Probiotics: Is There a Role for Routine Stool Cultures?

INTRODUCTION
The human gastrointestinal tract contains a large, complex, mostly unknown microbial population. Over 90% of the microorganisms known to be present by 16S rRNA sequencing cannot be cultured. Fecal cultures primarily reflect cultivable microorganisms predominantly in the descending colon and rectum. They do not accurately reflect the microbial populations in the cecum and distal small bowel. The normal intestinal microbiota consists almost entirely of obligate anaerobes. Accurate stool culture assessment of normal fecal flora requires meticulous anaerobic techniques and rapid processing. Fecal culture is a poor guide for evaluating the need for probiotic supplementation. Stool cultures are inadequate for the assessment of probiotic bowel colonization and therefore have little or no role in the selection and assessment of probiotics for supplementation.

THE NORMAL MICROFLORA - Known & Unknown
The intestines are home to a complex and dynamic assemblage of microorganisms. The normal human gastrointestinal microbiota is the most densely populated ecosystem known to science. Of the estimated 800 microbial species in the human gut, 30 to 40 make up 99% of the entire microbial population. Obligate anaerobes from the genera Bacteroides, Bifidobacterium, and Clostridium constitute the largest segment of the normal human microbiota. Lactobacillus species contribute an intermediate percentage while Enterobacteriaceae, staphylococci, enterococci, coliforms, yeasts and molds account for smaller numbers. While the cultivability of gastrointestinal microorganisms has traditionally been estimated as high, between 10 and 50%, it is becoming clear that a significant percentage of gastrointestinal microflora has yet to be cultured. In 2005, the National Institutes of Health GenBank® genetic sequence database held 1822 sequences of bacterial ribosomal RNA (16S rRNA) originating from the human gut; 1689 or 92.7% derived from uncultured microorganisms. Most of the normal gastrointestinal microbiota is a vast unknown that cannot be cultured by current techniques. The role of stool cultures in the understanding of gastrointestinal microflora appears to be very limited.

STOOL CULTURES & UNDERSTANDING NORMAL MICROFLORA
Although cultures of fecal material have been traditionally used to assess the gastrointestinal microflora, they have significant limitations to a full understanding of the bowel microbiology. Stool cultures at best reflect only the microbial populations present in the feces that can be cultured by current anaerobic and standard techniques. Stool cultures cannot reflect the microecology of the stomach, duodenum, and jejunum. The clinical data are conflicting as to whether fecal samples accurately reflect colon microbial populations. Culture-dependent studies of the luminal contents sampled from different parts of the colon following sudden death found that the cecal pH is much lower than in the distal colon. The production of short-chain fatty acids in the cecum is 8-times that in the distal colon. Bacterial growth is much higher in the cecum than in the distal colon. One culture-dependent study specifically compared cecal fluid cultures with stool cultures. Cecal fluid was collected by an intestinal tube inserted nasally and compared to meticulous anaerobically collected fecal samples. Fecal cultures had a significantly higher number of total anaerobes with higher numbers of both Bacteroides and Bifidobacterium species than did cecal fluid cultures. Facultative anaerobes comprised 25% of the anaerobic microbiota in cecal fluid cultures, but merely 1% in stool cultures. Fecal cultures yielded significantly fewer Lactobacillus species than could be cultured directly from cecal fluid. Fecal cultures are such a poor tool to evaluate the colonic microflora that they have been largely abandoned by investigators in favor of newer technologies. Sophisticated 16S rRNA-based fluorescent probe techniques hold the promise for greater understanding of the intestinal microbiota. In one elegant study, 16S rRNA-based fluorescent probes were used to compare the microflora found on colon and terminal ileum biopsies with the microbiota found in feces. This study found that the composition of the fecal microflora was similar to the microflora found in the terminal ileum and colon. However, a second study using denaturing gradient gel electrophoresis (DGGE) analysis of amplified 16S rRNA to compare the Lactobacillus populations found on colon biopsies with fecal populations produced conflicting results. The study disclosed that Lactobacillus species were similarly distributed throughout the colon, but the predominant microbial populations associated with the colonic mucosa varied significantly from those in the feces. With the available data, it is reasonable to conclude that stool cultures do not accurately reflect intestinal microbial populations. Sophisticated 16S rRNA-based techniques have yielded conflicting data on whether fecal microbiota accurately reflects the colonic and terminal ileal microflora.

ACCURATE STOOL CULTURES - Anaerobic Techniques Are Critical
More than 99% of the intestinal microflora consists of obligate and facultative anaerobes. Any culture-based assessment of these microorganisms must utilize stringent anaerobic techniques in order to accurately enumerate and identify the microbes in the feces. Studies that have employed culture-based techniques to assess the fecal microflora have utilized rigorous anaerobic techniques. In the study that compared cultures of cecal contents with stool cultures, subjects defecated into a special box that allowed immediate introduction of the stool into an anaerobic chamber for processing. In an extensive study evaluating the fecal microflora in populations at high risk for colon cancer, fecal samples were placed into plastic bags flushed with oxygen-free carbon dioxide within 5 minutes of collection and quickly placed into anaerobically sterilized dilution solution. In a study comparing the fecal microbiota of...
healthy young adults, healthy elderly people, and antibiotic-treated elderly people, fecal samples were processed under anaerobic conditions within 1 hour of defecation. The above examples highlight the two critical techniques required for accurate fecal cultures: anaerobic handling and rapid processing. One study has specifically evaluated the survival of Bifidobacterium species under various conditions. The investigators found that the time for a 90% reduction in viable bifidobacteria (T90) in phosphate-buffered saline was less than 8 hours for B. longum at 8°C and approximately 25 hours for B. adolescentis at 4°C. The authors concluded that because the survival of bifidobacteria is so limited, samples should be processed within 3 hours. The accuracy of stool culture-dependent techniques to assess intestinal anaerobic bacterial populations that do not use meticulous anaerobic techniques and rapid sample processing is highly questionable. Stool culture results expressed in an arbitrary, undefined 0-4+ format have never been standardized or validated and have little clinical relevance. The results of stool cultures processed without anaerobic technique are highly variable and not reproducible, rendering them useless as a tool to assess clinical interventions.

CONCLUSION

When fecal material is assiduously collected, rapidly processed, and cultured with careful anaerobic technique, the results primarily reflect cultivable microbes predominating in the descending colon and rectum. Stool cultures do not reflect the microflora of the small intestine and cecum. Fecal cultures that report semiquantitative numbers of Lactobacillus and Bifidobacterium species are poor guides to the need for and possible benefits of probiotics. Stool cultures cannot accurately assess probiotic bowel colonization.

STOOL CULTURES & PROBIOTICS

Stool cultures are used by many practitioners to assess the need for probiotic supplements and to evaluate the response to a probiotic regimen. This is an unreliable and questionable practice. Probiotics are usually lactic acid bacteria in the genera Lactobacillus and Bifidobacterium. Lactobacillus species are the predominant microflora in the small intestines. Stool cultures do not reflect the microbiota of the small bowel and its characterization has been hindered by sampling difficulties. Current understanding of intestinal Lactobacillus populations derive from post mortem studies of people suffering sudden death, sampling by intubation or aspiration at surgery, nasal or oral intubation and aspiration techniques, and ingestion of automatic capsules that can sample intestinal contents. Bifidobacterium species vie for predominance with Bacteroides species in the colon. As strict anaerobes, Bifidobacterium colony counts are unlikely to be accurate unless rapid, anaerobic processing is used. Absent or low numbers of Bifidobacterium species on stool cultures are more likely to represent poor technique than to reflect their actual numbers. The results of routinely collected stool cultures are a poor guide to the need for probiotics. The use of routine stool cultures to assess response to probiotics is based in part on a misconception that consumption of probiotics can repopulate the bowel microflora with “friendly” microorganisms. Most of the Lactobacillus species used as probiotics are not indigenous to the human gut. They are ingested with food or as supplements and once they are no longer consumed, they pass out of the body over days. Probiotics such as L. acidophilus, L. casei, L. rhamnosus, and L. plantarum will not be cultivable once they are no longer ingested. Even indigenous Lactobacillus strains, such as L. johnsonii, may not persist in fecal cultures once ingestion has ceased. Stool cultures have been shown to have a poor sensitivity to detect probiotics while they are being consumed. In a study that compared the rectal biopsies with stool cultures in detecting L. rhamnosus during consumption, the probiotic could be detected in 88% of rectal biopsy cultures compared to only 20% of stool cultures. The investigators concluded that fecal samples are not adequate to assess probiotic colonization.

References

Are stool cultures for intestinal microflora accurate?

Stool cultures may be accurate if they are carefully collected, appropriately handled, and rapidly processed. Lactobacillus and Bifidobacterium species, the two major healthful bacterial genera in the intestines, are anaerobic microorganisms requiring no oxygen to support their metabolism. Oxygen is toxic to strict anaerobic organisms. The Lactobacillus species are facultative or aerotolerant anaerobes and tolerate some exposure to air. Bifidobacterium species are strict anaerobes and will die after exposure to air. In order for stool cultures to accurately assess anaerobic microorganisms such as bifidobacteria, the stool must be immediately sampled and stored in anaerobic conditions. The sample must then be rapidly stored. In most research studies, the stool samples have been delivered to the laboratories within hours, often within 1 hour, of collection. If the stool sample has not been collected and processed this way, the stool culture results for Lactobacillus and Bifidobacterium populations will probably be inaccurate. It is highly likely that stool culture results indicating no or few Lactobacillus and Bifidobacterium populations are inaccurate, related to poor sampling technique, and lack of anaerobic storage and processing. Stool cultures for the microaerophilic, pathogenic bacterium, Campylobacter jejuni are also likely to be inaccurate unless the sample is placed in a deep, airtight container, chilled, and transported to a laboratory within 24 hours. Stool cultures for hardier, aerobic species, such as Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa, are generally more likely to be accurate although the results may be biased by conditions of handling and storage as well as the time elapsed before the sample arrives at the laboratory.

Are stool culture results a good way to decide on probiotic use?

Routine stool cultures provide very poor guidance for the selection and use of probiotics. Under the best sampling and processing conditions, stool cultures do not accurately reflect microbial populations in the small bowel and cecum. Routine stool cultures for probiotic species are inaccurate. Clinical stool cultures rarely, if ever, identify the species and strains of Lactobacillus and Bifidobacterium species. Practically this means that no or low populations of probiotic microbial genera reported on stool cultures are more likely to reflect poor sampling and processing techniques than real population numbers. Also, stool culture reports indicating “normal” or “healthy” Lactobacillus and Bifidobacterium populations reveal nothing about the diversity of the different species. For example, the highly beneficial L. plantarum, is only found in 25% of people consuming the highly processed Western diet, while it is present in 100% of people consuming traditional plant and fruit-based diets. Even when intestinal Lactobacillus and Bifidobacterium populations are reported as “normal”, clear benefit from consuming colonizing lactobacilli, such as L. rhamnosus, L. casei, and L. acidophilus, has been shown.

Are stool culture results a good way to assess or follow probiotic benefits?

Stool culture results are a very poor way to assess probiotic effectiveness. Routine stool culture results are likely to be inaccurate. If a person is taking a probiotic with bifidobacteria and the stool culture results indicate no or few bifidobacteria, the odds are high that the results are wrong and the bifidobacteria present could not be cultured due to inappropriate sample collection, storage, and processing. Furthermore, research has clearly shown that stool culture is a highly inaccurate test for probiotic colonization of the intestines. One study found that the probiotic species L. rhamnosus could only be detected in 20% of stool cultures while it was found in 88% of rectal biopsies in the same patients. Stool cultures have no role in the assessment and follow-up of probiotic use.

What do the bacterial culture classifications of “Beneficial flora”, “Imbalances”, and “Dysbiotic flora” mean?

These are misleading and simplistic classifications used on reports of stool culture results. Bifidobacterium, Lactobacillus, and E. coli are often called “beneficial flora” or “friendly bacteria”. While Bifidobacterium and Lactobacillus species are certainly important members of a healthy intestinal microflora, the implication is that these are the major beneficial microorganisms. At least half of the intestinal microflora species are unknown and are probably beneficial. The different microbial species are highly likely to synergistically interact to promote health in ways that are only beginning to be understood. It is misleading and inaccurate to call E. coli beneficial or friendly. E. coli is a pathogenic bacterium. Some E. coli strains are highly pathogenic causing life-threatening disease. Others are normal, commensal intestinal inhabitants, but capable of causing infections such as urinary tract infections. Aerobic E. coli never make up a significant proportion of the intestinal microflora, 99% of which is anaerobic. The health benefits of the vast majority of E. coli strains are at most unclear and may not exist. Sometimes stool culture reports refer to “imbalanced flora” as microorganisms that are commensal, but supposedly not pathogenic. This category is inaccurate and misleading. An imbalance in intestinal microflora refers to an abnormal distribution of populations within the microflora such as may occur after antibiotic therapy. An example is the excessive growth of Clostridium or Candida species that may follow an abrupt decline in the numbers of Bifidobacterium caused by antibiotics. Imbalances cannot possibly refer to the presence of one or more species. Also, some stool culture reports erroneously imply that pathogenic species, such as Klebsiella pneumoniae, are not pathogenic. The term dysbiosis means an alteration in the composition of the normal microflora. Calling specific microbial species dysbiotic flora is not accurate. For example, Pseudomonas aeruginosa is a pathogen that rarely colonizes the intestines. It is not a “dysbiotic” bacterium. High populations of pathogenic microorganisms such as Clostridium, Candida, and Enterobacteriaceae imply intestinal dysbiosis, but they are not “dysbiotic flora.”

What does the 0 to 4+ gradation of stool microorganisms mean?

These are arbitrary, subjective, largely undefined reporting classifications. Microorganisms are normally quantified by the number of organisms per gram of stool. Often 3+ or 4+ numbers of Lactobacillus and Bifidobacterium species are defined as healthy levels. However, it is not clear how healthy levels are defined or determined. The populations of these and other intestinal microorganisms vary from person to person, by age, by ethnic group, and by geographic location. Bifidobacteria are more commonly isolated from younger people than from older people. Individuals who appear healthy have been described as having no bifidobacteria on meticulous stool culture. The idea that everyone has the same “healthy level” of lactobacilli and bifidobacteria is simply wrong. In the case of other microorganisms, the 0 to 4+ gradations remain undefined, have no reference ranges, and are unvalidated. One can only presume that 4+
Klebsiella means there are more Klebsiella growing than there are with a 1+ result. It is not known exactly how many organisms have been cultured and what the difference between 4+ and 3+ or 3+ and 2+ is. Klebsiella may be cultured from the stool of 40% of the population. The clinical significance of 3+ or 4+ Klebsiella counts is unknown.

**Do routine stool cultures have any value?**
Routine stool cultures for normal intestinal microflora have no value. Stool can be cultured for pathogens such as Salmonella, Shigella, E. coli O157, Yersinia, and Campylobacter, although in the case of Campylobacter jejuni, the sample must be handled properly and sent to a laboratory within 24 hours. Stool cultures for Clostridium have little clinical value because the organism is fastidious and difficult to grow. It is more important to test for clostridial toxin A and B. However, the toxins are unstable and breakdown. A stool sample for clostridial toxin should be sent to a laboratory within 24 hours.

**Are there any stool tests that are useful for evaluating intestinal microflora health?**
A number of stool tests can provide important information to assist the clinician in the evaluation of the intestinal microbial balance. The intestinal barrier function test measures IgG, IgA, and IgM antibody titers to select dietary proteins, intestinal bacteria, and Candida strains and provides important information about the integrity of intestinal barrier function. A healthy, balanced intestinal microflora is important to normal barrier function and an abnormal test may suggest an altered microbial balance. However, the test is not specific. An elevated fecal pH suggests diminished microflora production of short-chain fatty acids. Total fecal short-chain fatty acids and butyrate levels may be measured. Abnormally low levels are suggestive of diminished intestinal lactic acid bacteria. However, there may be dietary factors that explain high fecal pH and low short-chain fatty acid levels. The results of these tests must be carefully assessed and evaluated by the clinician. There is no single test that provides a definitive assessment of the intestinal microflora.