

## Microbial Ecology and Dysbiosis in Human Medicine

John A. Catanzaro N.D. and Lisa Green B.Sc.

### Abstract

Microbial ecology and dysbiosis are topics worthy of study. This review article will be the first of a two-part series that will discuss normal flora and threats to its ecology resulting in dysbiosis. Microorganisms are generally required to attain critical population increases before they threaten the host. The research on the role of bacterial microorganisms which are considered to be part of normal flora and their importance in inhibiting potential pathogens will be discussed. This article also explores potential threats to healthy microbial flora, including dietary influences, anxiety and depression, and pathogenic bacteria and fungi. Defense mechanisms and their role in preventing translocation of infection from the GI tract to distal sites are also discussed. The role of probiotics in keeping a balanced microbial flora will be the subject of a future article. (Alt Med Rev 1997; 2(3):202-209)

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### Human Microbial Ecology

The human body maintains a rich and diverse microbial flora. Virtually every surface of the body having contact with the outside world has been colonized by various bacteria and fungi. The term "normal flora" implies that the microbes in question live with us symbiotically and generally cause no harm. However, each individual's dietary and lifestyle habits, environment, body chemistry, and immune system influence the floral balance of the body, and what is considered normal for one person may cause a pathogenic condition in another. The following discussion involves the various types of bacteria that colonize the oral cavity and gastrointestinal system, their metabolic effects upon each other and the body, and how their populations may be influenced by external factors such as diet, stress, antibiotics, and biotherapeutic agents.

The mouth, nose, and throat are colonized by Actinomyces, Neisseria, Bacteroides, Lactobacilli, Fusobacterium, Streptococcus, Staphylococcus, Corynebacterium, spirochetes, yeasts and others. There are an average of 10<sup>9</sup> microbes per ml in saliva. Anaerobic bacteria colonize at the gumline and between the teeth. More anaerobic than aerobic bacteria are found in the mouth, and especially high numbers of Fusobacterium and spirochetes have been implicated in periodontal disease. Both Streptococci and Lactobacilli play a major role in dental caries by converting oligosaccharides from the diet into dextran and levan, which cause plaque formation on the teeth. Fluoride and vitamin B6 reduce the numbers of these bacteria in the mouth, reducing the potential for cavities.<sup>1</sup>

The stomach harbors few bacterial species, and those that survive the highly acidic pH of gastric juices are primarily acid tolerant Lactobacilli and yeasts which are constituents of the oral flora.<sup>2</sup> Other bacteria that pass through the stomach are most likely destroyed by hydrochloric acid.<sup>1</sup> Evidence for this is that the duodenum, the part of the small intestine adjacent to and separated from the stomach by the pyloric sphincter, has a very low bacterial count of an average of 10<sup>5</sup> organisms per ml of intestinal juice.<sup>1</sup> The pyloric sphincter is usually partly open which allows chyme to enter the small intestine;<sup>3</sup> and the type of bacteria which are found consistently are the same oral type found in the mouth and stomach.<sup>1</sup> HCl, which is secreted by parietal cells of the stomach, kills bacteria by dissolving plasma membranes.<sup>3</sup> Studies have shown that, in conditions associated with decreased stomach acid, more bacteria are present in the gastric mucosa, including fecal microbes not normally found there. If normal acidity is maintained in conditions of gastritis or gastric ulcers, microbial presence remains at a low level.<sup>1</sup> As an exception, *Helicobacter pylori* is a microaerophilic gram negative bacilli which is uniquely adapted to survive in the stomach mucosa. This organism causes chronic gastritis and peptic ulcers by impairing the mucosal integrity of the stomach, and is usually treated with antibiotics. In the realm of natural therapeutic agents, the use of berberine sulfate, deglycyrrhizinated licorice, bismuth, bentonite, and L-glutamine has been reported for H.pylori-induced gastritis.<sup>4</sup>

The small intestine is relatively free of bacterial populations until the ileum approaches the ascending colon, where some fecal type bacteria are believed to migrate. The somewhat acidic nature of the

intestinal juice as well as the peristaltic movement of the small intestine are believed to be responsible for the low numbers of bacterial colonization.<sup>5</sup>

Dysbiosis of the small intestine may be caused by a number of factors, including surgical trauma, low acidity, environmental and emotional stress, and altered intestinal transit, which can facilitate the overgrowth of bacteria and contribute to malnutrition. In addition, reduced mucosal immunity can further contribute to increasing potentially pathogenic bacterial populations of the bowel. Secretory IgA is believed to be the first line of defense against potential harmful invaders of the mucosal flora.

The small intestine is the primary site of food absorption, and the metabolism of microbes can interfere with this process. Bacteria can prevent the uptake of vitamin B12 in the distal ileum, causing B12 deficiency and megaloblastic anemia. The lost dietary B12 can be recovered bound to bacteria in the feces of patients with bacterial overgrowth.<sup>5</sup> Urinary concentrations of indole and indican, which are degradation products of tryptophan, are increased in patients with small bowel overgrowth.<sup>1</sup> Amino acid absorption can be impaired while fecal nitrogen is increased and serum proteins lowered.<sup>5</sup> These changes, associated with bacterial overgrowth, can contribute to the dysfunction of mucosal cells, impairing protein and carbohydrate absorption.

In maldigestion, certain enzyme activity can be impaired as a result of degradation. Degradation of digestive enzymes by bacterial proteases have been shown to occur by certain species such as *Bacteroides fragilis*, *Clostridium perfringens*, and *Streptococcus faecalis*.<sup>5</sup> This may further contribute to enzyme insufficiency and maldigestion.

Two diseases of the small intestine which are complications of bacterial overgrowth and which cause malabsorption syndromes are tropical sprue and blind loop syndrome. In addition, increased occurrences of bacterial overgrowth are seen as a result of antibiotic overuse, antibiotic misuse, and depleted probiotic species. In cases of tropical sprue, a disease found geographically in the Caribbean area, significant colonization of Enterobacteriaceae occurs in the small bowel in a higher proportion than anaerobes, with the adherence of bacteria to mucosal cells. Some enterotoxin-producing species such as *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Escherichia coli* have been isolated from jejunal aspirates of patients with tropical sprue. These bacteria have been shown in animal models to cause fluid and electrolyte secretion in the small bowel. Fatigue, chronic diarrhea, weight loss, and nutrient depletion (e.g. vitamin B12 deficiency) are common in cases of tropical sprue.

In blind loop syndrome, a portion of the small intestine is dilated and does not empty during peristalsis as does the rest of the intestine. Bacteria remain and propagate in this area causing enteritis and penetration of the bacteria into the intestinal epithelium, where obligate anaerobes rather than facultative anaerobes tend to grow.<sup>5</sup> Malabsorption is prominent in this disease, with bacterial deconjugation of bile acids preventing fatty acid and monoglyceride absorption, causing steatorrhea.<sup>1</sup> In both of these conditions, antibiotics improve gastrointestinal symptoms, confirming that the conditions are due to bacterial overgrowth of certain species.

Bowel dysbiosis is also characterized by many of the same symptoms as the two previous mentioned conditions. Decreased HCl, digestive enzyme deficiency, and imbalances in short and long chain fatty acids can all contribute to bacterial overgrowth of the small and/or large bowel. Malabsorption, maldigestion, and malnutrition can all result from disturbance or disruption of the bowel microbial ecology. It behooves clinicians to be mindful of this delicate balance when prescribing treatment.

Identifying potential pathogenic bacteria is not an easy task. The feces contain about 400 billion bacteria per gram of dry weight, which accounts for about 40% of fecal volume. These organisms are representative of the population of the ascending, transverse, and descending colon as well as the rectum. There are 400-500 different species of bacteria in the human intestinal tract at any given time.<sup>6</sup> See Table 1 for the most prevalent bacterial genera in humans. The clinician should keep in mind that bacterial overgrowth manifests differently among individuals. What may provoke symptoms in one person may not be a problem for another. This may be due to intact immune surveillance which can increase resistance to infection.

Within a week, a breast-fed infant develops a fecal flora primarily consisting of *Bifidobacterium*. Its prominence is facilitated by a disaccharide amino sugar which is required as a growth factor by the bacteria, and which is present in mother's milk.<sup>2</sup> Other bacteria seen in the infant flora to a lesser degree include *Lactobacillus*, *Escherichia*, *Streptococcus*, and *Staphylococcus*. As the diet changes, the intestinal flora also changes. Gram negative anaerobes are observed when meat is introduced into the diet.<sup>1</sup> Persons who consume meat exhibit proportionately higher numbers of *Bacteroides* and other gram negative anaerobes in comparison with those on a vegetarian diet.

When bacterial overgrowth occurs in the small intestine, changes in the colonic flora also occur. The number of gram negative anaerobes remains unchanged, but the proportion of Bifidobacterium is greatly reduced and although there may be an increase in aerobes such as Enterobacteriaceae, Erococcus, Staphylococcus, Proteus, and Clostridium, the major increase is within the Enterobacteriaceae family and include Klebsiella and Enterobacter. The overgrowth of this fecal flora seems to be associated with an increased pH of gastric juice, and although the larger numbers do not in themselves indicate a disease state, opportunistic infection and complications in the small intestine are interrelated.<sup>1</sup>

Pathologic states that are associated with increased bacterial overgrowth include the presence of Clostridium perfringens, which produces a number of exotoxins and an enterotoxin which cause food poisoning, cellulitis, and antibiotic associated diarrhea. This organism can also be found in increased numbers in the feces of rheumatoid arthritis patients.<sup>1</sup> Clostridium difficile is another organism which causes antibiotic-associated diarrhea. It also causes pseudomembranous colitis by producing a toxin which promotes necrosis and ulceration of the colonic mucosa. The bacteria is able to proliferate when a patient has been treated with antibiotics, especially clindamycin and ampicillin. These drugs eliminate facultative anaerobes which compete with Clostridium difficile for growth.<sup>5</sup> Saccharomyces boulardii, Lactobacillus and Bifidobacterium are biotherapeutic agents that have been used successfully to prevent antibiotic-associated diarrhea caused by Clostridium difficile infection.<sup>7</sup>

Abnormal proliferation of microbes in the bowel are regulated by the organisms themselves. Facultative anaerobes create a reduced environment by utilizing oxygen, therefore creating a hospitable environment for anaerobes. Metabolic products of anaerobic bacteria (and facultatives to some extent) are short chain fatty acids such as acetic, propionic and butyric acid which can inhibit bacterial proliferation by entering the bacterial cell at low pH and inhibiting metabolism.<sup>8</sup> Some bacterial strains produce antibiotic substances that can directly reduce populations of potential pathogenic bacterial species. Bacteriocins are examples of antibiotic substances that are produced by some bacterial species. E. coli produces a substance known as colicine, which prevents growth of other species, and can autoregulate its own population. See Table 2 for information explaining some of the direct and indirect inhibitory effects which indigenous microorganisms have on potential pathogens.

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### **Dietary Influence on Bowel Microflora**

The diet has significant influence on microbial populations in the bowel, although the most profound changes occur when an individual consumes a chemically defined, non-bulk diet. A study was conducted on individuals who consumed this type of diet for almost two weeks. Researchers concluded that extremely oxygen sensitive anaerobes as well as Bifidobacterium disappeared in these individuals. The major changes observed were an increased number of aerobes, primarily E. coli, and a decrease of Lactobacillus in these individuals. In researching how diet affects colon cancer, British and American subjects on a typical Western diet with a high proportion of meat in their diet were compared with Indian and Ugandan subjects who consumed strict vegetarian diets. Subjects with significant amounts of meat in the diet were found to have many more gram negative, non-spore forming anaerobes such as Bacteroides in their feces, whereas subjects who consumed a vegetarian diet were found to have a higher proportion of Streptococci and Enterobacteriaceae. Therefore, the anaerobe to aerobe bacterial ratio was higher in those people consuming a typical Western diet, manifesting in individuals as an increased risk of colon cancer.<sup>10</sup>

Other studies have observed that despite changes in microfloral composition, major groups of organisms remain the same under different dietary conditions, and the shift in specific population numbers does not provide enough evidence that animal protein consumption contributes to colon cancer.<sup>10</sup> There is evidence, however, that bacteria may have mutagenic activity which may further increase procarcinogenic activity. Procarcinogenic substances such as food preservatives, dyes, additives, or pollutants can be transformed into carcinogenic substances by bacterial enzymes beta-glucuronidase, beta-galactosidase, beta-glucosidase, nitro-reductase, azoreductase, 7-alpha dehydroxylase, and cholesterol dehydrogenase. Persons on a high meat diet showed greater beta-glucuronidase activity than those on a meatless diet. Bacterial beta-glucuronidase hydrolyzes glucuronidase, contributing to the toxicity of compounds which have been previously detoxified by liver glucuronidation. Bacterial nitroreductase reduces nitrogenous compounds to aromatic amines via nitrosamines and n-hydroxy compounds, which are suspected mutagens. Azoreductase promotes the reduction of azo food dyes to substituted phenyl and naphthyl amines, which are potent carcinogens.<sup>5</sup>

Laboratory rats which have been experimentally fed beef and Lactobacillus have reduced fecal amounts of beta-glucuronidase, azoreductase, and nitroreductase. This suggests that Lactobacilli may influence the metabolic activity of colonic flora and inhibit cancer potential.<sup>5</sup>

Kruis et al reported significant increases in gut transit time, fermentative colonic bacterial activity, and intestinal bile acids in healthy subjects who were fed a diet high in refined sugar. The lengthened transit time caused an increase in secondary bile acid concentration, which may be associated with the development of colorectal cancer. High sugar intake and increased gut fermentation are also associated with and implicated in the formation of gallstones and Crohn's disease.<sup>11</sup>

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### **Anxiety, Depression, Transit and Changes in the Microflora**

In terms of affecting gastrointestinal symptoms, emotional stress and anxiety also play a role. Anxiety is associated with increased bowel frequency, and depressed patients tend to be constipated.<sup>12</sup> The composition of the flora is very sensitive to changes in the host's intestinal tract. The greatest differences in composition of colonic flora seem to be caused by anger-stress situations. Emotional reactions trigger changes in intestinal motility and enzyme, bile, and/or mucin secretion. Mucin is an important substrate for many intestinal microorganisms.<sup>6</sup> The theory of a link between disturbed bowel function and psychological factors is also strengthened by the increased psych morbidity seen in hospitalized patients with irritable bowel syndrome.<sup>13-15</sup> The intestinal transit time differences between anxious and depressed patients and the correlation of depression scores with prolonged transit time show an effect of mood on bowel motility. Intestinal transit time can alter symbiotic relationships of the bowel microflora.<sup>12</sup>

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### **Defense and Translocation**

Bacterial presence stimulates defensive factors in the intestinal wall. Germ-free or antibiotic-treated animals have thinner intestinal walls due to reduction in the normal amounts of lymphatic and reticuloendothelial tissue. When germ-free animals are inoculated with fecal type bacteria, these tissues rapidly develop.<sup>8</sup> Continued use of antibiotics which reduces the number of normal flora can cause susceptibility by reducing the defenses of the intestinal wall and enabling adherence and growth of pathogenic bacteria. Penicillin, clindamycin, and metronidazole eliminate certain obligate anaerobes, allowing gram negative facultative anaerobes to thrive and overpopulate, then translocate from the gastrointestinal tract.<sup>8</sup> Translocation and subsequent sepsis is a problem in immunocompromised hosts, especially in conditions where intestinal permeability is altered.<sup>16</sup> Facultative anaerobes such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* are likely to translocate to the mesenteric lymph nodes; and translocation is especially promoted by abnormally high numbers of these organisms.<sup>8,16</sup> Patients who receive immunosuppressive chemotherapy in addition to antibiotics are especially at risk. Translocation has also been known to occur apart from trauma or any other known etiology. For example, *Candida albicans* was administered orally to healthy patients and, within three hours of inoculation, the *Candida* had translocated into the blood stream.<sup>17</sup> In cases like these, the possibility of impaired mucosal immune defense must be considered. As stated earlier, emotional factors, environmental factors and dietary habits can all significantly influence mucosal symbiosis. However, we must not ignore the possibility of genetic predisposition as a possible cause. Translocation has been known to occur in seemingly healthy individuals.<sup>17</sup> Continual study is necessary for clinicians to understand the process of translocation and immunological surveillance.

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### **Conditions Favoring Bacterial Overgrowth**

The maintenance of a stable host flora equilibrium involves host defenses, environmental factors, and bacterial interactions. These host mechanisms include acid secretion, intestinal peristalsis, and a properly functioning ileocecal valve. In addition, intestinal immunoglobulin secretion and a mucosal barrier preventing bacterial adherence are of vital importance in minimizing pathogenic bacterial populations. Intestinal motility is another major host defense against bacterial overgrowth. However, the relationship between enteric flora and intestinal motility is not clearly understood. Delayed transit time and achlorhydria are two common manifestations in individuals with bacterial overgrowth. Antibiotics clearly alter the bowel flora, creating an environment favoring proliferation and adhesion of

pathogens to the bowel mucosa. However, it is interesting to note that broad spectrum antibiotics generally do not disturb the anaerobic bacteria. Finally, bacteria are involved in their own regulation. There are mechanisms that include competition for nutrients, production of growth promoters and/or inhibitors by bacteria, and the creation of an anaerobic environment by oxygen-utilizing microorganisms. Bacteria may transfer antibiotic resistance to each other as well as secrete substances with antibiotic activity.18-20

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### **Clinical Features**

Clinical aspects of bacterial overgrowth are predictable once pathophysiology is understood. However, it must be emphasized that many individuals harbor bacterial overgrowth without symptoms. Clinical features in symptomatic individuals can include megaloblastic anemia, weight loss, malnutrition, diarrhea, constipation, bowel cramping, and chronic joint pain. Rare presentations may include night blindness, abnormal bleeding, hypocalcemic tetany, osteomalacia, peripheral neuropathy, and edema. In cases where there is strong suspicion of bacterial overgrowth, comprehensive bacteriology and digestive analysis are important diagnostic tools. In addition, parasitology, measuring urinary bacterial metabolites, and breath testing may all be useful adjuncts in diagnosis.18,20-22 Intestinal permeability and immunological surveillance testing need further clinical study as to their efficacy in clinical diagnosis.

Probiotics and their benefits in human medicine will be discussed in a future article.

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