Escherichia coli bacteraemia, the most common bacteraemia from 1990 to 1999, and ampicillin resistance have remained at a high level at approximately 55% of cases (Hammad & Shimamoto, 2010). Gentamicin resistance increased from 1.7% in 1990 to 3% in 2000, and ciprofloxacin resistance has been increasing from extremely low levels of less than 1% to 5% in 2000. S. aureus is the second most commonly reported bacteraemia (Ogunshe, 2008; Oudhuis et al., 2011). Resistance to meticillin, which indicates fluoro- acillin resistance, has been significantly growing since the early 1990s (Dancer, 2008).

Strains of Salmonella that are resistant to antimicrobial agents have become a worldwide health problem. A distinct strain of Salmonella enterica serotype Typhimurium, known as definitive type 104 (DT104), is resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline, and has become a major cause of illness in humans and animals in Europe, especially in the UK (Glynn et al., 1998). Orman et al. (2002) revealed that the progressive acquisition and accumulation of plasmid-mediated resistance determinants occurred from 1984 to 1998 in nontyphoid Salmonella isolates of the most prevalent serovars from Argentina. It is suggested that antimicrobial-resistance mechanisms in these bacteria may have been the consequence of plasmid exchange between Salmonella enterica serovar Typhimurium and E. coli or Shigella flexneri and/or spreading of mobile elements from the nosocomial environment. Fey et al. (2000) provided additional evidence that antibiotic-resistant strains of Salmonella in the United States evolve primarily in livestock. Resistance to ceftriaxone, the drug of choice for invasive Salmonella disease, is a public-health concern, especially with respect to children, since fluoroquinolones, which can also be used to treat this disease, are not approved for use in children.
Antibiotics and the reduction of microbiome diversity

Despite the wide consumption of antibiotics, few studies have measured the effects of such drugs on the quantity or variety of bacteria in the mammalian intestine. A microbiome is defined as an entirety of microbes that contains genetic elements, as well as environmental interactions in a specific environment. Micro-organisms that live within or on the human body are called microbiota, and their genomes that act together as a living system are known as the human microbiome. The human intestine is inhabited by 100 trillion micro-organisms that represent each of the three domains of life (Nayak & Mukherjee, 2011). Several intestinal bacteria are regarded as mutualistic; they help maintain and improve regular mammalian physiology, appropriate digestion, epithelial cell function, metabolism, enteric nerve function, angiogenesis and the immune system. Patients with inflammatory bowel disease or allergies have altered intestinal bacteria, although bacterial communities in the intestine promote normal immune homeostasis, which indicates that microbial populations may affect disease pathogenesis (Gueimonde & Collado, 2012; Peterson et al., 2008; Tuohy et al., 2009; Ventura et al., 2009). The infant gut microbiota undergoes dramatic alterations during the first 2 years of life. Antibiotic treatment is one of the most significant factors that influence the development and acquisition of this population. Fouhy et al. (2012) evaluated the short-term recovery of the infant gut microbiota following antibiotic treatment. They compared the gut microbiota of nine infants who underwent parenteral antibiotic treatment with ampicillin and gentamicin (within 48 h of birth) by using high-throughput sequencing (employing both 16S rRNA- and rpoB-specific primers) and quantitative PCR (Fouhy et al., 2012).

They revealed that the gut microbiota of the antibiotic-treated infants had significantly lower proportions of Actinobacteria (P = 0.00001) and the associated genus Bifidobacterium (P = 0.0132), as well as the genus Lactobacillus (P = 0.0182), and significantly higher proportions of Proteobacteria (P = 0.0049), than the untreated controls 4 weeks after the cessation of treatment. By week 8, the Proteobacteria levels remained significantly higher in the treated infants (P = 0.0049), but the Actinobacteria, Bifidobacterium and Lactobacillus levels had recovered and were similar to those in the control samples. Despite this recovery of total Bifidobacterium numbers, rpoB-targeted pyrosequencing revealed that the number of different Bifidobacterium species present in the antibiotic-treated infants was reduced. It is, thus, obvious that the mutual use of ampicillin and gentamicin in early life can significantly affect the evolution of the infant gut microbiota.

The mammalian GI microbiota consists of various collections of bacteria in a native environment. For the host health maintenance, these various GI microbes are considered important, since they have substantial metabolic and defensive performances, for instance, assisting the host in the immune system, epithelium development and nutrient extraction; and against pathogens, they are also considered as a natural defence (Jia et al., 2008).

Most antibiotics are either administered orally or injected, so that they circulate through the host system and affect the whole microbiota. Next-generation sequencing technologies enabled the determination of the effects of antibiotics on mammalian microbiota with the use of conserved phylogenetic markers (e.g. the 16S rRNA gene sequence) for making taxonomic assignments. For example, 16S rRNA gene sequence analysis of the entire community illustrated that most bacterial taxa in the gut were influenced by the administration of ciprofloxacin to humans, leading to diversity, evenness and decreased richness (Dethlefsen et al., 2008; Looft & Allen, 2012).

It is clear that agricultural and medical antibiotic consumption is beneficial. Nevertheless, the collateral consequences are complex and numerous. It is noted that the prediction of negative side effects, for instance, the promotion of antibiotic resistance, is of importance, but other variations in the host and its microbiota are difficult to predict. The range of unpredicted effects of antibiotic therapy possibly goes beyond the simple growth inhibition of susceptible strains. Thus, these bioactive organic compounds should be considered as multi-activity signalling molecules. After birth, nearly every surface of the human body is occupied by a various and rich commensal microbe community. This microbiota has perpetual and significant impact on physiological development and human health, involving prevention of pathogen invasion, dietary and nutritional processing, and the maturation of the immune system. Consequently, both long-term and short-term influences on the commensal microbiota and its encoded microbiome are expected to be produced by antibiotic treatment to avoid or remove a pathogenic infection at nearly any body site (Collado et al., 2012).

Pervasive deviations in the microbial community structure and persistent increases in antibiotic resistance among sampled cultivatable species are caused by antibiotic treatment, which may be attributed to the rapid increase of antibiotic-resistant strains in low basal levels in the microbiota. This increase is also caused by the horizontal transfer of antibiotic-resistance genes. The application of meta-genomic functional selection, high-throughput sequencing on microbiota exposed to antibiotics and novel cultivation methods ought to facilitate a complete comprehension of the processes that result in the association between the resistant microbiome and antibiotics, and its effect on clinical problems related to antibiotic resistance (Engelbrekson et al., 2006; Mikkelsaa, 2011). The main causative agent of peptic ulcer disease and gastric cancer is the bacterium Helicobacter pylori and antibiotic treatment is the typical treatment for this bacterium. Jakobsson et al. (2010) studied short- and long-term effects of metronidazole and clarithromycin treatment, the therapy regimen usually used against H. pylori, on the indigenous microbiota in the throat and in the lower intestine. They found that the
microbial communities of untreated control subjects were relatively stable over time, while dramatic shifts were observed 1 week after antibiotic treatment, with reduced bacterial diversity in all treated subjects in both locations. Additionally, they revealed that high levels of the macrolide resistance gene ermA were found after 4 years treatment. This study highlights the importance of a restrictive antibiotic usage in order to prevent subsequent treatment failure and the potential spread of antibiotic resistance (Jakobsson et al., 2010). In addition, antibiotic usage strongly affects microbial intestinal metabolism and thereby impacts human health. Perez-Cobas et al. (2013) demonstrated that antibiotics targeting specific pathogenic infections and diseases may change gut microbial ecology and interactions with host metabolism at a much higher level than previously assumed. Microbial ecology research studies have provided convincing data for the crosstalk between members of the autochthonous microbiota and the host immune system. The tight interrelationship between microbiota and host mucosal cells is mediated by microbial metabolites that are in charge of bacterial cell-to-cell communication by quorum-sensing mechanisms, and also through the activation of eukaryotic cells following secretion of host defensins and modulation of cytokine expression profiles. All these host functions can be positively influenced by probiotic bacteria of human origin. However, few requirements for evaluating these strains for use in humans have been set according to their composition and metabolic activity (Mikelsaar et al., 2011).

**Antibiotic effects on the human metagenome**

The study of the metagenome, the genetic material recovered from environmental samples, is called metagenomics. This broad field may also be considered as environmental genomics, community genomics or ecogenomics. The initial environmental gene sequencing involved cloning specific genes (often the 16S rRNA gene) to generate a variety of profiles in a natural sample, whereas traditional microbiology and microbial genome sequencing and genomics depend on cultivated clonal cultures. Such work showed that most of microbial biodiversity has been missed by cultivation-based methods (Belda-Ferre et al., 2012; Turpin et al., 2011).

Recently conducted studies utilize ‘shotgun’ Sanger sequencing or massively parallel pyro-sequencing to obtain balanced samples of all genes from all sampled community members. Its capability to disclose the previously concealed variety of microscopic life makes metagenomics a powerful lens for seeing the microbial world that has the potential to revolutionize our understanding of the whole living world (Garcia-Mazcorro et al., 2011). Although the composition and mechanisms of microbial communities remain mysterious, such communities still serve a key function in preserving human health.

Most of the bacterial types forming part of the biosphere have not been cultivated at all. Consequently, comprehensive research on bacterial communities involves the utilization of non-culture-based methods, which are known as metagenomics. Nonetheless, the issue of which antibiotics are regarded as pollutants, which might have significant impact on the structure of natural bacterial populations, has not been taken into account. Hence, the use of non-culture-based techniques (metagenomics) is essential for describing the alterations in bacterial populations confronted with antibiotic contamination in a more precise manner (Gueimonde & Collado, 2012; Tuohy et al., 2009; Ventura et al., 2009).

Metagenomics, since it is a more complete technique compared to culture-based methods, is presently utilized for describing the microbial population’s structure. The describing of the microbiota, through two complementary approaches, could be addressed as: the analysis of microbial species distribution or the analysis of genes distribution (Ventura et al., 2009).

**Probiotics as living drugs (alternative medicine)**

The concept of probiotics can be traced back to 1908 when Eli Metchnikoff, a Nobel Prize winner, proposed that ‘ingested lactobacilli can displace toxin producing bacteria, promoting health, prolonging life’. Lilly and Stillwell introduced the term ‘probiotics’ in 1965. Probiotics are considered as microbially derived factors that stimulate the growth of other organisms. By emphasizing the requirement of viability for probiotics, Roy Fuller (Fuller, 1989) highlighted the useful effect of probiotics on the host. As non-digestible substances, probiotics have an important physiological effect on the host by provoking the growth or activity of the limited indigenous bacteria. Species of Lactobacillus and Bifidobacterium are the most frequently utilized probiotics. Probiotics have different types, the health benefits of which are determined by their function in the gut. Several major probiotics have been identified through their genus, species and strain level, including Lactobacillus, Bifidobacteria, Saccharomyces boulardii, Streptococcus thermophilus, Enterococcus faecium and Leuconostoc. The lactic acid bacteria consisting of Lactobacillus species that have been utilized for food preservation by fermentation for thousands of years can serve a twofold function by acting as agents for food fermentation and can possibly impart health benefits (Ogue-Bon et al., 2011; Siew-Wai et al., 2010).

Various infections, particularly infections of mucosal surfaces such as the gut and vagina, have been treated using biological agents (biotherapeutic agents or probiotics). The value of traditional treatments diminished after the discovery and progress of antibiotics. However, alternatives to antibiotics are required to fight the increasing number of infections emerging because of the extreme consumption of antibiotics (Butler et al., 2012).

Probiotics aid the naturally occurring gut microbiota. Diarrhoea caused by antibiotics has been prevented by using some probiotic preparations. Researchers have documented...
probiotic effects on various GI and extra-intestinal disorders, such as irritable bowel syndrome (Hoveyda et al., 2009), vaginal infections (Reid et al., 2009), inflammatory bowel disease (Damaskos & Kolios, 2008) and immune enhancement (Gill & Prasad, 2008).

Dairy products and probiotic-fortified foods are the most common forms of probiotics. Capsules, tablets and sachets, including bacteria in freeze-dried form, are also available. Depending on the strain and product, the dose of probiotics needed differs. A fixed dose that is required for probiotics is impossible to consider. In fact, the dosage should be in accordance to the human studies showing the health benefit of the microbial ecosystems and mucosal immunity. Through motivating both the non-immune mechanisms and the mucosal immune mechanisms by antagonism/competition with potential pathogens, the probiotics influence the intestinal ecosystem. These phenomena mediate the most effective probiotic impacts, for instance, decreasing the severity and incidence of diarrhea.

By destroying the activity of particular bacterial enzymes that may raise the levels of pro-carcinogens, probiotics lessen the risk of colon cancer in animals. Different probiotic strains, such as *Lactobacillus reuteri* ATCC 55730 (Cimperman et al., 2011), *Lactobacillus rhamnosus* GG (Teran et al., 2009), *Lactobacillus casei* DN-114 001 (Merenstein et al., 2010) and *Saccharomyces cerevisiae* (Snydman, 2008), have been discovered to adhere to mucosal cells; it may intervene in mucin secretion and receptor competition. *Saccharomyces boulardii* and lactobacilli have the potential to be utilized in this situation. Bacteria and yeast, including the bacteria *Lactobacillus* spp. (Ruszczynski et al., 2008) (*L. rhamnosus* GG), an *Enterococcus* (Al-Nassir et al., 2008) and the non-pathogenic yeast *Saccharomyces boulardii*, have been investigated in AAD prevention (Barc et al., 2008). A meta-analysis of probiotics in AAD prevention evaluated nine controlled trials that showed that both lactobacilli and *Saccharomyces boulardii* can stop AAD. However, the mechanisms of action of probiotics are not evidently realized. The probable mechanisms involve competition for nutrients, stimulation of immunity, production of antimicrobial materials, and the inhibition of mucosal and epithelial adherence of pathogens. More mechanisms might involve immunomodulation of gut-associated lymphoid tissue, effects on mucus secretion and receptor competition. *Lactobacillus* GG has been discovered to adhere to mucosal cells; it may strive for nutrients and yield a substance that avoids bacteria and alters faecal glucuronidase, which might be an intestinal bacterial metabolism marker. The efficiency of probiotics in stopping AAD in children has been proven by the results of six placebo-controlled randomized controlled trials involving 766 children. Compared with the placebo, treatment with probiotics reduced the danger of AAD from 28.5 to 11.9 %. So, probiotics decrease the danger of AAD in children. The consumption of probiotics, which are live microbial food ingredients and valuable to health, is a preventive measure. The postulation that in several cases diarrhoea is connected to the disturbance in the normal intestinal microflora is regarded as the rationalization for the probiotics consumption in AAD.

AAD differs in frequency, but can occur in up to 39% of hospitalized patients given antibiotics because of an intense change in the normal colonic flora. Alterations in the faecal flora result in changes in colonic carbohydrate digestion, reduce short-chain fatty acid absorption and induce osmotic diarrhoea, although the pathophysiology underlying these changes remains poorly understood. The results of AAD include a longer hospital stay (8 days on average), a higher cost of care ($1280 to 2560 ($) to 4000 per stay), a fivefold increase in the incidence of other nosocomial infections, and a threefold increase in death (ranging from 0.7 to 38%).

Probiotic impact in children has been the focus of many studies (McFarland, 2008; Ruszczynski et al., 2008). The control groups received placebo and antibiotics in all trials, and probiotics were given together with antibiotics in the test subjects. The meta-analysis is indicative that probiotics can be implemented to stop AAD, and that *Saccharomyces boulardii* and lactobacilli have the potential to be utilized in this situation. Bacteria and yeast, including the bacteria *Lactobacillus* spp. (Ruszczynski et al., 2008) (*L. rhamnosus* GG), an *Enterococcus* (Al-Nassir et al., 2008) and the non-pathogenic yeast *Saccharomyces boulardii*, have been investigated in AAD prevention (Barc et al., 2008). A meta-analysis of probiotics in AAD prevention evaluated nine controlled trials that showed that both lactobacilli and *Saccharomyces boulardii* can stop AAD. However, the mechanisms of action of probiotics are not evidently realized. The probable mechanisms involve competition for nutrients, stimulation of immunity, production of antimicrobial materials, and the inhibition of mucosal and epithelial adherence of pathogens. More mechanisms might involve immunomodulation of gut-associated lymphoid tissue, effects on mucus secretion and receptor competition. *Lactobacillus* GG has been discovered to adhere to mucosal cells; it may strive for nutrients and yield a substance that avoids bacteria and alters faecal glucuronidase, which might be an intestinal bacterial metabolism marker. The efficiency of probiotics in stopping AAD in children has been proven by the results of six placebo-controlled randomized controlled trials involving 766 children. Compared with the placebo, treatment with probiotics reduced the danger of AAD from 28.5 to 11.9 %. So, probiotics decrease the danger of AAD in children. The consumption of probiotics, which are live microbial food ingredients and valuable to health, is a preventive measure. The postulation that in several cases diarrhoea is connected to the disturbance in the normal intestinal microflora is regarded as the rationalization for the probiotics consumption in AAD.

Antibiotic-associated disease
As living organisms, probiotics, particularly *L. rhamnosus* GG and the yeast *Saccharomyces boulardii*, have beneficial therapeutic effects when ingested. The advantage of these probiotics in the prevention of antibiotic-associated diarrhoea (AAD) has been proven through controlled trials. The effectiveness of *Saccharomyces boulardii* (Goldin & Gorbach, 2008) or *L. rhamnosus* GG (Snydman, 2008) in AAD prevention evaluated nine controlled trials involving 766 children. Compared with the placebo, treatment with probiotics reduced the danger of AAD from 28.5 to 11.9 %. So, probiotics decrease the danger of AAD in children. The consumption of probiotics, which are live microbial food ingredients and valuable to health, is a preventive measure. The postulation that in several cases diarrhoea is connected to the disturbance in the normal intestinal microflora is regarded as the rationalization for the probiotics consumption in AAD.

No important dissimilarity in the duration of diarrhoea between groups has been reported by Correa (*P*=0.253). However, administration of probiotics may pose some danger, although adverse effects seem to be rare. The majority of complications occur in severely immune-compromised subjects or in patients with other life-threatening illnesses managed in intensive-care areas. Although the utilization of probiotics in immune-competent subjects seems to be safe,
whether they can be utilized in the prevention of AAD in immune-compromised patients still remains unclear (Botina et al., 2011; Lonnermark et al., 2010).

Probiotic effects on epigenetic alterations
The study of the mechanisms or pathways that initiate and retain heritable patterns of gene expression and gene function without altering the DNA sequence is referred to as epigenetics. This definition is parallel to that of genome, which refers to the complete set of genetic information enclosed in the DNA of an organism. Some epigenetic pathways, for example, DNA methylation and histone modifications, have been identified by researchers (Nakao, 2001; Bastow et al., 2004).

Alterations in the epigenetic pathways are implicated in common human diseases. Cancer epigenetic changes have been recorded in nearly every step of tumour development. Less DNA methylation (hypo-methylation) results in chromosome instability and initiates oncogenes. A malignant cell can have 20–60% less genomic methylation than its normal counterpart. By contrast, the silencing of tumour suppressor genes may be initiated by excessive DNA methylation (hyper-methylation) (Iacobuzio-Donahue, 2009).

Epigenetic markers have been evaluated by medical researchers as a tool for primary cancer diagnosis and clinical-outcome prediction. Therapeutics based on epigenetic strategies are also implemented for cancer prevention or treatment. Increasing evidence has shown that the development of particular human diseases, such as neurodevelopmental disorders (Abel & Zukin, 2008), cardiovascular diseases (Orlová & Smith, 2010), type 2 diabetes (Park et al., 2008), obesity (Blaut & Bischoff, 2010; Gluckman & Hanson, 2008) and infertility (Rajender et al., 2011), is mainly caused by epigenetic changes.

Epigenetics includes genetic control by elements other than an individual’s DNA sequence. To determine which proteins are transcribed, epigenetic changes switch genes on or off. Epigenetics is involved in several normal cellular processes. One way to switch genes off, which can also contribute to differential expression, is epigenetic silencing. Epigenetic changes can be responsible for some disease states despite being required for normal development and health. Abnormal activation or silencing of genes can be caused by the disruption of any of the three systems that help the epigenetic changes. Disturbances of this kind have been connected to cancer and disease syndromes, involving mental retardation and chromosomal instabilities (Canani et al., 2011).

Cancer was regarded to be connected to epigenetics. Researchers discovered that diseased tissue from patients with colorectal cancer had less DNA methylation than normal tissue from the same patients. Abnormally high gene activation by shifting the arrangement of chromatin can be caused by the loss of DNA methylation, because methylated genes are typically switched off (Kumar et al., 2013).

The link between the gastric mucosa and allergy is becoming progressively clearer. The permeation of antigens across the gut lining has been the primary implicating factor in food hypersensitivities, which manifest as allergies. Probiotics positively affect the GI mucosa in a number of ways, many of which are currently being clarified. Thus, probiotics affect the host defence mechanisms in three important ways. First, probiotics promote the structural integrity of the GI barrier through its adhesive properties (Sengupta et al., 2013). Second, probiotics bind antigens; thus, reducing their immunogenicity (Delcenserie et al., 2010). Third, probiotics reduce inflammation of the gut by the balancing of pro- and anti-inflammatory cytokines (Shadnoush et al., 2013). Probiotics stop IgE-associated allergy until age 5 in Caesarean-delivered children, but not in the total cohort over the past decades (Kuitunen et al., 2009). Allergy is a major health issue because the rate of children with allergies in the westernized world has significantly increased. This increase has been attributed to the so-called ‘hygiene hypothesis’.

Many farm studies have demonstrated that child exposure to large numbers of microbes is linked to fewer allergic diseases at school age. The use of farm milk may independently protect against the development of allergies (Kuitunen et al., 2012).

The gut microbiome and hygiene indulgence
The hygiene hypothesis has been an evolving concept in immunology for more than 20 years. This hypothesis suggests that experiencing an environment that is tremendously clean with a lesser microbial problem than the environments we developed in and dwelled in for a majority of human history upsets the commonly regulated balance of the immune system and tips it toward an allergy predilection (Kinross et al., 2011). Based on observations showing that allergies are less widespread amongst children who have older siblings, lived on farms, attended day care or had pets, in comparison with children expecting to have less exposure to bacterial and viral diseases, soil organisms and commensal bacteria early in life, the hypothesis was expanded. Children who did not have such exposures are expected to develop allergies as they age.

At birth, we all have the necessary blood cells that provide our innate immunity; however, interaction with foreign bodies (antigens) is required to provide antibodies, which is our ‘learned immunity’. This assumption is consistent with the idea that children with hygienic backgrounds and a limited exposure to antigens from bacteria have a greater predisposition to suffer from allergies, including asthma. This correlation between hygiene and allergy has been dubbed the hygiene hypothesis. Probiotics may offer the primary-care physician a safe and efficacious method for treating allergy (Yazdanabakhsh et al., 2002; Okada et al., 2010). Based on the hygiene hypothesis, the decrease in infections explains the greater occurrence of allergic and autoimmune diseases in advanced countries. The occurrence
of autoimmune and allergic diseases is related to the level of sanitary conditions at both the national and individual levels. Collective hygiene is also essential. However, the sanitary quality of drinking water and food (cold chain), vaccination (as mentioned above), or the excessive use of antibiotics that affect gut flora may be more important than individual hygiene (Bach & Chatenoud, 2012). Riboulet-Bisson et al. (2012) revealed that *Lactobacillus salivarius* UCC118 administration had an effect on genus *Firmicutes* members in pig and mice. This effect was not observed when the mutant strain was administered, and was, thus, associated with bacteriocin production. Surprisingly, they showed that *L. salivarius* UCC118 administration and production of Abp118 in both models had an effect on Gram-negative micro-organisms, although Abp118 is usually not active in *vitro* against this group of micro-organisms. Thus, *L. salivarius* UCC118 administration has a significant but subtle impact on mouse and pig microbiota, by a mechanism that seems at least partially bacteriocin-dependent.

**Culture of microbiome in newborn gut**

Saliva is a complex fluid that serves a number of significant functions. The antibacterial effect attributed to lysozyme content is one of the initially recognized properties of human saliva. According to many studies, saliva contains various antibacterial substances, such as lactoperoxidase, antibodies, lactoferrin, leukocytes and cationic proteins (Hattori & Taylor, 2009; Kho et al., 2014). Periparturient licking by females of their anogenital and mammary areas and wound licking are considered as the two behavioural patterns connected to licking where the antibacterial impact of saliva might be crucial. Licking of the anogenital and mammary areas before parturition is obvious in dogs, rodents and cats. During late pregnancy, the shift of grooming from other parts of the body to the anogenital and mammary areas has similarly been quantitatively recognized for rats. The intestinal bacterial flora that is regarded as protective against opportunistic pathogens does not exist in newborn mammals with a sterile gut. In case of being exposed before colostrum ingestion, the newborns are in an endangered state to some of the more dangerous strains of *E. coli* regularly discovered in faeces. Finally, licking their wounds instantly after being hurt or during the curing process is a common observation among carnivores and rodents that have constant cutaneous injuries (Johnson & Versalovic, 2012).

Physically washing the wound of external material, such as tissue debris and bacterial contaminants, using the tongue is another obvious function. The licking of wounds would be mainly adaptive in decreasing bacterial infection if the saliva of the animals has antibacterial agents that are effective against potential wound-tissue pathogens. The hygienic function of maternal licking of the mammary and anogenital areas in protecting newborns from these diseases is facilitated by the bactericidal effects of saliva. *E. coli* and *Streptococcus canis*, along with coagulase-positive *Staphylococcus* and *Pseudomonas aeruginosa*, are among the common wound contaminants of dogs. Wound contamination by *E. coli* and *Streptococcus canis* can be reduced by wound licking and the use of saliva (Gilchrist et al., 2010; Guarner et al., 2006).

**Conclusion**

The intestinal microbiota of the host can be modulated through the consumption or administration of probiotic micro-organisms that establish an excellent means of remediation and deterrence against a variety of intestinal disorders and infections. Given the lack and hazards of antibiotics, including reduction of microbiome diversity and antibiotic resistance, the use of probiotics instead of antibiotics is becoming increasingly acceptable.

**Future perspective**

The human body is a ‘supra-organism’ and the GI flora has been referred to as ‘a virtual organ of the human body’. The majority of probiotics in use have been designed for the GI tract, while there is now an impetus to progress the field to encompass other regions of the body.

Lactobacilli derived from the endogenous flora of normal donors, as probiotics in functional foods or as vaccine carriers, displayed similar properties in *vitro* but strain-specific effects under *in vivo* conditions.

Probiotics, in the future, will be expanded in order to address microbiota-associated conditions that are outside the limits of the *micro-organisms* utilized as probiotics today. Micro-organisms, which have been genetically modified, may supply effective epitopes for improving natural immune responses, restoring antigen-specific tolerance and oral vaccine delivery. The components of the cell surface, which supply a potential strategy for the treatment of inflammatory intestinal disorders, can be changed by probiotic bacteria. The transplant of faecal microbiota into the duodenum of diabetic patients with the metabolic syndrome enhanced their sensitivity to insulin. A more adequate approach in future studies will be provided by cocktails of defined microbes with vital functionalities. Nevertheless, particular valuable strains with outstandingly properties, for instance, *Faecalibacterium prausnitzii* (chronic gut inflammation), *Oxalobacter formigines* (kidney stones), etc., in well-designed clinical trials, ought to be more comprehensively investigated.

Efficient probiotic interventions in relation to microbiota-associated situations require a broad comprehension of the relationships between microbial, genetic and environmental effects within humans. This will contribute to the identification of the subset of patients responsive to gut microbiota manipulations and the optimal agents to apply in a specific subject. Through probiotics, the future of decreasing the risk of illness is bright and hopeful, but validated biomarkers for numerous target diseases are
deficient. Therefore, in the field of probiotic investigation, the defining of validated biomarkers needs to be advanced. In addition, in healthy populations, the measurement of meaningful physiological changes needs further investigation. The identification of subjects on the limits of a normal physiological range might be considered as a productive direction for research on healthy subjects. The consequence of extensive utilization of probiotic products regarding quality-of-life indicators and society-wide economics ought to be evaluated, in different communities, through endpoints involving the reducing of prevalent infectious diseases. Support for sustained research in the probiotic field of study could be provided through such information. Based on the effect of probiotics both locally in the GI tract and distantly at other sites of the body, studies regarding the immunomodulatory actions of probiotic bacteria should be performed to evaluate both their local and systemic effects on the upregulation of immune responses against infection and the downregulation of immune responses associated with allergic reactions.

Summary

- Probiotic therapy merits consideration in future trials.
- Trials of probiotic therapy must adequately address the risk of colonization with probiotics and their related infection.
- Trials of probiotic therapy should be well ordered, and researchers should carefully consider where to administer the probiotics. The cause-and-effect relationship in the pathogenesis of disease-related disorders in relation to the role of mutualistic microorganisms remains to be defined.
- Some probiotics can improve clinical outcomes for hard to treat diseases and some others deliver benefits for healthy individuals.
- Probiotics act by direct interaction with the host cells through immune signalling mechanisms and/or changing the composition and activities of the colonizing microbiota.
- There is a clear association of microbiome alterations with a progression from health to disease, but the causality and reversal of disease in response to probiotic-induced microbiome changes remain to be verified.

Acknowledgements

The financial support of the University Putra Malaysia and Tabriz University of Medical Sciences, Tabriz, Iran, are gratefully acknowledged.

References


and Streptococcus thermophilus for prevention of antibiotic–associated
Effect of antibiotic prescribing in primary care on antimicrobial
resistance in individual patients: systematic review and meta-analysis.
BMJ 340, c2096.
inflammatory bowel disease: microflora ‘on the scope’. Br J Clin
Pharmacol 65, 453–467.
Delcenserie, V., Martel, D., Lamoureux, M., Amiot, J., Boutin, Y. &
pervasive effects of an antibiotic on the human gut microbiota,
Dinleyici, E. C., Eren, M., Ozen, M., Yargic, Z. A. & Vandenplas, Y.
(2012). Effectiveness and safety of Saccharomyces boudardii for acute
Ellison, R. T., III (2006). Review: probiotics are effective for prevention
of antibiotic-associated diarrhea and treatment of Clostridium difficile
disease. ACP J Club 145, 46.
Engelbrektson, A. L., Korzenik, J. R., Sanders, M. E., Clement, B. G.,
Gluckman, P. D. & Hanson, M. A. (2008).
Effect of a multi-species synbiotic formulation on fecal bacterial
microbiota of healthy cats and dogs as evaluated by pyrosequencing.
Foudry, F., Guinane, C. M., Hussey, S., Wall, R., Ryan, C. A., Dempsey,
E. M., Murphy, B., Ross, R. P., Fitzgerald, G. F. & other authors
(2012). High-throughput sequencing reveals the incomplete, short-
term recovery of infant gut microbiota following parenteral antibiotic
Friedman, G. (2012). The role of probiotics in the prevention and
treatment of antibiotic-associated diarrhea and Clostridium difficile
applied bacteriology 66, 365–378.
Garcia-Mazcorro, J. F., Lanerie, D. J., Dowd, S. E., Paddock, C. G.,
Effect of a multi-species synbiotic formulation on fecal bacterial
microbiota of healthy cats and dogs as evaluated by pyrosequencing.
good or bad? Nitric Oxide 22, 104–109.
health benefits. In Bioactive Components of Milk, pp. 423–454. Edited by
Gluckman, P. D. & Hanson, M. A. (2008). Developmental and
epigeneic pathways to obesity: an evolutionary-development
perspective. Int J Obes (Lond) 32 (Suppl. 7), S62–S71.
Glynn, M. K., Bopp, Ch., Dewitt, W., Dabney, P., Mokhtar, M. &
Guarner, F., Bourdet-Sicard, R., Brandtzaeg, P., Gill, H. S., McGuirk,
Gueimonde, M. & Collado, M. C. (2012). Metagenomics and
probiotic-antibiotic combination therapy: assessment of antimicrobial
a new frontier of human biology. DNA Res 16, 1–12.
Hoveyda, N., Heneghan, C., Mahtani, K. R., Perera, R., Roberts, N. &
Glasiou, P. (2009). A systematic review and meta-analysis of
probiotics in the treatment of irritable bowel syndrome. BMC
Gastroenterol 9, 15.
Annu Rev Pathol 4, 229–249.
Jakobsson, H. E., Jernberg, C., Andersson, A. F., Sjölund-Karlsson,
therapy has differing long-term impacts on the human throat and
Johnson, C. L. & Versalovic, J. (2012). The human microbiome and
genesis: influencing the microbial ecosystem in the gut. Adv Appl
mucilage as a candidate substance for saliva substitute: in vitro study
of its viscosity and influences on lysozyme and peroxidase activities.
Gerontology 31, 34–41.
Kuitunen, M., Kukkonen, K., Juntunen-Backman, K., Korpela, R.,
prevent IgE-associated allergy until age 5 years in cesarean-delivered
maternal allergy and use of probiotics during pregnancy on breast
milk cytokines and food antibodies and development of allergy in
children until 5 years. Int Arch Allergy Immunol 159, 162–170.
Kumar, M., Nagar, R., Verma, V., Kumar, A., Kaur, N., Hemalatha, R.,
Lodise, T. P., Graves, J., Evans, A., Graffunder, E., Helmecke, M.,
vancomycin MIC and failure among patients with methicillin-
resistant Staphylococcus aureus bacteremia treated with vancomycin.
Lönnermark, E., Friman, V., Lappas, G., Sandberg, T., Berggren, S. &
Adlerberth, I. (2010). Intake of Lactobacillus plantarum reduces
certain gastrointestinal symptoms during treatment with antibiotics.
Lofft, T. & Allen, H. K. (2012). Collateral effects of antibiotics on
Merenstein, D., Murphy, M., Fokar, A., Hernandez, R. K., Park, H.,
Nsofu, H., Sanders, M. E., Davis, B. A., Niborski, V. & other authors
(2010). Use of a fermented dairy probiotic drink containing


