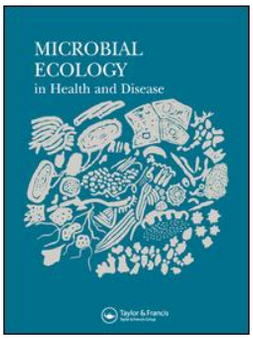

Taylor & Francis
Taylor & Francis Group

Microbial Ecology in Health and Disease

ISSN: (Print) 1651-2235 (Online) Journal homepage: informahealthcare.com/journals/zmeh20

Normal flora: diversity and functions

Lynne V. McFarland

To cite this article: Lynne V. McFarland (2000) Normal flora: diversity and functions, Microbial Ecology in Health and Disease, 12:4, 193-207, DOI: 10.1080/08910600050216183

To link to this article: https://doi.org/10.1080/08910600050216183

Normal flora: diversity and functions

Lynne V. McFarland

From the Department of Medicinal Chemistry, University of Washington, and Biocodex, Inc. Seattle, WA, USA

Correspondence to: Lynne V. McFarland, Ph.D., Biocodex, Inc., 1910 Fairview Avenue E, Suite # 208, Seattle, WA 98102, USA. Fax: 206-323-2968; E-mail: lvmcfarl@u.washington.edu

Microbial Ecology in Health and Disease 2000; 12: 193–207

Recent research into the therapeutic use of living organisms has focused attention on the impact of various disruptive factors (antibiotics, surgery, immunosuppression) and their impact on the host's normal flora. This review covers what is meant by 'normal flora', how the microecology differs by the niche in the body, type of diet, age and health status. In addition, the functions and tools used to investigate normal flora will be explored. The functions of the normal flora include digestion of substrates, production of vitamins, stimulation of cell maturation, stimulation of the immune system, aid in intestinal transit and colonization resistance. A variety of factors can disrupt the normal flora including age, diet, stress, illness and exposure to antibiotics. Research involving microecologic populations is difficult due to the challenge of unraveling the complex dynamics within a usually inaccessible niche, but progress is being made.

INTRODUCTION

In the past, the role that microorganisms played in the normal functioning of the body was not appreciated. In the early 1900's when Dr. Metchnikoff was credited with the discovery of the importance of intestinal flora, other physicians felt that the colon was totally unnecessary and often surgically removed them from their patients (1). The colon was described as 'a poisonous cess-pit infecting the body with rheumatism, tuberculosis, cancer and other diseases'. Today, we know that normal flora is a dynamic and complex mixture of microbes that have diverse functions including digestion of essential nutrients, maturation of intestinal physiology, stimulation of immune system, systemic effects on blood lipids and the inhibition of harmful bacteria. Current research techniques allow better evaluation of the specific bacterial and fungal microbes within various body sites. This paper revisits some popular conceptions about normal flora and updates them with regard to recent scientific findings.

WHAT IS 'NORMAL FLORA'?

Although the term 'normal flora' is commonly used, it is really a misnomer. Microbial flora has spatial and temporal complexity that differs by individual, body niche, age, geographic location, health status, diet and type of host (2, 3). Even within the same individual, the composition of the microbial flora can vary according to changes in diet, stress, sexual behavior, medication, hormonal changes and other host-related factors (3–7). With this caveat in mind, the field of 'normal flora' can be examined for common predominant types of flora present within body niches and shared functional traits.

The adult human body contains 10^{14} cells, of which only 10% compose the body proper and 90% are accounted for by members of the microflora (8). The predominating types of species in humans differ according to the body niche (oral cavity, skin, vagina, stomach, ileum, colon or urinary tract), as shown in Fig. 1 (3, 9–13). Normal flora found in the oral cavity has been found to vary by the area sampled (tooth enamel, tongue, gingivital surface, saliva) and the state of periodontal health (14, 15). The oral cavity contains a wide mixture of microbes, which are mainly anaerobic bacteria. Gagliardi et al. sampled normal flora in the healthy esophagus during upper endoscopy procedures in 30 patients and the predominant flora was found to be *Streptococcus viridans* (16). Lactobacilli and alpha-hemolytic *Streptococcus* species are frequently isolated on tonsils of healthy children (15, 17). *Lactobacillus* species that have the ability to adhere to mannose-containing receptors, such as *L. plantarum*, have a distinct advantage in surviving in the oral cavity (18). Results from different studies profiling predominant flora may be difficult to compare as subject age, sampling techniques (washing of the surface, aspirates or biopsies), diet, sampled location and microbiological assay techniques may produce significantly different results. Fewer bacteria exist in the stomach (usually below 10^3/g due to acidic lumen). *Helicobacter pylori* has been found in patients with peptic ulcers and gastric neoplasia, but is also found in 60% of healthy hosts, which casts suspicion that this microbe is always a cause for gastric disease (19–21). The concentration of

 ISSN 0891-060X

microbes increases as progression is made down the intestinal tract: small intestine ($\sim 10^4$/ ml contents), to 10^6–10^7/ ml at the ileocecal region and 10^{11}–10^{12}/g in the colon. The intestinal microflora consists of 10^{11} organisms/gram of feces with over 500 different species, ranging in concentrations from 10^2–10^{11}/ml luminal contents (22). Although the variety of organisms is complex, generally there are more anaerobic microbes than aerobes (9). The development of new techniques and genetic probes has allowed better characterization of the types of organisms that comprise the normal intestinal flora. Franks et al. developed six 16S rRNA-targeted oligonucleotide probes that can detect at least 66% of the anaerobic fecal flora in humans (23). When these probes were used to characterize the flora in nine healthy human volunteers, *Bacteroides* species accounted for 20% of the total fecal population, *Clostridium coccides* and *Eubacterium rectale* accounted for 29%, Gram-positive bacteria accounted for 12% and *Bifidobacterium* species accounted for 3% of the fecal flora. These probes may prove very valuable in the characterization of the microecologic profiles, but more research is needed (as discussed later).

Previous studies have reported that flora in the healthy vagina is typically a mixture of aerobic *Lactobacillus* species, including *L. jensenii*, *L. acidophilus* or *L. rhamnosus* (9). Two strains of lactobacilli (*L. crispatus* and *L. jensenii*) protect vaginal surfaces by producing H_2O_2, which inhibits the colonization of pathogenic anaerobes and mycoplasmas associated with bacterial vaginosis, *Neisseria gonorrhoeae* or other sexually transmitted diseases (5, 13). Vaginal flora has been shown to change over the menstrual cycle (4), sexual activity and hygiene habits (7, 24), and use of intravaginal microbicides (such as nonoxynol-4) (25).

However, studies show most healthy women (52–78%) have transient changes in vaginal flora (4, 7, 24). Some more recent prospective studies have shown only a minority (22–26%) of healthy women had a lactobacilli-predominant flora (5, 24). Thus, the characterization of vaginal flora is open to debate and requires additional prospective studies in well-defined populations of healthy women.

The process of the development of normal flora starts at birth. It is thought that colonization begins during parturition when the neonate's intestine is seeded with mostly Gram-positive facultative anaerobes from the vaginal microflora during delivery (22, 26, 27). Whether the vaginal flora in the last trimester is similar to vaginal flora when the woman is not pregnant is not known. However, Karvoven et al. documented that the vaginal flora collected from mothers after delivery was the same as flora found in the stools of neonates (27). Neonates born by caesarian section usually acquire their first microbes from the environment of the hospital nursery (26). Neonates are quickly colonized by facultative anaerobes (*E. coli* and *Streptococcus*), reaching concentrations of 10^8 to 10^{10}/g feces within 1–2 days (9, 26). Previous studies reported that anaerobic flora do not become established until the second month of life (9). The hypothesis for this observation was that when the newly seeded facultative microbes grew and produced a more anaerobic environment, this established an anaerobic environment suitable for anaerobes (9). However, these

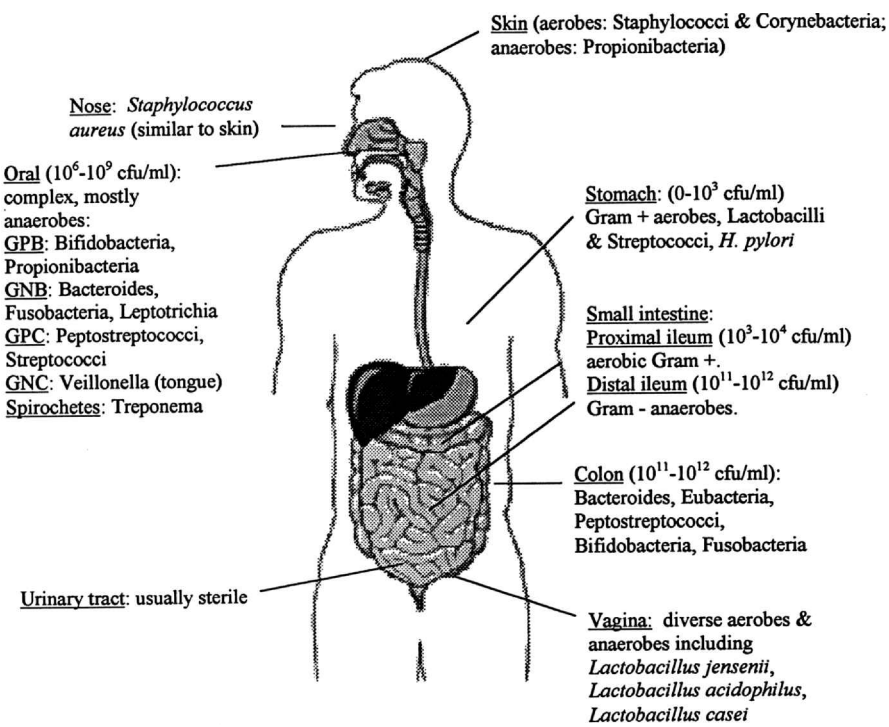

Fig. 1. Predominant flora in different niches of the human body. Compiled from references: (3), (9–13).

Table I

Microbial-associated characteristics (MAC) relating to different functions of the normal intestinal microecology

1.	Digestion of metabolizable substrates
2.	Colonization resistance
3.	Production of vitamins
4.	Development of attachment sites
5.	Induces development of the immune system
6.	Production of exogenous enzymes
7.	Stimulation of intestinal transit
8.	Maturation and turn-over of intestinal cells

Compiled from references: (22), (36–49).

conclusions were based on standard culturing techniques (9, 28, 29). Harmsen et al. compared newer techniques (FISH, 16S rRNA probes) with culturing techniques and found high counts were found by culturing only after 8–9 days, but FISH detected anaerobes by the second day (30). In addition, another study indicated that neonates can acquire strict anaerobes, such as *C. difficile*, within the first 2 days of stay on a neonatal ward (31). Therefore, the theory that colonization of the neonatal intestine by anaerobes is dependent upon facultative bacteria producing an anaerobic environment may be erroneous.

The type of diet largely influences the types of flora in pre-weaning infants. Several studies have reported that infants who are breast-fed have higher concentrations of bifidobacteria as compared to formula-fed infants (28, 29, 32, 33). Breast milk contains low protein content and high levels of oligosaccharides and glycoproteins, which are considered to be growth factors for bifidobacteria (26). Formula-fed infants have a more complex microbiota consisting of *Bifidobacterium*, *Bacteroides*, *Clostridium* and *Streptococcus* species (22, 28). However, other researchers have not found a significant difference in breast-fed and formula-fed infants (34).

Historically, the characterization of neonatal fecal flora has relied on culturing techniques, which are not able to detect non-culturable species and may not be able to distinguish different microbial populations. More recent techniques using 16S rRNA probes, which are able to amplify the bacterial genes and are followed by sequence analysis, are more sensitive than the traditional culturing techniques (23). Harmsen et al. used 16S rRNA probes and confirmed previous findings that breast-fed infants have predominant populations of bifidobacteria in their stools and formula-fed neonates had a more complex mixture of organisms (32). By 2 years of age, children have a similar complexity and range of microbes as adults (35). Differences in breast-fed and formula-fed microbial profiles found by different studies may be due to sampling techniques, time of collection, assay method, hospital practices or by the buffering capacity of different formulas (34).

FUNCTIONS OF THE NORMAL FLORA

Digestion

The functions of the normal flora have been called 'microflora-associated characteristics' (MAC) by several researchers (22, 36–38). These MAC (Table I) include digestion of metabolizable substrates, colonization resistance, vitamin production, mucosal cell development, immune system stimulation and intestinal transit regulation (36–49). An important role of the intestinal flora is the digestion of metabolizable substrates. A major source of nutrients is the upper intestinal tract and the available substrates may include dietary fibers, starches, oligosaccharides, sugars, some lipids and proteins. Another source of nutrients is within the colon itself and includes endogenous mucins, sloughed epithelial and enterocyte tissues, bacterial debris, bile acids and cholesterol. The types of metabolizable substrates are key in determining the type of flora present in the colon. The main products of bacterial digestion of non-absorbed dietary carbohydrates are short chain fatty acids (SCFAs). The SCFAs produced in the largest quantities by the normal flora include acetic, propionic and butyric acids (50). Acetic and propionic acids are rapidly absorbed and are a major source of energy, in addition to stimulating salt and water absorption (22, 51). Butyric acid has several functions including the maintenance of the integrity of the colonic epithelial layer, as a chief energy source for these cells and regulating cell growth and differentiation (22, 51). Treem et al. studied fecal SCFA in patients with Inflammatory Bowel Disease (IBD) (52). Fecal homogenates from 10 patients with IBD and 10 age-matched controls were compared as to the ability of the fecal homogenate to produce SCFA. Patients with IBD produced less total SCFA, less acetate acid and less propionic acid. Since normal flora is responsible for the production of SCFA, this study may indirectly link normal flora disruption with IBD. However, Treem et al. did not characterize the flora responsible, nor compare the microbial profiles of patients with IBD and controls. In some rare instances, the role for a member of the normal flora has been identified for a specific function. *Oxalobacter formigenes* is a normal anaerobe in the colon responsible for regulating the breakdown of oxalic acid and it has been demonstrated that patients with recurrent calcium oxalate kidney stone formation problems do not harbor this useful bacterium (53). Normal flora are also involved in the conversion of primary bile salts. The identification of the genes encoding conjugated bile salt hydrolases has been identified in a strain of *L. johnsonii* (54). Usually the role for a specific member of the normal flora is not limited to one function, rather the role is usually to interact with other members of the flora for more complex functions (such as colonization resistance).

Colonization resistance

Colonization resistance is the first line of defense against invasion by exogenous, pathogenic organisms or indigenous opportunistic organisms and the normal flora is responsible for this formidable task (8, 55). Even though the focus here is the intestinal tract, it should be remembered that colonization resistance plays an important role at other body sites (oral, skin, vagina, etc.). Colonization resistance is a dynamic phenomenon that may differ dramatically by microbial species, type of host, diet and other host factors. Colonization resistance has been found to be an extremely effective natural barrier against such pathogens as *C. difficile*, *Salmonella*, *Shigella*, *Pseudomonas*, pathogenic *E. coli* strains, *Candida albicans* and others (8, 56–58).

Autochthonous flora (indigenous species that normally inhabit a given ecologic niche) may become disrupted and allochthonous species (not normally present) are then able to colonize the site. Colonization with allochthonous bacteria may or may not result in disease, as the colonizing organism may not be a pathogenic species. Early evidence for colonization resistance arose from the observation that, in germ-free animals (animals with no flora), animals were extremely sensitive to colonization with pathogens and subsequently developed disease at higher rates than animals with intestinal flora (57). This increased susceptibility could be corrected if fecal flora or mixtures of bacteria and yeasts were administered (38). Germ-free animals and animals that have been given certain antibiotics are able to be colonized by exogenous bacteria at doses 1000 to 100 000 fold less than in animals with normal flora present (11). Freter and Abrams found the concentration of *Shigella flexneri* depends upon the degree of normal flora present in the gut. In germ-free mice, *S. flexneri* is present at 10^9/g, but if the mice are pre-colonized with a mixture of anaerobic bacteria, *S. flexneri* is held to 10^5/g and when *E. coli* was added to the mixture, the levels dropped to 10^3/g (59). Romond et al. showed that bifidobacteria, given to gnotobiotic mice, prevented the colonization by *E. coli* (60).

Table II

Mechanisms of action for colonization resistance

Mechanism	Factor	Factors active against
Production of inhibitory substances	Bacteriocins	*Salmonella*, *Shigella*
Toxic metabolic endproducts	Hydrogen peroxide	*Chlamydia*, *Gardnerella*
Adverse microenviroments	Acidic endproducts, short chain fatty acids	*S. aureus*, *E. coli*
Nutrient or substrate depletion	Monomeric glucose	*Clostridium difficile*
Attachment interference	Non-toxigenic strains of *E. coli*	enterotoxigenic *E. coli*
Immune system stimulation	Secretory IgA	rotavirus, *C. difficile*

Compiled from references: (11), (13), (41), (55), (59), (60), (63), (67), (68), (75), (80).

The role of colonization resistance has been well studied in the case of the resistance to *C. difficile*. *C. difficile* readily colonizes neonatal animals and human babies at the time when there is scarce flora established, but is cleared from the intestines once a more mature microecology develops (55). In the adult host, colonization resistance adequately prevents infection by *C. difficile* unless the microbial barrier is disrupted (61). Once the flora is disrupted, *C. difficile* colonizes the intestines, produces two major toxins and may cause overt disease including diarrhea, colitis, pseudomembranous colitis or toxic megacolon (62, 63). If protective flora is seeded back into the intestines (either by fecal infusion of normal stools or selected biotherapeutic organisms), colonization of *C. difficile* may be prevented and the disease does not develop (64).

The mechanism of colonization resistance is dynamic and complex (Table II). Bacteria comprising the normal flora are capable of producing substances and antimicrobial peptides that are inhibitory against colonizing bacteria (40, 65). Bacteriocins are a group of anti-bacterial proteins produced by intestinal flora that have a broad inhibitory spectrum including many Gram-positive and Gram-negative bacteria. Lactobacilli bacteria have been shown to produce a bacteriocin called reuterin, which is inhibitory *in vitro* for *Salmonella*, *Shigella*, *Clostridium* and *Listeria* species (66). Although an intriguing *in vitro* finding, it has not been shown that these bacteriocins reach concentrations inhibitory to pathogens in the intestinal lumen, thus the clinical significance of bacteriocins is not known.

Normal flora may also produce other metabolic endproducts that are inhibitory to other microbes. Most notable is hydrogen peroxide (H_2O_2) produced under anaerobic conditions by several strains of normal flora (66). The presence of H_2O_2 results in peroxidation of lipid membranes, increased bacterial membrane permeability, destruction of bacterial nuclear acids in bacterial strains that do not possess catalase. The vaginal tract is usually predominantly colonized with lactobacilli (Fig. 1) and H_2O_2 producing *Lactobacillus* strains have been found in 75% of vagina samples from healthy women. Vaginal colonization with *Lactobacillus* strains that produce H_2O_2 has been shown to be protective of infections caused by *Chlamydia trachomatis*, *Gardnerella vaginalis*, *Ureaplasma urealyticum* and the development of bacterial vaginosis (13). In women with bacterial vaginosis, these strains of H_2O_2 producing lactobacilli are absent and, instead, high concentrations of *Gardnerella vaginalis* and anaerobes are present (66). Another protective mechanism is the production of a low pH

environment, which may be inhibitory for certain pathogens. The production of acids as an end product of carbohydrate metabolism is common in many species of the normal flora and is inhibitory against Gram-positive and Gram-negative bacteria. Several pathogens, including *Staphylococcus aureus*, *Salmonella*, *E. coli*, and *Bacillus cereus*, are inhibited by acids produced by normal flora such as lactobacilli and bifidobacteria. A common end product of microbial fermentation is short chain fatty acids (SCFA). The presence of these SCFA have been shown to be inhibitory to nonindigenous bacteria (40). Rolfe showed in a hamster model of *C. difficile* disease that SCFA levels inhibited *C. difficile* growth (40). As newborn hamsters age, they start to produce high levels of acetic, butyric and propionic acids by day 16–19. Growth of *C. difficile* was significantly reduced when levels of these SCFA increased (40). If normal flora are disrupted, decreased levels of SCFA result and pathogenic microbes may take advantage of this decrease and reproduce to levels that induce disease. To date however, the identification of which specific SCFA or mixtures of SCFAs are responsible for the inhibition of pathogens has not been demonstrated.

Competition for nutrients may be another mechanism for colonization resistance. As it is extremely difficult to assess the levels of specific nutrients in the interior of the colon, most of the research on nutrient depletion has been done using continuous flow culture techniques. Wilson et al. inoculated normal flora from a mouse into a continuous flow culture and found one or more of the flora competed more successfully for monomeric glucose, N-acetylglucosamine and sialic acid, resulting in significantly reduced levels of *C. difficile* (67). Sweeney et al. found that even small numbers of an ingested *E. coli* strain F-18 could supplant established flora, as this strain utilized an available nutrient (gluconate) more efficiently than the other microbes present in the system (68). However, continuous flow cultures are extremely dependent on culturing and incubation parameters and the applicability of these results is unclear.

Normal flora may also produce extracellular enzymes that are inhibitory or interfere with pathogen attachment. A yeast (*Saccharomyces boulardii*) has been shown to produce a protease that destroys toxin A and toxin B receptor sites in rabbit ileal models for *C. difficile* disease (69). The toxins of *C. difficile* act by inactivating Rho proteins that keep the cytoskeleton of the intestinal enterocyte intact, thereby distorting the cellular morphology leading to fluid loss and diarrhea (70). Rho proteins are also involved in yeast budding processes (reproduction), thus this yeast may produce the protease to protect itself against soil *Clostridia* that may produce similar toxins to *C. difficile*, but in the human, the protease may coincidentally protect the host against infection with *C. difficile*.

Survival in various body niches necessitates the attachment to receptor sites, especially in the colonic lumen where peristalsis sweeps unattached microbes and undigested debris away. Competition for attachment sites is a successful mechanism to inhibit colonization of pathogenic microbes. Colonization with non-enterotoxin producing *E. coli* has been reported to prevent the subsequent infection with enterotoxin strains of *E. coli* in several mouse and pig animal models (40). Fuller reviews several studies in animals showing positive interference with attachment of normal flora when an non-indigenous microbe is ingested (71). A variety of host factors are also involved with colonization resistance including secretory IgA levels, peristaltic movement, production of mucus, epithelial or enterocyte turnover (40).

An area of intense research is to determine which member or members of the numerous species of microbes present in the intestine are responsible for colonization resistance. Most studies support the role of anaerobic flora as the major microbes responsible for colonization resistance (11, 56). Hazenberg et al. found a mixture of human anaerobic flora restored colonization resistance against *P. aeruginosa* in the germ-free mice model (57). Giuliano et al. showed cefoxitin increased the numbers of fecal enterobacteriaceae, enterococci and yeasts in human volunteers (72). The increase in all three groups of these aerobic organisms may be due to the suppression of selected anaerobic bacteria by cefoxitin. Leonard et al. reported that ceftriaxone decreased anaerobic flora in mice and human volunteers and colonization resistance was also depressed in ceftriaxone treated animals (73). Clindamycin is effective against anaerobes and has been shown to impair colonization resistance (58). Some antibiotics that are effective against anaerobes, but are only present at low levels in the gut (tinidazole, cephradine), do not impair colonization resistance (58). Specific members of the normal flora have also been studied including *Bifidobacteria longum*, peptostreptococci and *Clostridium cocleatum*. Romond et al. tested *Bifidobacterium longum* in germ-free mice model (60). Pre-colonization of germ-free mice with *E. coli* delayed colonization by *B. longum* for one month, compared to a colonization time of only 24 hours in the totally germ-free animals. Herias et al. observed that peptostreptococci reduced translocation of *E. coli* in germ-free rat model by priming the immune system (74). Boureau et al. showed that a normal member of the mouse intestinal flora, *C. cocleatum*, inhibited the growth of pathogenic *C. difficile* (75). *C. cocleatum* was found to produce a variety of glycosidases that attacked intestinal oligosaccharides. As the peptide core of the mucin layer in the intestine is protected by oligosaccharides, this bacteria may interfere with the attachment of *C. difficile* but further study is needed. Bourlioux et al. found a *Ruminococcus* species that prevented *C. perfringens* colonization in a gnotobiotic animal model (76). Although these studies have identified

potential candidates for species which may be responsible for colonization resistance; it is more likely that a complex mixture of many microbes is responsible.

A note of caution must be exercised with these animal studies, as the behavior of microbial species has been found to differ depending upon which animal is studied (including human). Wong et al. evaluated the mouse as a model for studying microbial ecology interactions that may occur in humans (77). Human strains fed to mice usually survived, with the exception of *Bacillus* and *Lactobacillus* species. They also found that in the mouse, human organisms only produced 25% of the expected organic acids, which may suggest a change in metabolism when human strains colonize the mouse. Resistance to a challenge strain (*Salmonella*) was preserved, indicating that the strains maintain their ability to produce colonization resistance (77).

Production of vitamins

Intestinal flora are also involved in the production of vitamins including panthothenic acid (B5), biotin (vitamin H), pyridoxine (vitamin B6) and menaquinone (Vitamin K2) (66, 78, 79). Without the intestinal flora, these vitamins would not be produced or in some cases, not broken down into an absorbable form. Intestinal flora can usually provide the daily minimum requirement for many of the vitamins in humans. Microbes that are added to foods (such as fermented diary products) may also increase the dietary levels of these vitamins (66). Vitamins B12, niacin, riboflavin and thiamin are also made by intestinal flora, but are not absorbed in the colon. The value of microbially supplemented foods is that the Vitamin B12 is ingested and it can be absorbed in the small intestine and utilized.

Attachment

Attachment to the intestinal mucosa is an important survival trait for organisms in the intestinal tract. The presence of intestinal flora has been shown to stimulate the production of epithelial glycoconjugates, which may be receptors for some pathogenic bacteria (22). Umesaki et al. documented that the presence of a strict anaerobe, *B. thetaiotaomicron* and a segmented filamentous bacterium were associated with fucosylated glycoconjugates in the small intestinal tract (80). Colonic mucins are a class of high molecular weight glycoproteins, which are secreted by the mucosa and exocrine glands. Mucin is present in the lumen and functions as a lubricant, a modulator of water and electrolyte absorption, may aid in attachment of microbes and protects the mucosa from injury (81). A large number of oligosaccharide side-chains of mucin glycoproteins aids in the stability of the mucin layer. Carlstedt-Duke et al. found antibiotics including bacitracin, clindamycin and vancomycin resulted in altered mucin degradation in healthy human volunteers (82). The effect on mucin required 5 weeks before it was restored to pre-antibiotic levels, which may indicate the length of time that it takes for normal flora to recover from antibiotic exposure. The most abundant intestinal species (*Bacteroides*) does not possess the glycosidases necessary for mucin degradation and only 1% of normal flora species can degrade mucin. Hoskins et al. identified five strains of mucin oligosaccharide chain-degrading bacteria from healthy humans, of which three were *Ruminococcus* strains and two were *Bifidobacterium* strains (83). Mucin-degrading bacteria represent a distinct subset of normal flora with a specific function.

Stimulation of the immune system

Normal flora also induces the maturation of the gut-associated lymphoid system (GALT). The intestinal flora provides an array of antigenic stimulants to the GALT cells, affecting both local and systemic levels (22). Gnotobiotic mice have been shown to have fewer intraepithelial lymphocytes, plasma cells and Peyer's patches than mice with intact intestinal flora (78). When gnotobiotic mice are immunized, the only local immune response is secretory IgA, however, mice with intact intestinal flora also respond with IgM and IgG (40, 41). Intestinal flora may also be involved in the development of tolerance to antigens (42). Herias et al. gave germ-free rats *E. coli* alone or a mixture of *E. coli*, *Lactobacillus acidophilus* and a strain of an obligate anaerobe (*Peptostreptococcus*) and observed two effects (74). The peptostreptococci reduced translocation rates of *E. coli* and increased serum anti-*E. coli* antibodies. It may be that peptostreptococci act as an immune system primer to other bacterial antigens, thereby leading to a decrease in translocation. Further evidence that normal flora may act as an immune primer was found in a study using BALB/c mice. Pulverer et al. found that normal flora releases low molecular weight substances that interact with MALT (mucosa associated lymphoid tissue) and these substances appear to be essential for an adequate immune response (43). Antibiotic decontamination in a mouse model resulted in a decreased immune response. Thus, normal flora may have an important role as an immune system primer.

Intestinal transit

The presence of intestinal flora has been shown to stimulate peristalsis or otherwise involve the enteric nervous system (44, 45, 78). In gnotobiotic mice models, the small intestine has thinner walls and is smaller than in mice with intestinal flora (78). Husebye et al. studied germ-free rats (who have enlarged caeca) and found a slower progress of chyme through the intestinal tract when compared to rats with a normal microbiota (46). He found that the mechanism may be through the enteric nervous system, rather than stimulating motility by a direct action on intestinal smooth muscle. This may explain why colonic bacteria are

Table III

Geographical differences in intestinal microflora in humans

Microorganisms or groups of microorganisms	English/mixed Western diet (London)	Ugandan vegetarian diet (in London)	Americans/ mixed Western diet	Japanese/vegetarian diet	Japanese/mixed Western diet
Total anaerobes	10.1	9.3	10.2	9.9	11.5
Total aerobes	8.0	8.2	7.5	9.4–9.8	9.6
Facultative anaerobes				7.2*	4.8*
Enterococci	5.7*	7.0*	5.5	8.4	8.4
Bacteroides	9.7*	8.2*	9.8	10.1	11.1
Bifidobacteria	9.9*	9.3*	10.0	8.2	9.5
Lactobacilli	6.0*	7.2*	7.3	5.7	4.0
Clostridia	4.4–5.0	4.0–4.6	4.4	5.1–9.7	9.5
Yeasts	1.3	3.1*			

* Indicates a significant difference between groups as reported in the original publication (Adapted from reference 3).

able to influence transit through the small intestine in gnotobiotic mice. Pothoulakis et al. found that one intestinal response to *C. difficile* involved events relating to the neural cascade (84). The role of normal flora on the enteric nervous system requires further study and may have promise for therapeutic intervention by medications that inhibit the neural response.

Colonic physiology and maturation

It has been shown that the presence of intestinal flora stimulates the maturation and turnover rates in colonic epithelial cells. The surface layer of the mucosa in the intestines is replaced every 2–3 days, which allows basal stem cells to migrate up the crypt, presenting differentiated mature cells that are involved in nutrient absorption and mucin secretion. In germ-free mice (gnotobiotic), the rate of cell turnover was found to be significantly slower than mice with established microflora (78, 80). Bry et al. also found that normal flora induced enterocyte cell turnover (85).

FACTORS THAT INFLUENCE NORMAL FLORA

Age

Early infancy (< 2 years old) is a time of flux in the composition of the normal intestinal flora, as this is the time that the microecology is becoming established. The diet in pre-weaned babies is largely influenced by the diet (breast or formula fed) as previously discussed. Mitsuoka et al. characterized the *Lactobacillus* flora in four age ranges: infants < 7 months, children 4–6 years, adults 20–64 years and elderly 65–86 years old (86). Infants were found to usually be colonized by three types of resident lactobacilli, and in the older ages both number of different species and transient nature increased. Normal flora in older adults may also differ from younger adults. Postmenopausal women have been found to have increased numbers of fungi, clostridia and lactobacilli compared to women who are pre-menopausal (3). Other studies have shown variation of flora in the elderly, but whether this variation is due to age or medical exposures, or due to illnesses was unclear (87, 88).

Geography

There are numerous studies which report differences in normal flora depending upon geographical location. Benno et al. compared the fecal flora in elderly Japanese living in rural areas or urban centers (89). On the whole, the diversity and counts of fecal flora were similar. Urban Japanese were found to have significantly less *Bifidobacterium adolescentis*, but significantly more total anaerobic bacteria, bacilli and *Clostridium* spp. than rural Japanese. Benno et al. attributed these slight changes to a different diet (high fiber) in the rural population, but did not support this hypothesis with data on dietary patterns. Sepp et al. also cultured neonates at 1 week born in Estonia and Sweden (90). Estonian neonates had significantly higher counts of staphylococci (coagulase negative), enterococci and enterobacteri compared to Swedish neonates. Different feeding habits and differences in antibiotic use in these two countries was proposed to explain these differences, but further study is needed.

As shown in Table III, the composition of normal flora reported in geographical populations are not due to genetic differences as originally thought, but to differences in diet (3). For example, when intestinal flora is compared for English people living in London and eating a mixed western diet against Ugandans living in London and eating vegetarian diets, the English had more bfidobacteria and bacteroides but less enterococci, lactobacilli and yeasts than Ugandans. Vegetarian diets are associated with fewer anaerobes and higher counts of facultative and aerobic microbes (3). Generally, one individual has a fairly constant profile of microbes that they can consider as 'normal flora' unless the person is exposed to antibiotics or other factors that disrupt the flora, but inter-individual differences of normal flora may vary considerably.

The comparison of differences in normal flora for diverse geographic populations is complicated (and may be completely obscured) by vast differences in diet, culturing techniques, degree of sophisticated technology and different study populations. What is needed are studies which follow similar populations (same age range and sex), using identical microbiologic techniques and thoroughly documenting dietary constituents, in order to compare differences seen in normal flora of different countries.

Diet

Diet has been shown to change the flora in several animal studies, but there is little direct evidence from human studies other than studies with calcium, sugars or fiber manipulation (40). Dietary calcium precipitates cytotoxic substances such as bile acids resulting in less cytolysis. A decreased luminal cytotoxicity may help to reinforce endogenous flora. Bouvee-Oudenhoven et al. found when calcium supplemented diets were given to rats *Salmonella enteritidis* colonization was reduced as was translocation (91). Fructo-oligosaccharides (FOS), found in bananas, onions, asparagus and artichokes, are fermented mostly by *Bifidobacterium* species. Increased ingestion of FOS at doses of 4 g/d was found to increase levels of bifidobacteria in the intestines in human volunteers, but resulted in excessive flatus at doses over 20 g/day (92, 93). Buddington et al. also studied dietary fructo-oligosaccharides in 12 healthy volunteers (39). FOS was found to increase numbers of total anaerobes and bifidobacteria in this small study. Sucrose was found to increase *Bacteroides* species and inulin was found to increase mostly bifidobacteria (92). Different types of fiber have found to result in altered levels of bifidobacteria, lactobacilli and fungi in rats and pigs (94–96). Some types of fermentable fibers may support the growth of normal flora, yielding SCFA and decreased colonic pH, which can act to inhibit the growth of certain pathogens, such as *C. difficile* (95, 97). Tea polyphenols given to pigs resulted in increased levels of lactobacilli and decreased levels of bacteroides (98).

Evidence that diet influences normal flora in adults is sparse, but numerous studies have been done in young infants. Previous studies have shown that breast-fed infants have been found to harbor mostly bifidobacteria. In contrast, formula-fed infants are colonized with a wider variety of species, namely enterobacteria, bacteroides and clostridia (40). A recent study by Heavey and Rowland reported that high levels of bifidobacteria were seen in infants fed formula, but only for formulas that had a low buffering capacity (34). Rubaltelli et al. studied the effect of an adapted formula (high maltose) on the intestinal colonization of bifidobacteria compared to breast-fed infants (33). Breast-fed babies were again found to have more bifidobacteria (48%) than formula-fed (15%) infants, even as early as the fourth day of life. Unfortunately, controlled studies that document how well-defined diets can change normal flora have not been done.

Stress

Stress is frequently cited as a major influence on intestinal function, but the evidence for its impact on normal flora is limited. A frequently quoted reference to document the impact that daily stress has on normal flora may be misleading (99). This study was done with postoperative patients, so that the disruption of the normal flora may have been due to antibiotics or medications associated with the surgery itself and not due to stress (99). Studies linking stress and the Irritable Bowel Syndrome (IBS) often do not provide data on normal flora (100). Bochkov et al. studied the fecal flora of cosmonauts during training and space flight and concluded that stress affected the ability of the normal flora to mount effective colonization resistance (101). Stress may decrease hydrochloric acid production in the stomach allowing for the growth of coliforms and bacteroides (102). There is no direct evidence that stress causes a significant shift in normal flora populations.

Antibiotics

The effect of antibiotics on intestinal flora has been well studied, mainly because of the frequent occurrence of disease associated with antibiotic use. Antibiotics are often used to treat a specific illness without considering how the antibiotic will impact the normal flora (11). A common use of antibiotics is to decontaminate the intestines in preparation for intestinal surgery. As it was observed that the etiology of some post-surgical infections was of intestinal origin, it was thought that decontaminating the intestines with antibiotics might reduce the risk. However logical this tactic appeared it has not been productive, largely due to underestimating both the protective role of normal colonic flora and the profound disruptive impact of broad-spectrum antibiotics. Tetteroo et al. studied selective gut decontamination and found patients experienced 'rebound colonization' with potentially pathogenic organisms after surgery (103). Of 135 patients studied, 20 patients had rebound colonization with a nosocomial aerobic pathogen. Lingnau et al. studied 357 patients with trauma who had topical and gut decontamination using two mixtures of antibiotics and compared them to trauma patients who were not given mixtures of antibiotics (104). Treatment with the antibiotics did reduce colonic bacteria, but had no impact on the incidence of expected post-surgical infections (pneumonia, sepsis or organ failure). Unfortunately, the identification of the inhibited microbes was not performed. A follow-up study showed that gut decontamination did result in higher rates of oxacillin-resistant *Staphylococcus aureus* (105). Terg et al. studied four different dose regimens for ciprofloxacin in 29 patients with cirrhosis (106) but found no statistical differences by dose.

Most studies of antibiotics and their impact on flora have been directed at either the antibiotic's potential to kill

pathogenic bacteria (and not normal flora) or the influence antibiotics have on the development of antibiotic resistance. However, there are a few studies on the impact antibiotics have on normal flora. A summary of antibiotics by the impact on the normal flora is given in Table IV. Thijm et al. studied mice treated with ampicillin or epicillin or cephradine and observed the rates in which resistant strains of *E. coli* colonized as the measure of colonization resistance (107). Both penicillins resulted in a loss of colonization resistance to *E. coli*. Oral treatment with cephradine did not influence normal flora or effect colonization resistance. No direct measures on specific strains of normal flora were done.

Most of the studies of normal flora and antibiotics have been done using healthy volunteers. Barza et al. studied 20 healthy subjects, of whom 16 were given 4 antibiotics intravenously and 4 were given no antibiotics (108). Only those subjects exposed to antibiotics (7/16) were later found to be colonized with Gram-negative bacilli. Van Nispen et al. studied trovafloxacin on the effect on normal fecal flora in 19 healthy male subjects (109). In comparison with the seven men who received placebo, the 12 who received trovaloxacin had significantly reduced rates of enterobacteriaceae, but there were no differences in Gram-positive cocci, anaerobes or yeast populations. Vollaard et al. studied the effect of amoxycillin, erythromycin and roxithromycin on the colonization resistance in healthy volunteers (110). Amoxycillin decreased colonization resistance (evidenced by increased numbers of enterobacteriaceae and yeasts). Vollaard followed this study with another in 6 healthy volunteers when he defined impairment of colonization resistance by significant increase in the numbers of fecal yeasts, Gram-negative bacteria or by increased colonization by a 'challenge strain' (111). Thomakos et al. studied the effect of cefamandole, cefuroxime and cefoxitin on *Candida albicans* colonization in 28 surgical patients (112). Thomakos found that 10 days treatment with either three of these antibiotics resulted in increased numbers of *C. albicans* in the feces. Unfortunately, he did not study other types of normal flora. Van de Leur et al. suggested that before giving neutropenic patients ciprofloxacin to decontaminate the colon and reduce disease, the effect of low dose ciprofloxacin should be studied when the flora has been disturbed (by another antibiotic) in healthy subjects (113). Therefore, he then treated five healthy volunteers with ciprofloxacin for 20 days and then ciprofloxacin combined with clindamycin for 14 days. Fecal samples were analyzed for Gram-negative bacilli, enterococci, yeasts and antibiotic levels. Not surprisingly, the addition of a second antibiotic was found to dramatically increase the acquisition of Gram-negative bacilli. When low-dose ciprofloxacin was used alone, no increases in Gram-negative bacilli were noted. Edlund et al. studied 20 healthy human volunteers who received cefuroxime axetil (500 mg/d for 1 week) and ten of those who additionally received vancomycin (500 mg/d) for the following 7 days (114). Cefuroxime resulted in rapid decrease of anaerobic *Bifidobacterium* and *Lactobacillus* species, but increased the numbers of three species of *Enterococcus*. Vancomycin resulted in a rapid decline of *Enterococcus faecium*, *E. faecalis* and *E. durans*, *Bacteroides* and *Clostridium* species and a significant increase in vancomycin-resistant *Enterococcus gallinarum* and *E. casseliflavus*. Van der Auwera et al. also documented that 64% of human volunteers given vancomycin carried vancomycin-resistant enterococci as part of their intestinal flora (115).

These studies show clearly that antibiotics may disrupt normal intestinal flora and may predispose patients to disease from opportunistic pathogens. Recovery of the colonization resistance brought on by antibiotic exposure may take weeks to months. Hashimoto et al. exposed rats to 6 days of broad-spectrum antibiotics and found that, by the seventh day, fecal anaerobic levels decreased, bile acids

Table IV

Influence of antibiotics on normal flora from human volunteer studies

Antibiotic tested	Daily dose	Number of volunteers	Effect	Reference
Clindamycin	800 mg bid	10	↓ anaerobes	(136)
Erythromycin	500, 1000 mg, tid	12	↓ anaerobes, ↓ aerobic GNB, No effect on enterococci or GPB	(137)
Ciprofloxacin	50, 100, 200 mg bid	10	No significant effect by dose	(138)
Cefoxitin, cefoxitin or cefamandole	na	28*	↑ in yeasts	(112)
Ciprofloxacin	20 mg	5	↓GNB	(113)
Ciprofloxacin (and clindamycin)	(300mg)	5	↑ Cipro resistant GNB	
Trovafloxacin	200 mg bid	12	↑ *E. coli*	(109)

* in surgical patients.

Abbreviations: GNB = Gram-negative bacilli, GPB = Gram-positive bacilli, na = data not available, bid = twice a day, tid = three times a day.

decreased and the cecum enlarged in size (116). After antibiotics were stopped, fecal microbes recovered to their initial counts within a week, but the restoration of bile acid and cholesterol metabolism did not return to baseline until 3 weeks later. Unfortunately, no other measures of colonization resistance were made. Larson and Borriello found that the susceptibility to *C. difficile* in hamsters exposed to clindamycin was prolonged (74 days) compared to a relatively short period of susceptibility (2 days) if the hamsters were given ampicillin (117). Although many antibiotics have their activity tested against pathogenic organisms or on selected populations of normal flora, few studies have measured their impact directly on colonization resistance.

TOOLS AND MODELS

Selective culture techniques

Routine biochemical assays can identify specific species and these traditional methods are standard and well-known (118). However, the results will only quantitate culturable bacteria and cannot identify unknown types of microbes nor test microbial interactions or protective functions. Routine microbiological culturing will underestimate both the number and diversity of flora and in addition, frozen samples may result in less sensitive results (119).

Continuous flow cultures

In an attempt to control some of the interactive factors present in microecologies within various body sites, reliance upon continuous flow cultures has become established. Although the results may depend more on culture specifications than the microbial interactions, this technique is popular. Bernhardt et al. studied the growth of *C. albicans* using a continuous flow culture (120). This method exhibited a 'colonization resistance' effect on *C. albicans*. Macfarlane et al. used a three-stage compound continuous culture system to study normal flora (121). Stage 1 models for the proximal colon and Stages 2 and 3 model the distal colon. Metabolism, bacterial interactions and changes in the diet and environmental conditions may be studied using this system. Kontula et al. used SHIME (simulator of human intestinal microbial ecosystem) to study flora changes and observed changes in fatty acids and gas production and enterococci numbers (122). Continuous flow cultures have the advantage of being able to tightly control various factors (nutrient concentration, types of species, etc.) but whether the results can be extrapolated to the intact intestinal microecosystem is uncertain.

Serotyping, plasmid profiles, antibiotic resistance patterns

Monoclonal antibodies have been used to enumerate *Bacteriodes* species present in human fecal flora samples (123). These techniques do not require culturing, so less work is required, but these assays still cannot identify unknown species if there is no known biomarker. Corthier et al. also used luciferase gene biosensors to detect *Lactococcus lactis* (124). Serotyping and plasma profiles have been helpful to differentiate different strains of similar bacterial and fungal species. The most practical use for these techniques may be in the tracking of outbreaks. However, these techniques cannot quantitate levels of different microbes nor provide any functional measurements.

16 S ribosomal RNA typing

Cumulative databases allow placement of unknown species using 16S ribosomal RNA fingerprint techniques (125). This technique is applicable for both culturable and non-culturable microbes and is becoming increasing popular as a research tool. Use of polymerase chain reaction technologies are increasing, but it is costly. Dore et al. used 16S rRNA targeted oligonucleotide probe to detect and quantify *Bacteroides* species in human fecal samples (126). Franks et al. used 16S rRNA-target oligonucleotide probes to quantify predominant groups of anaerobic bacteria in human fecal samples (23). These probes were able to detect at least two-thirds of the anaerobic fecal flora.

Several researchers report good agreement between 16S rDNA probe identification and culturing techniques (32, 127, 128). But other researchers have noted DNA probes can detect more anaerobes than by culturing techniques (30, 129). Several advantages of the newer probe techniques are that frozen stools may be used, and non-cultivable species may be detected. Standard culturing has advantages of being inexpensive and widely available.

When 16S rDNA probes are used to characterize the species composition of a population, several sources of bias may arise. Bacterial strains may react differently to the processing required by this technique. Bacteria will be detected more frequently if they are susceptible to lysing, amplication and permeability (129).

Functioning analysis

The above probes all share the disadvantage of not guaranteeing that the species identified by the probe may not be the microbe responsible for a specific microbial associated function. Also, as colonization resistance results from the interactions of many microbes, the above probes do not measure these interactions. Measuring the function of the target organ as a whole corrects for this disadvantage. Collinder et al. and others have suggested using MACs (Microflora-Associated Characteristics) to study colonization resistance (36–38, 130). MACs include: degradation of mucin, conversion of cholesterol to coprostanol, bilirubin to urobilinogens (to study hepatic-intestinal interactions), inactivation of tryptic activity (to study pancreatic-intestinal interactions), degradation of b-aspartylglycine (found in disturbed flora) and production of SCFA. Several types of indicators of functions can be

measured, including bacterial enzymes and bacterial metabolite levels. The metabolite levels include NH_3 levels (breakdown product of protein and urea), phenols and cresols (amino acid catabolism), and the breath hydrogen test (118, 131).

Animal models

Gnotobiotic models have been used to test normal flora and disruptive factors (132, 133). Wilson et al. used gnotobiotic mice to test the ability of hamster flora for the protection for *C. difficile* and *E. coli* (134). Segmented filamentous bacteria restored diffuse GALT in gnotobiotic animals (80). Bourlioux et al. used gnotobiotic animals to study colonization resistance to *C. perfringens* linked to a *Ruminococcus* species and colonization resistance to *C. difficile* linked to mucin interactions (76).

Mouse models with normal flora are typically used by first shocking the intestine with a dose of antibiotics, then testing with various microbial species. Sweeney et al. shocked mice with streptomycin and then exposed them to a human fecal isolate of *E. coli* F18 (68). He found that *E. coli* F18 occupies a distinct niche in the large intestine (defined by the presence of gluconate). Animal models have the advantage of studying flora *in situ*, but are less easy to control, can be expensive and the results may not be typical of the human microecosystem.

CONCLUSIONS AND FUTURE CHALLENGES

'Normal flora' is a dynamic ecosystem with a wide variety of microbes and functions. These functions provide one of the more powerful sentinels against infection by opportunistic pathogens and enable the body to function efficiently. The disruption of normal microecology by a wide variety of factors may have dire consequences. Colonization by multiple bacterial species makes it difficult to distinguish specific contributions by individual species to symbiotic interactions. Most of the indigenous species are obligate anaerobes and are difficult to culture and identify and quantitate. The development of more precise tools and models will aid our understanding of this complex bionetwork.

The challenge with studies on normal flora is that it exists as a complex and diverse system that may be difficult to sample and impossible to identify every microbial species. Intestinal normal flora are composed of a mixture of identifiable bacteria, fungi and viruses, but there are many non-cultivable organisms. For over twenty years, it has also been acknowledged that microbiologic studies of flora from stool samples may not accurately reflect the flora or metabolic state inside the intestinal tract (2). Microbial flora in the feces represent only those microbes who are not attached to mucin or the mucosa and have survived intestinal digestion, transit and dehydration processes. Metabolic activity and species composition also differ for the distal and proximal segments of the intestine. Marteau et al. overcame this difficulty by sampling jejunal flora directly by using a lumen tube with a proximal occluding balloon (135). This allows sampling of the flora at the desired location within the intestine. However, this technique is invasive and not as simple as culturing feces, but is well tolerated by subjects. The use of selective media and polymerase chain reactions (PCR) probes of 16S rDNA have enhanced the detection of fastidious organisms and should provide valuable assistance to future studies of normal flora.

REFERENCES

1. Gordon R. The alarming history of medicine. New York: St. Martin's Press, 1993: 164.
2. Savage DC. Microbial ecology of the gastrointestinal tract. Annu Rev Microbiol 1977; 31: 107–33.
3. Salminen S, Isolauri E, Onnela T. Gut flora in normal and disordered states. Chemotherapy 1995; 41: 5–15.
4. Keane FE, Ison CA, Taylor-Robinson D. A longitudinal study of the vaginal flora over a menstrual cycle. Int J STD AIDS 1997; 8: 489–94.
5. Martin HL Jr, Richardson BA, Nyange PM, Lavreys L, Hillier SL, Chohan B, Mandaliya K, Ndinya-Achola JO, Bwayo J, Kreiss J. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J Infect Dis 1999; 180: 1863–8.
6. Marshall JC. Gastrointestinal flora and its alterations in critical illness. Curr Opin Clin Nutr Metab Care 1999; 2: 405–11.
7. Priestley CJ, Jones BM, Dhar J, Goodwin L. What is normal vaginal flora? Genitourin Med 1997; 73: 23–8.
8. Hooper LV, Bry L, Falk PG, Gordon JI. Host-microbial symbiosis in the mammalian intestine: exploring an internal ecosystem. BioEssays 1998; 20: 336–43.
9. Hentges DJ. The anaerobic microflora of the human body. Clin Infect Dis 1993; 16: S175–80.
10. Hagiage M. La flora intestinale de l'équilibre au déséquilibre. Paris: Vigot, 1994: 25.
11. Vollaard EJ, Clasener HAL, van Saene HKF, Muller NF. Effect on colonization resistance: an important criterion in selecting antibiotics. DICP 1990; 24: 60–6.
12. Reid G, Bruce AW, McGroarty JA, Cheng K-J, Costerton JW. Is there a role for lactobacilli in prevention of urogenital and intestinal infections? Clin Microbiol Rev 1990; 3: 335–44.
13. Hillier SL. The role of naturally occurring and exogenously supplied lactobacilli in the human vagina. Microbial Ecology in Health and Disease 1998; 10: 193.
14. Listgarten MA. Structure of the microbial flora associated with periodontal health and disease in man., A light and electron microscopic study. J Periodontol 1976; 47: 1–18.
15. Stjernquist-Desatnik A, Holst E. Tonsillar microbial flora: comparison of recurrent tonsillitis and normal tonsils. Acta Otolaryngol 1999; 119: 102–6.
16. Gagliardi D, Makihara S, Corsi PR, Viana A, de T, Wiczer MV, Nakakubo S, Mimica LM. Microbial flora of the normal esophagus. Dis Esophagus 1998; 11: 248–50.
17. Marushko IuV. The colonization resistance of the tonsils in healthy and frequently ill children. Lik Sprava 1998; 1: 115–7.

18. Ahrne S, Nobaek A, Jeppsson B, Adlerberth I, Wold AE, Molin G. The normal Lactobacillus flora of healthy human rectal and oral mucosa. J Appl Microbiol 1998; 86: 88–94.
19. Smith VC, Genta RM. Role of *Helicobacter pylori* gastritis in gastric atrophy, intestinal metaplasia, and gastric neoplasia. Microsc Res Tech 2000; 48: 313–20.
20. Quartero AO, Numans ME, de Melker RA, de Wit NJ. In-practice evaluation of whole-blood *Helicobacter pylori* test: its usefulness in detecting peptic ulcer disease. Br J Gen Pract 2000; 50: 13–6.
21. Parente F, Cucino C, Bollani S, Imbesi V, Maconi G, Bonetto S, Vago L, Porro GB. Focal gastric inflammatory infiltrates in inflammatory bowel diseases: prevalence, immunohistochemical characteristics, and diagnostic role. Am J Gastroenterol 2000; 95: 705–11.
22. Salminen S, Bouley C, Boutron-Ruault M-C, Cummings JH, Franck A, Gibson GR, Isolauri E, Moreau M-C, Roberfroid M, Rowland I. Functional food science and gastrointestinal physiology and function. Br J Nutr 1998; 80: S147–71.
23. Franks AH, Harmsen HJM, Raangs GC, Jansen GJ, Schut F, Welling GW. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. Appl Environ Microbiol 1998; 64: 3336–45.
24. Schwebke JR, Richey CM, Weiss HL. Correlation of behaviors with microbiological changes in vaginal flora. J Infect Dis 1999; 180: 1632–6.
25. Rosenstein IJ, Stafford MK, Kitchen VS, Ward H, Weber JN, Taylor-Robinson D. Effect on normal vaginal flora of three intravaginal microbicidal agents potentially active against human immunodeficiency virus type 1. J Infect Dis 1998; 177: 1386–90.
26. Collins MD, Gibson GR. Probiotics, prebiotics and synbiotics: approaches for modulating the microbial ecology of the gut. Am J Clin Nutr ; : S 1999; 69: 1052S–7.
27. Karvonen AV, Vesikari T, Casas IA. *Lactobacillus* and *Lactobacillus reuteri*: mother-child vinculum at birth and during early age of infants. Microbial Ecology in Health and Disease. 1998; 10: 204.
28. Benno Y, Sawada K, Mitsuoka T. The intestinal microflora of infants: composition of fecal flora in breast-fed and bottle-fed infants. Microbiol Immunol 1984; 28: 975–86.
29. Bezirtzoglou E, Romond C. Effect of the feeding practices on the establishment of bacterial interactions in the intestine of the newborn delivered by cesarian section. J Perinat Med 1989; 17: 139–43.
30. Harmsen HJ, Gibson GR, Elfferich P, Raangs GC, Wildeboer-Veloo AC, Argaiz A, Roberfroid MB, Welling GW. Comparison of viable cell counts and fluorescence in situ hybridization using specific rRNA-based probes for the quantification of human fecal bacteria. FEMS Microbiol Lett 2000; 183: 125–9.
31. McFarland LV, Surawicz CM, Greenberg RN, Bowen KE, Melcher SA, Mulligan ME. Possible role of cross-transmission between neonates and mothers with recurrent *Clostridium difficile* infections. AJIC Am J Infect Control 1999; 27: 301–3.
32. Harmsen HJM, Wildeboer-Veloo ACM, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, Welling GW. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr 2000; 30: 61–7.
33. Rubaltelli FF, Biadaioli R, Pecile P, Nicoletti P. Intestinal flora in breast- and bottle-fed infants. J Perinat Med 1998; 26: 186–91.
34. Heavey PM, Rowland IR. The gut microflora of the developing infant: microbiology and metabolism. Microbial Ecology in Health and Disease 1999; 11: 75–83.
35. Ducluzeau R. Development, balance and role of the microbial flora of the newborn. Annales Pediatriques (Paris) 1993; 40: 13–22.
36. Norin KE, Gustafsson BE, Lindblad BS, Midtvedt T. The establishment of some microflora associated biochemical characteristics in feces from children during the first years of life. Acta Paediatr Scand 1985; 74: 207–12.
37. Midtvedt AC, Carlstedt-Duke B, Norin KE, Saxerholt H, Midtvedt T. Development of five metabolic activities associated with the intestinal microflora of health infants. J Pediatr Gastroenterol Nutr 1988; 7: 559–67.
38. Gustafsson A, Berstad A, Lund-Tonnesen S, Midtvedt T, Norin E. The effect of faecal enema on five microflora-associated characteristics in patients with antibiotic-associated diarrhoea. Scand J Gastroenterol 1999; 34: 580–6.
39. Buddington RK, Williams CH, Chen SC, Witherly SA. Dietary supplement of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects. Am J Clin Nutr 1996; 63: 709–16.
40. Rolfe RD. Colonization resistance. In: Mackie RI, White BA, Isaacson RE, eds. Gastrointestinal microbiology, vol 2. New York: Chapman & Hall, Inc., 1997: 501–36.
41. Moreau MC, Bisetti N, Dubuquoy C. Immunomodulating properties of a strain of *Bifidobacterium* used as probiotic on the faecal and cellular intestinal IgA anti-rotavirus responses in mice. In: Functional Foods Proceedings. Royal Society of Chemistry, 1998.
42. Moreau M, Gaboriau-Routhiau V. The absence of gut flora, the doses of antigen ingested and aging affect the long-term peripheral tolerance induced by ovalbumin feeding in mice. Research Immunology 1996; 147: 49–59.
43. Pulverer G, Ko HL, Beuth J. Microflora-associated defense stimulating factors. Scand J Gastroenterol 1997; 32: 107–11.
44. Guandalini S. Enteric nervous system: intestinal absorption and secretion. J Pediatr Gastroenterol Nutr 1997; 25: S5–6.
45. Midtvedt T. Gastro-intestinal motility. In: Heidt PJ, Rusch V, Van der Waaij D, eds. Old Herborn University Seminar Monograph, Vol 9. Herborn-Dill: Inst Microecology & Biochem, 1997: 1–7.
46. Husebye E. The stimulatory influence of the intestinal microflora on gastro-intestinal motility and myoelectric activity of small intestine. In: Heidt PJ, Rusch V, Van der Waaij D, eds. Old Herborn University Seminar Monograph, Vol 9. Herborn-Dill: Inst Microecology & Biochem, 1997: 41–52.
47. Conway PL. Microbial ecology of the human large intestine. In: Gibson GR, Macfarlane GT, eds. Human colonic bacteria: role in nutrition, physiology, and pathology. Boca Raton, FL: CRC Press, 1995: 1–24.
48. Buts J-P, Bernasconi P, Vaerman J-P, Dive C. Stimulation of secretory IgA and secretory component of immunoglobulins in small intestine of rats treated with *Saccharomyces boulardii*. Dig Dis Sci 1990; 36: 251–6.
49. Buts J-P, de Keyser N, de Raedemaeker L. *Saccharomyces boulardii* enhances rat intestinal enzyme expression by endoluminal release of polyamines. Pediatr Res 1994; 36: 522–7.
50. Cummings JH. Short chain fatty acids. In: Gibson GR, Macfarlane GT, eds. Human colonic bacteria: role in nutrition, physiology, and pathology. Boca Raton, FL: CRC Press, 1995: 101–30.
51. Clausen MR, Bonnen H, Tvede M, Mortensen PB. Colonic fermentation to short-chain fatty acids is decreased in antibiotic-associated diarrhea. Gastroenterology 1991; 101: 1497–504.

52. Treem WR, Ahsan N, Kastoff G, Hyams JS. Fecal short-chain fatty acids in patients with diarrhea-predominant irritable bowel syndrome: in vitro studies of carbohydrate fermentation. J Pediatr Gastroenterol Nutr 1996; 23: 280–6.
53. Sidhu H, Holmes RP, Allison MJ, Peck AB. Direct quantification of the enteric bacterium *Oxalobacter formigenes* in human fecal samples by quantitative competitive-template PCR. J Clin Microbiol 1999; 37: 1503–9.
54. Elkins CA, Savage DC. Identification of genes coding conjugated bile salt hydrolase and transport in *Lactobacillus johnsonii* 100-100. J Bacteriol 1998; 80: 4344–9.
55. Wilson KH. The microecology of *Clostridium difficile*. Clin Infect Dis 1993; 16: S214–8.
56. Apperloo-Renkema HZ, van der Waaij BD, van der Waaij D. Determination of colonization resistance of the digestive tract by biotyping of Enterobacteriaceae. Epidemiol Infect 1990; 105: 355–61.
57. Hazenberg MP, Bakker M, Verschoor-Burggraaf A. Effects of the human intestinal flora on germ-free mice. J Appl Bacteriol 1981; 50: 95–106.
58. Nord CE, Kager L. The normal flora of the gastrointestinal tract. Neth J Med 1984; 27: 249–52.
59. Freter R, Abrams GD. Function of various intestinal bacteria in converting germfree mice to the normal state. Infect Immun 1972; 6: 119–26.
60. Romond MB, Haddou Z, Mielcareck C, Romond C. Bifidobacteria and human health: regulatory effect of indigenous bifidobacteria on *Escherichia coli* intestinal colonization. Anaerobe 1997; 3: 131–6.
61. McFarland LV. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. Dig Dis 1998; 16: 292–307.
62. Borriello SP. Pathogenesis of *Clostridium difficile* infection. J Antimicrob Chemother 1998; 41: 13–9.
63. Guerrant RL, Steiner TS, Lima AAM, Bobak DA. How intestinal bacteria cause disease. J Infect Dis 1999; 179: S331–7.
64. Fekety R. Guidelines for the diagnosis and management of *Clostridium difficile*-associated diarrhea and colitis. Am J Gastroenterol 1997; 92: 739–50.
65. Boman HG. Gut microflora. ASM News 2000; 66: 57.
66. Naidu AS, Bidlack WR, Clemens RA. Probiotic spectra of lactic acid bacteria (LAB). Critical Reviews in Food Science and Nutrition 1999; 38: 13–126.
67. Wilson KH, Perini F. Role of competition for nutrients in suppression of *Clostridium difficile* by the colonic microflora. Infect Immun 1988; 56: 2610–4.
68. Sweeney NJ, Klemm P, McCormick BA, MollerNielsen E, Utley M, Schembri MA, Laux DC, Cohen PS. The *Escherichia coli* K-12 gntP gene allows *E. coli* F-18 to occupy a distinct nutritional niche in the streptomycin-treated mouse large intestine. Infect Immun 1996; 64: 3497–503.
69. Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C. *Saccharomyces boulardii* protease inhibits the effects of *Clostridium difficile* toxins A and B in human colonic mucosa. Infect Immun 1999; 67: 302–7.
70. Just I, Fritz G, Aktories K, Giry M, Popoff MR, Boquet P, Hegenbarth S, von Eichel-Streiber C. *Clostridium difficile* toxin B acts on the GTP-binding protein Rho. Journal of Biological Chemistry 1994; 269: 10706–12.
71. Fuller R. Probiotics 2. Applications and practical aspects. London: Chapman & Hall. 1997: 21.
72. Giuliano M, Barza M, Jacobus NV, Gorbach SL. Effects of broad-spectrum parenteral antibiotics on composition of intestinal microflora of humans. Antimicrob Agents Chemother 1987; 31: 202–6.
73. Leonard F, Andremont A, Leclerq B, Labia R, Tancrede C. Use of β-lactamase-producing anaerobes to prevent ceftriaxone from degrading intestinal resistance to colonization. J Infect Dis 1989; 160: 274–80.
74. Herias MV, Midtvedt T, Hanson LA, Wold AE. Increased antibody production against gut-colonizing *Escherichia coli* in the presence of the anaerobic bacterium Peptostreptococcus. Scand J Immunol 1998; 48: 277–82.
75. Boureau H, Decre D, Carlier JP, Guichet C, Bourlioux P. Identification of a *Clostridium cocleatum* strain involved in an anti-*Clostridium difficile* barrier effect and determination of its mucin-degrading enzymes. Res Microbiol 1993; 144: 405–10.
76. Bourlioux P. What is currently known about the molecular mechanisms of colonisation resistance. Anaerobe 1997; 3: 179–84.
77. Wong WC, Hentges DJ, Dougherty SH. Adequacy of the human faecal microbiota associated mouse as a model for studying the ecology of the human intestinal tract. Microbial Ecology in Health and Disease 1996; 9: 187–98.
78. Rolfe RD. Interactions among microorganisms of the indigenous intestinal flora and their influence on the host. Rev Infect Dis 1984; 6: S73–9.
79. Silverman H, Romano J, Elmer G. The vitamin book. New York: Bantam Books, 1999.
80. Umesaki Y, Okada Y, Matsumoto S, Imaoka A, Setoyama H. Segmented filamentous bacteria are indigenous intestinal bacteria that activate intraepithelial lymphocytes and induce MHC class II molecules and fucosyl asialo GM1 glycolipids on the small intestinal epithelial cells in the ex-germ-free mouse. Microbiol Immunol 1995; 39: 555–62.
81. Kindon H, Pothoulakis C, Thim L, Lynch-Devaney K, Podolsky DK. Trefoil peptide protection of intestinal epithelial barrier function: cooperative interaction with mucin glycoprotein. Gastroenterology 1995; 109: 516–23.
82. Carlstedt-Duke B, Hoverstad T, Lingaas E, Norin KE, Saxerholt H, Steinbakk M, Midtvedt T. Influence of antibiotics on intestinal mucin in healthy subjects. Eur J Clin Microbiol 1986; 5: 634–8.
83. Hoskins LC, Agustines M, McKee WB, Boulding ET, Kriaris M, Niedermeyer G. Mucin degradation in human colon ecosystems. J Clin Invest 1985; 75: 944–53.
84. Pothoulakis C, Castagliuolo I, LaMont JT. Nerves and intestinal mast cells modulate responses to enterotoxins. News Physiol Sci 1998; 13: 58–63.
85. Bry L, Falk PG, Midvedt T, Gordon J. A model for host-microbial interactions in an open mammalian ecosystem. Science 1996; 273: 1380–3.
86. Mitsuoka T, Hayakawa K, Kimura N. Die faekalflora bei menschen. III. Mitteilung: die zusammensetzung der laktobazillenflora der verschiedenen altersgruppen. Zentralbl Bakteriol Orig A 1975; 232: 499–511.
87. Valenti WM, Trudell RG, Bentley ND. Factors predisposing to oropharyngeal colonization with gram-negative bacilli in the aged. N Engl J Med 1978; 298: 1108–11.
88. McFarland LV, Surawicz CM, Stamm WE. Risk factors for *Clostridium difficile* carriage and *C. difficile*-associated diarrhea in a cohort of hospitalized patients. J Infect Dis 1990; 162: 678–84.
89. Benno Y, Endo K, Mizutani T, Namba Y, Komori T, Mitsuoka T. Comparison of fecal microblora of elderly persons in rural and urban areas of Japan. Appl Environ Microbiol 1989; 55: 1100–5.
90. Sepp E, Loivukene K, Koljalg S, Naaber P, Mikelsaar M, Bjorksten B. Comparison of intestinal microflora of Estonian and Swedish neonates. Microbial Ecology in Health and Disease 1998; 10: 203.

91. Bouvee-Oudenhoven IM, Termont DS, Weerkamp AH, Faassen-Peters MA, Van der Meer R. Dietary calcium inhibits the intestinal colonization and translocation of Salmonella in rats. Gastroenterology 1997; 113: 550–7.
92. Gibson GR. Dietary modulation of the human gut microflora using prebiotics. Br J Nutr 1998; 80: S209–12.
93. Bouhnik Y, Vahedi K, Achour L, Attar A, Salfati J, Pochart P, Marteau P, Flourie B, Bornet F, Rambaud J-C. Short-chain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. J Nutr 1999; 129: 113–6.
94. Kuda T, Goto H, Yokoyama M, Fujii T. Fermentable dietary fiber in dried products of brown algae and their effects on cecal microflora and levels of plasma lipid in rats. Fisheries Science 1998; 64: 582–8.
95. May T, Mackie RI, Fahey GC Jr, Cremin JC, Garleb KA. Effect of fiber source on short-chain fatty acid production and on the growth and toxin production by *Clostridium difficile*. Scand J Gastroenterol 1994; 29: 916–22.
96. Elmer GW, Martin SW, Horner KL, McFarland LV, Levy RH. Survival of *Saccharomyces boulardii* in the rat gastrointestinal tract and effects of dietary fiber. Microbial Ecology in Health and Disease 1999; 11: 29–34.
97. Ward PB, Young GP. Dynamics of *Clostridium difficile* infection. Control using diet. Adv Exp Med Biol 1997; 412: 63–75.
98. Hara H, Orita N, Hatano S, Ichikawa H, Hara Y, Matsumoto N, Kimura Y, Terada A, Mitsuoka T. Effect of tea polyphenols on fecal flora and fecal metabolic products of pigs. J Vet Med Sci 1995; 57: 45–9.
99. Dal Nogare AR, Toews GB, Pierce AK. Increased salivary elastase precedes gram-negative bacillary colonization in postoperative patients. Am Rev Respir Dis 1987; 135: 671–5.
100. Drossman DA, McKee DC, Sandler RS, Mitchell CM, Cramer EM, Lowman BC, Burger AL. Psychosocial factors in the irritable bowel syndrome. A multivariate study of patients and nonpatients with irritable bowel syndrome. Gastroenterology 1988; 95: 701–8.
101. Bochkov IA, Lizko NN, Semina NA, Borunov NL, Lurko LP. Peculiarities of cosmonauts fecal flora. Aviakosm Ekolog Med 1998; 32: 25–8.
102. Lidbeck A, Nord CE. Lactobacilli and the normal human anaerobic microflora. Clin Infect Dis 1993; 16: S181–7.
103. Tetteroo GW, Wagenvoort JH, Bruining HA. Bacteriology of selective decontamination: efficacy and rebound colonization. J Antimicrob Chemother 1994; 34: 139–48.
104. Lingnau W, Berger J, Javorsky F, Lejeune P, Mutz N, Benzer H. Selective intestinal decontamination in multiple trauma patients: prospective, controlled trial. J Trauma 1997; 42: 687–94.
105. Lingnau W, Berger J, Javorsky F, Fille M, Allerberger F, Benzer H. Changing bacterial ecology during a five year period of selective intestinal decontamination. J Hosp Infect 1998; 39: 195–206.
106. Terg R, Llano K, Cobas SM, Brotto C, Barrios A, Levi D, Wasen W, Bartellini MA. Effects of oral ciprofloxacin on aerobic gram-negative fecal flora in patients with cirrhosis: results of short- and long-term administration, with daily and weekly dosages. J Hepatol 1998; 29: 437–42.
107. Thijm HA, van der Waaij D. The effect of three frequently applied antibiotics on the colonization resistance of the digestive tract of mice. J Hyg (Lond) 1979; 82: 397–405.
108. Barza M, Giuliano M, Jacobus NV, Gorbach SL. Effect of broad-spectrum parenteral antibiotics on 'colonization resistance' of intestinal microflora of humans. Antimicrob Agents Chemother 1987; 31: 723–7.
109. Van Nispen CHM, Hoepelman AIM, Rozenberg-Arska M, Verhoef J, Purkins L, Willavize SA. A double-blind, placebo-controlled, parallel group study of oral trovafloxacin on bowel microflora in healthy male volunteers. Am J Surg ; : S 1998; 176: 27S–31.
110. Vollaard EJ, Clasener HA, van Griethuysen AJ, Janssen AJ, Sanders-Reijmers AJ. Influence of amoxycillin, erythromycin and roxithromycin on colonization resistance and on appearance of secondary colonization in healthy volunteers. J Antimicrob Chemother 1987; 20: 131–8.
111. Vollaard EJ, Clasener HA, Janssen AJ. Co-trimoxazole impairs colonization resistance in healthy volunteers. J Antimicrob Chemother 1992; 30: 685–91.
112. Thomakos N, Maraki S, Liakakos T, Macheras A, Kanavaki S, Marinis E, Sehas M, Margioris AN, Samonis G. Effect of cefamandole, cefuroxime and cefoxitin on yeast fecal flora of surgical patients. Chemotherapy 1998; 44: 324–7.
113. Van de Leur JJJPM, Vollaard EJ, Janssen AJHM, Dofferhoff ASM. Influence of low dose ciprofloxacin on microbial colonization of the digestive tract on healthy volunteers during normal and during impaired colonization resistance. Scand J Infect Dis 1997; 29: 297–300.
114. Edlund C, Barkholt L, Olsson-Liljequist B, Nord CE. Effect of vancomycin on intestinal flora of patients who previously received antimicrobial therapy. Clin Infect Dis 1997; 25: 729–32.
115. Van der Auwera P, Pensart N, Korten V, Murray BE, Leclercq R. Influence of oral glycopeptides on the fecal flora of human volunteers: selection of highly glycopeptide-resistant enterococci. J Infect Dis 1996; 173: 1129–36.
116. Hashimoto S, Igimi H, Uchida K, Satoh T, Benno Y, Takeuchi N. Effects of beta-lactam antibiotics on intestinal microflora and bile acid metabolism in rats. Lipids 1996; 31: 601–9.
117. Larson HE, Borriello SP. Quantitative study of antibiotic-induced susceptibility to *Clostridium difficile* enterocecitis in hamsters. Antimicrob Agents Chemother 1990; 34: 1348–53.
118. Papasouliotis K, Sparkes AH, Gruffydd-Jones TJ, Cripps PJ, Harper EJ. Use of the breath hydrogen test to assess the effect of age on orocecal transit time and carbohydrate assimilation in cats. Am J Vet Res 1998; 59: 1299–302.
119. Bonten MJM, Nathan C, Weinsein RA. Recovery of nosocomial fecal flora from frozen stool specimens and rectal swabs. Comparison of preservatives for epidemiological studies. Diagn Microbiol Infect Dis 1997; 27: 103–6.
120. Bernhardt H, Wellmer A, Zimmermann K, Knoke M. Growth of *Candida albicans* in normal and altered faecal flora in the model of continuous flow culture. Mycoses 1995; 38: 265–70.
121. Macfarlane GT, Macfarlane S, Gibson GR. Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon. Microb Ecol 1998; 35: 180–7.
122. Kontula P, Jaskari J, Nollet L, De Smet I, von Wright A, Poutanen K, Mattila-Sandholm T. The colonization of a simulator of the human intestinal microbial ecosystem by a probiotic strain fed on a fermented oat bran product: effects on the gastrointestinal microbiota. Appl Microbiol Biotechnol 1998; 50: 246–52.
123. Corthier G, Muller MC, L'Haridon R. Selective enumeration of *Bacteroides vulgatus* and *B. distasonis* organisms in the predominant human fecal flora by using monoclonal antibodies. Appl Environ Microbiol 1996; 62: 735–8.

124. Corthier G, Delorme C, Ehrlich SD, Renault P. Use of luciferase genes as biosensors to study bacterial physiology in the digestive tract. Appl Environ Microbiol 1998; 64: 2721–2.
125. Bergeron MG, Ouellette M. Preventing antibiotic resistance through rapid genotypic identification of bacteria and of their antibiotic resistance genes in the clinical microbiology laboratory. J Clin Microbiol 1998; 36: 2169–72.
126. Dore J, Sghir A, HannequartGramet G, Corthier G, Pochart P. Design and evaluation of a 16S rRNA-targeted oligonucleotide probe for specific detection and quantitation of human faecal Bacteroides populations. Systematic and Applied Microbiology 1998; 21: 65–71.
127. Langendijk PS, Schut F, Jansen GJ, Raangs GC, Kamphuis GR, Wilkinson MHF, Welling GW. Quantitative fluorescence *in situ* hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. Appl Environ Microbiol 1995; 61: 3069–75.
128. Wilson KH, Blitchington RB. Human colonic biota studied by ribosomal DNA sequence analysis. Appl Environ Microbiol 1996; 62: 2273–778.
129. Welling GW, Elfferich P, Raangs GC, Wildeboer-Veloo AC, Jansen GJ, Degener JE. 16S ribosomal RNA-targeted oligonucleotide probes for monitoring of intestinal tract bacteria. Scand J Gastroenterol Suppl 1997; 222: 17–9.
130. Collinder E, Lindholm A, Midtvedt T, Norin E. The usefulness of microbial-associated characteristics (MACs) for studies of microbial intestinal functions in different species. Microbial Ecology in Health and Disease 1998; 10: 203–4.
131. Goldin BR, Gorbach SL. The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. Am J Clin Nutr 1984; 39: 756–61.
132. Onderdonk AB, Cisneros RL, Bartlett JG. *Clostridium difficile* in gnotobiotic mice. Infect Immun 1980; 28: 277–82.
133. Umesaki Y, Sakata T, Yajima T. Abrupt induction of GDP-fucose:asialo GM1 fucosyltransferase in the small intestine after conventionalization of germ-free mice. Biochem Biophys Res Commun 1982; 105: 439–43.
134. Wilson KH, Sheagren JN, Freter R, Weatherbee L, Lyerly D. Gnotobiotic models for study of the microbial ecology of *Clostridium difficile* and *Escherichia coli*. J Infect Dis 1986; 153: 547–51.
135. Marteau P, Vaerman J-P, Dehennin J-P, Bord S, Brassart D, Pochart P, Desjeux J-F, Rambaud J-C. Effects of intrajejunal perfusion and chronic ingestion of *Lactobacillus johnsonii* strain La1 on serum concentrations and jejunal secretions of immunoglobulins and serum proteins in healthy humans. Gastroenterol Clin Biol 1997; 21: 293–8.
136. Heimdahl A, Nord CE. Effect of phenoxymethylpenicillin and clindamycin on the oral, throat and faecal microflora of man. Scand J Infect Dis 1979; 11: 233–42.
137. Kaukoranta-Tolvanen S-S, Renkonen O-V, Gordin A, Tamm L, Mannisto PT. Effect of erythromycin acistrate and erythromycin stearate on human colonic microflora. Scand J Infect Dis 1989; 21: 717–20.
138. Meijer-Severs GJ, van Santen E, de Vries-Hospers HG. Low-dose ciprofloxacin for selective decontamination of the digestive tract in human volunteers. Eur J Clin Microbiol Infect Dis 1990; 9: 285–7.