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DIGESTIVE TRACT MICROBIOTA IN HEALTHY VOLUNTEERS

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Zilberstein B, Quintanilha AG, Santos MAA, Pajecki D, Moura EG, Alves PRA, Maluf Filho F, Souza JAU de, Gama-Rodrigue J. Digestive tract microbiota in healthy volunteers. Clinics. 2007:62(1):47-54.

PURPOSE: The aim of this study was to standardize the methods of sample collection of mucus from the digestive tract and to determine the microbiota in healthy volunteers from Brazil, collecting samples from the mouth, esophagus, stomach, duodenum, jejunum, ileum, colon, and rectum.
METHODS: Microbiota of selected healthy volunteers from the oral cavity (n=10), the esophagus (n=10), the upper digestive tract (n=20), and the lower digestive tract (n=24) were evaluated through distinct collection methods. Collection methods took into account the different sites, using basic scraping and swabbing techniques, stimulated saliva from the oral cavity, irrigation-aspiration with sterile catheters especially designed for the esophagus, a probe especially designed for upper digestive tract, and a special catheter for the lower digestive tract.
RESULTS: (i) Mixed microbiota were identified in the oral cavity, predominantly Gram-positive aerobic and anaerobic cocci; (ii) transitional flora mainly in the esophagus; (iii) *Veillonella* sp, *Lactobacillus* sp, and *Clostridium* sp in the stomach and duodenum; (iv) in the jejunum and upper ileum, we observed *Bacteroides* sp, *Proteus* sp, and *Staphylococcus* sp, in addition to *Veillonella* sp; (v) in the colon, the presence of "nonpathogenic" anaerobic bacteria *Veillonella* sp (average 10^5 UFC) indicates the existence of a low oxidation-reduction potential environment, which suggests the possibility of adoption of these bacteria as biological markers of total digestive tract health.
CONCLUSIONS: The collection methods were efficient in obtaining adequate samples from each segment of the total digestive tract to reveal the normal microbiota. These procedures are safe and easily reproducible for microbiological studies.

KEYWORDS: Bacteria Anaerobic. Bacteria Aerobic. Fungi. Colony count microbial. Gastrointestinal tract.

INTRODUCTION

Interest in the microbiologic composition of the normal digestive tract began in the 19th century with Pasteur, who believed that a symbiotic relationship between man and bacteria was essential to life. However, at the beginning of the 20th century, several authors including Metchnikoff et al[1] believed that microbiota competed with the host organism for nutrients. The development of experimental models to study the relationship between microbiota and the human organism in totally germ-free laboratory animals demonstrated the role of the normal microflora in vitamin synthesis, metabolism of nitrogen compounds and lipids, and especially their participation as a barrier against the invasion of pathogenic microorganisms.[2-4] In 1964, Donaldson[5] standardized the quantitative analysis of bacteria in the feces of healthy volunteers, and in 1966, Kalser[6] described an aspiration sampling technique for studying the microbiota of the jejunum and ileum. There have been few advances in collection methods since then; however, there

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have been some improvements in cultivation techniques and the use of genetic probes for identification of the microorganism.[7]

Over the last 20 years, there has been an increased interest in the study of the microbiota and their role in diseases of the digestive system including neoplasias,[8,9] intestinal inflammatory disease,[10,11] diarrhea associated with antibiotic use,[12-14] and bacterial translocation.[15,16] However, one of the difficulties in performing conclusive repeatable studies has always been the lack of a standard collection method that can be used to establish a uniform control group of healthy individuals against which the different diseases could be compared.

This shortcoming becomes even more important when there is the intention to study the eventual modification of the normal microbiota caused by or as a consequence of digestive diseases.[11,17]

The aim of this study was to standardize collecting sample methods from the different segments of the digestive tract in healthy volunteers in order to better characterize their microbiota.

PATIENTS AND METHODS

A multidisciplinary prospective investigation was developed involving the Digestive Surgery Division and the Gastrointestinal Endoscopy Unit of the São Paulo University Medical School, and the Institute of Biomedical Sciences of the University of São Paulo.

The study was approved by the Ethic Committees and informed consent was obtained from all volunteers.

The different segments of the digestive tract were studied in separate groups of healthy volunteers. Sample collection methods were specifically developed for each segment. Healthy individuals were selected based on the following exclusion criteria: use of antibiotics and/or anti-inflammatory drugs in the preceding 90 days, dental prostheses, smoking, alcoholism, diabetes, presence of malignant tumors, scleroderma, previous surgical procedures on the digestive tract, or any other problem that could affect the digestive tract, the esophagus, stomach, intestines, or specifically the mouth flora; a dental caries index (CPO – D) greater than 2.6[18] was also was also an exclusion criterion.

Standardized collection method from the mouth. Ten healthy individuals were prospectively studied, including 5 men and 5 women, with a mean age of 33.3 years. Samples for microbiological study were collected from saliva, the back of the tongue, and supragingival and subgingival bacterial biofilm. A sterile Petri dish was used for saliva collection after stimulation by masticatory mouth movements. From the back of the tongue, a sterile cotton swab was used with a front-to-back movement. The supragingival bacterial biofilm was collected from the vestibular face of the first lower left molar and from the subgingival vestibular face of the contralateral first molar with a periodontal (Gracey) curette (Figure 1). The collected materials were placed in a test tube containing phosphate-buffered saline for immediate transport.

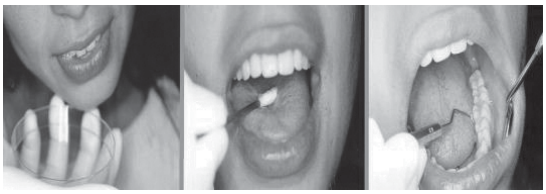

Figure 1 - Saliva; back of the tongue; sub-gingival biofilm

Standardized collection method from the esophagus. Ten individuals were prospectively studied, including 6 women and 4 men, with a mean age of 43.4 years. Volunteers were selected among those who were going to undergo an upper digestive tract endoscopy. After local mouth anesthetic with 10% lidocaine spray and intravenous sedation with diazepam (3-5 mg), a nº14(Fr) Levine catheter was introduced with extreme care into the mouth through an nº7.5 or 29 Fr orotracheal tube. The catheter was pushed forward to touch the esophagus lumen, where 10 mL of saline was injected using a 20 mL syringe connected to its proximal end; aspiration was then performed. The collected material was transported in a phosphate buffer saline solution (1 mL/9 mL) to the microbiology laboratory within 1 hour (Figure 2). Upper digestive endoscopy was then immediately performed to identify any abnormality of the upper digestive tract.

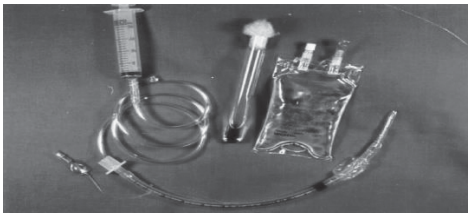

Figure 2 - Levine nº 14 catheters; 20 ml syringe; phosphate buffer solution (1ml/9ml); 10 ml of saline solutions; nº 7.5 orotracheal cannula

Standardized collection method from the stomach, duodenum, jejunum, and proximal ileum. This was performed in 20 other volunteers: 8 men and 12 women with a mean age of 42 years. All individuals presented normal upper digestive tract endoscopies. A flexible silicone sampling probe was used with a small (3 mm) distal opening and with a lead weight bound in its extremity.[19-23] This

probe was introduced by mouth and swallowed until it reached the stomach, being located by radioscopy. The first sample was collected, aspirating 1 mL of mucus with a 20 mL syringe connected to its proximal end. The probe was allowed to migrate by peristaltic movement to the more distal segments and was periodically monitored by radioscopy (Figure 3). The same probe was used for collection of material in each intestinal segment. The evaluation of the exact site from which the mucus was obtained was determined by the length of the probe introduced and its location through radioscopy. To avoid contamination and eventual false results, the probe was washed out several times, and the contents were discarded before the actual collection of mucus. A series of 1-mL samples was taken from the duodenum, proximal jejunum, distal jejunum, and proximal ileum. The samples were placed into the test tubes with 9 mL of phosphate buffer saline solution and taken to the microbiology laboratory within the first hour.

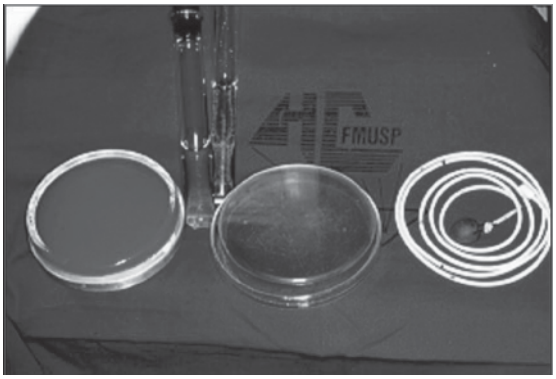

Figure 3 - Agar-blood; Bile esculin (BBE); Tioglicolato; Flexible silicone sampling probe with distal orifice and lead weight bound to extremity

Standardized collection method from distal ileum, colon, and rectum. This method was performed in another 24 volunteers, 10 men and 14 women, with a mean age of 53 years, through colonoscopy after previous colon preparation with a 10% mannitol solution until the stool was liquid, clear, and free of residues.[24] Colonoscopy was performed at least 5 hours after the colonic preparation to avoid interference in the determination of the microbiologic contents of the colonic mucus.[25] Colonoscopy was performed after sedation with diazepam (to 10 mg) and meperidine (to 100 mg). The colonoscope was introduced into the distal ileum, checking the integrity of the lower digestive tract (distal ileum; cecum; ascending, transverse, descending, and sigmoid colon; and rectum). A proper catheter, especially developed for this purpose, was used to pass through the working channel of the colonoscope to collect the samples of mucus from the different segments of the lower digestive tract. This catheter, obtained from a polyethylene probe, was especially designed, being 2.20 m long and 8 Fr in diameter; it was internally coated with silicone (Figure 4). The distal end of the catheter was covered by a protective membrane; this was ruptured at the time of collection by inflation with air. Sample volume was always 0.1 mL, determined by step marks on the distal end of the catheter[5,2,25-27] that could be visualized by the colonoscope. A fresh sterile catheter was used at each site of the lower digestive tract for collecting the samples. After collection, the samples were taken to the microbiological laboratory in a sterile Eppendorf tube containing 0.9 mL of VMGA III (viability-maintaining transport medium).[28]

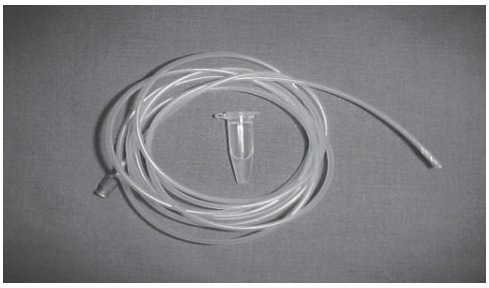

Figure 4 - Catheter for sampling ependdorff tube

Culture media

The collected materials from all segments were placed in selective culture media for aerobic and anaerobic microorganisms and yeasts.[29] The following culture media were used: Sabouraud agar *, MacConkey agar**-blood, *Enterococcus*-selective agar**, reinforced *Clostridium* medium** ; Phenylethylalcohol agar*, *Veillonella* medium*, Brain-Heart infusion (BHI) agar* + vitamin K + hemin + streptomycin, Chapman-Stone medium**, *Bacteroides fragilis* bile-esculin agar medium (BBE) *, *Bifidobacterium* medium, *Propionibacterium* medium*, BHI* + extract of yeast, Blood-trypticase soy agar (TSA) * + vitamin K + hemin (*DIFCO. St. Louis, MO, USA; **MERCK DIAGNOSTICA, RJ, Brazil).

Statistical analysis

Descriptive analysis of findings from the mouth, esophagus, and upper digestive tract was done. The chi square (X^2) test was used to verify the difference in distribution of each bacterial species in the various regions of the lower digestive tract. The expected frequency of each bacterial species in different regions of the lower digestive tract was calculated using nonparametric tests.[30]

The results of the concentration of microorganisms were expressed in units of colony formation/mL (ucf) expressed in logarithm base 10 ($\log_{10}$). Significance was assigned to a P value of < 0.05.

Table 1 - Frequency and locations of microorganisms in the oral cavity

Microorganism	Saliva (%)	Tongue (%)	Superior Biofilm (%)	Inferior Biofilm (%)
Actinomyces sp	40	30	30	20
Anaerobic rods (Gram- pig)	30	10	50	10
Anaerobic rods (Gram- npg)	10	0	0	0
Candida sp	20	30	10	0
Corynebacterium sp	70	40	20	20
Escherichia coli	10	20	20	0
Fusobacterium sp	60	0	40	0
Lactobacillus sp	90	50	30	0
Neisseria sp	0	40	0	0
Neisseria sp	0	40	0	0
Peptostreptococcus sp	70	50	50	20
Propionibacterium sp	20	30	60	30
Staphylococcus sp (coag-)	50	30	10	10
Streptococcus sp (alpha- hemolytic)	100	90	90	80
Streptococcus sp (gamma- hemolytic)	20	10	0	10
Veillonella sp	100	60	80	70

sp = species; pig = pigmented; npg = nonpigmented; coag- = coagulase negative

RESULTS

Mouth microbiota. Table 1 shows the frequency and locations of microorganisms found in the mouth. Mixed microbiota were identified here, with as predominance of Gram-positive aerobic and anaerobic cocci

Esophagus microbiota. Table 2 shows the frequency and average concentration of microorganisms found in the esophagus. It can be seen that in this segment the flora is transitional.

Stomach, duodenum, jejunum, and proximal ileum microbiota. Table 3 shows the mean concentrations and prevalence of the different microorganisms found in each segment of the upper digestive tract**.** *Veillonella* sp, *Lactobacillus* sp, and *Clostridium* sp are predominant in the stomach and duodenum; while *Bacteroides* sp, *Proteus* sp, and *Staphylococcus* sp, in addition to *Veillonella* sp are predominant in the jejunum and upper ileum;

Distal ileum, colon, and rectum microbiota. Table 4 shows the different microorganisms found in each segment. The most distinctive feature here is the presence of "nonpathogenic" anaerobic bacteria *Veillonella* sp (average 10^5 UFC)

Table 5 shows the association between microorganisms and sites of the lower digestive tract.

Table 2 - Frequency and concentration of microorganisms in the esophagus

	Frequency (%)	Concentration (ufc/mL)
Streptococcus sp	40	10^1
Staphylococcus sp	20	10^2
Corynebacterium sp	10	10^2
Lactobacillus sp	10	10^2
Peptococcus sp	10	10^1

sp = species

DISCUSSION

The complex interactions between the different microorganisms or between them and healthy hosts or their interaction in digestive diseases are poorly understood. The study of the microbiota in humans starts with the need for standardizing sample collection methods from the different segments of the digestive tract, including availability of different technical resources and ethical issues. This base is necessary when we intend to compare different studies.

This investigation describes the sample collection methods from different segments of the digestive tract. This methodology is characterized by the following characteristics: (i) it is innocuous for patients; (ii) it allows material to be collected from restricted sites, avoiding contamination; (iii) it uses available materials with accessible prices, and (iv) it is easily reproducible.

Material sampling from the mouth[18,31,32] and esophagus[33] was done by modifying previously validated methods already described in the literature. For the stomach, duodenum, jejunum (proximal and distal), and proximal ileum, similar methods were used.[19-22] Finally, samples from the distal ileum, colon, and rectum were collected using a new method developed by the authors.[28]

The oral cavity presented very diverse microbiota due to the different anatomical sites and constant exposure to the external environment. The method of collecting saliva without using chewing gum or paraffin tablets gave better results than in previous studies,[34-38] suggesting that the technique used in this study is more sensitive and causes fewer alterations to indigenous flora. Results from the back of the tongue were similar to those of other studies.[35-38]

Periodontal Gracey curettes were preffered for sampling from the supra- and subgingival biofilm. Our results were

Table 3 - Mean concentration and prevalence of microorganism in the upper digestive tract

Site	STOMACH		DUODENUM		JEJUNUM Proximal		JEJUNUM Distal		ILEUM Proximal	
Microorganisms	MC	%	MC	%	MC	%	MC	%	MC	%
Bacillus sp			4	9.1						
Bacteroides sp					2	9.1	3	9.1		
Bacteroides sp (npg)	2	16	2	9.1	3.5	36.4	3	45.5	4.5	40.0
Bifidobacterium sp			3	9.1	3	9.1				
Candida sp			2	9.1	3	18.2			1	10.0
Clostridium ramosum										
Clostridium sp										
Clostridium sp (gel-)	4	33.3	2	27.3	2.5	18.2	5	27.3	3.5	20.0
Clostridium sp (gel+)										
Corynebacterium sp	3	25	3	18.2	2	9.1	5.5	18.2	3	30.0
Escherichia coli	5.5	16	2.5	18.2	5.5	18.2	6	36.4	3	30.0
Enterobacter sp	7	8.3			5	27.3	7	27.3	7	50.0
Enterococcus faecalis			4	9.1						
Enterococcus sp	7	8.3	2	27.3	4.5	18.2	6	18.2	5	20.0
Eubacterium lentum										
Eubacterium sp										
Fusobacterium fusiformes	3	8.3					1	9.1		
Fusobacterium sp	2	8.3	4	9.1	2	18.2	2	18.2	3	10.0
Klebsiella pneumoniae										
Klebsiella sp	3.5	16	2	45.5	3.5	18.2	7	36.4	9	10.0
Lactobacillus acidophillus										
Lactobacillus sp	4	41.6	2	27.3	3	45.5	4	27.3	4.5	20.0
Leptotrichia sp					1	9.1				
Peptococcus sp	3.5	16	2	9.1	3	9.1	3	27.3	4	30.0
Peptostreptococcus sp	5	8.1							3	10.0
Propionibacterium sp	3	8.1	2.5	18.2		9.1	2	36.4		
Proteus sp	5	8.1	2	45.5	4	45.5			6	20.0
Pseudomonas sp										
Rodothorula sp	4	8.1	1	9.1			4	18.2	4.5	20.0
Sarcinia lútea										
Staphylococcus sp	2	8.1	1	9.1		18.2	2	45.5	3	50.0
Staphylococcus sp (coag-)			3	27.3						
Streptococcus sp (alpha hemolytic viridans group)	4	2.5			2	27.3	4	9.1	5	10.0
Torulopsis sp			4	9.1						
***Veillonella* sp**	4	41.6	3	45.5	4	54.5	4	63.6	4	50.0

MC = mean concentration (UFC/$\log_{10}$); % = prevalence; sp = species; npg = nonpigmented; gel+ = gelatinase positive; gel- = gelatinase negative; npg = nonpigmented; coag- = coagulase negative

similar to those reported in the literature,[32,38,39] validating this method.

From a microbiological point of view, the esophagus has always been considered to be a saliva secretion passageway with transitory microbiota similar to the mouth and of little interest in research. However, when alimentary stasis occurs, as in neoplasia or achalasia,[40,41] there is an excessive increase in bacterial growth, with the risk of serious infections after surgery or endoscopy.[42,43]

Sample collection for studying the microbiota of the esophagus cannot be performed with catheters that pass through the mouth because of the risk of contamination during their insertion. The use of sterile catheters that pass through the working channel of an endoscope is expensive and does not permit aspiration of sufficient thick material from an esophagus with stasis.

Gagliardi et al[33] developed a similar collection method to that used in our study. However, by taking the samples before endoscopic examination, we avoided possible contamination during the introduction or removal of the endoscopic device. This small technical modification enabled us to obtain fewer positive cultures (40% vs 66%) with similar strains of bacteria, with predominantly Gram positive aerobes and without Gram negative aerobes, as described by Gagliardi in 13% of cases.

The samples obtained from the stomach for microbiological study presented no problems because of the ease of access, and because gastric pH is responsible for the destruction of most swallowed bacteria, all of which facilitates the isolation of the appropriate microorganisms.[19]

In the stomach, there was a predominance of *Lactobacillus* sp, *Veillonella* sp, and *Clostridium* sp, which are all resistant to the acid environment, demonstrating potential residual microflora that could develop down to the lower regions of the digestive tract.

Other species such as *Escherichia coli, Peptococcus* sp,

Klebisiella sp, *Bacteroides* sp (npg) start an ascending curve of growth or "re-population" of the lower sites of the digestive tract, being dormant in the stomach because of the acidic environment.

Sample collection from small intestine segments is technically more difficult because of difficulties relating to access and to the standardization of specific locations. Nevertheless, it is of major interest because of the range of diseases associated with alterations in its microbiota.[44,45]

Shiner[46] developed a stainless steel capsule that only opens at collection time to aspirate the contents of the jejunum; this avoids contamination during its passage, but it is very complex to use. Kalser et al[6] developed a double lumen polyvinyl catheter with a mercury weight on the distal end for taking samples starting 75 cm from Treitz's ligament to near the distal ileum and cecal valve. However, there was no mechanism to protect from contamination. Their results were very similar to those of Shiner. [46]

This method was tested in our midst by Machado et al,[19] who collected samples of the jejunum liquid in patients suffering from Chagasic achalasia. It was also used by Quintanilha et al[20,21] for qualitative evaluation of microbiota alterations in the proximal jejunum of patients with Chagasic megacolon, both before and after surgery.

A similar method has been used by different authors in the study of bacterial translocation in critical patients. Belov et al[47] evaluated sepsis mediators (TNF and IL-1) using jejunal aspiration in septic shock patients. Pardo et al[16] demonstrated a reduction in bacterial overgrowth and translocation in cirrhotic patients using cisapride. Bernhardt et al[48] studied *Candida* sp colonization in digestive tracts of critical patients under long-term antibiotic treatment.

In this study, the method of collecting mucus from the upper digestive tract was used to evaluate healthy volunteers with the aim of establishing a normal pattern that could serve as a control in studies of microbiotic changes in disease states. Even though the sample group was small, we obtained similar results to those described by Kalser[6] and Shiner.[46]

It must be said that sample collection from the jejunum and proximal ileum is an uncomfortable procedure for the patient. Therefore, recruiting healthy volunteers is not easy; this makes it difficult for researchers to recruit control groups for their studies.

The study of lower digestive tract microbiota is a major challenge because of the high concentration and variety of microorganisms pertaining to the indigenous and transitory microbiota.[49] In early trials in which researchers attempted to study the microbiology of dregs,[4,5] they were unable to differentiate indigenous from transitory microbiota or determine the different levels of bacteria in the different segments of the lower digestive tract. Studies that have employed sample collection during laparotomy[50] have not respected the physiological conditions of the patient, because the quantitative study was prejudiced due to dilution of mucus during collection.

The need for more precise and reliable methods motivated us to develop a special catheter to collect adequate volume of undiluted mucus from specific regions of the lower digestive tract during colonoscopy using an aseptic technique. This is an efficient method as shown by the large variety of aerobic, anaerobic, microaerophile, and facultative microorganisms, and yeasts obtained. The results show a stable increase in anaerobic microorganisms (*Veillonella* sp, *Peptococcus* sp, and *Fusobacterium* sp) with the capacity to digest amino acids but with little ability to ferment carbohydrates. These and other similar microorganisms comprise a distinct metabolism group in the large intestine.[4]

There was also the constant presence of other bacteria with average concentrations of 10^5 such as *Clostridium* sp (gel-) *Corynebacterium* sp, *E. coli*, *Enterobacter* sp, *Klebisiella* sp, *Lactobacillus* sp, *Propionibacterium* sp, *Proteus* sp, and *Veillonella* sp; there were others at lower concentrations.

Bacterial types were characterized colonizing restricted areas of the lower digestive tract with average concentrations of 10^5 (Table 5); the following could be interpreted as "biological markers" of health: *Fusobacterium* sp in the rectum; *Peptococcus* sp in the sigmoid; and *Enterococcos* sp in the transverse colon. *Bacteroides* sp tend to reside in the more proximal regions, gradually decreasing in prevalence in the sigmoid and rectum.

CONCLUSION

The presented sampling collection methods are safe and efficient for obtaining suitable samples of mucus for qualitative and quantitative microbiologic studies, revealing an environment of low oxidation-reduction (redox) potential.

These results open the possibility for many other studies in this area, using low-risk and highly reliable methodology, to define the role of microbiota in the other gastrointestinal diseases as well as for standardization of future prophylactic treatment[50,51] in gastroenterology.

ACKNOWLEDGMENTS

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RESUMO

Zilberstein B, Quintanilha AG, Santos MAA, Pajecki D, Moura EG, Alves PRA, Maluf Filho F, Souza JAU de, Gama-Rodrigue J. Microbiota no trato digestivo em voluntários saudáveis. Clinics. 2007:62(1):47-54.

OBJETIVO: Padronizar os métodos de coleta do muco do trato digestivo e determinar a microbiota, em voluntários saudáveis no Brasil, coletando amostras da boca, esôfago, estômago, duodeno, jejunos e íleo, cólons e reto.
MÉTODOS: A microbiota de voluntários saudáveis foi avaliada através de diferentes métodos de coleta: cavidade oral (n=10 voluntários), do esôfago (n=10), do trato digestivo alto (n=20) e do trato digestivo baixo (n=24). Métodos de coleta foram adotados em cada sítio restrito, usando derramar saliva, técnica de esfregar a mucosa e saliva estimulada da cavidade oral, irrigação-aspiração, cateteres específicos designados para o esôfago, sonda especial para o trato digestivo alto e cateteres especiais para o trato digestivo baixo.
RESULTADOS: Identificados: (i) na cavidade oral, microbiota mista, predominando cocos aeróbios e anaeróbios Gram positivos; (ii) no esôfago, flora transitória; (iii) no estômago e duodeno, *Veillonella* sp, *Lactobacillus* sp and *Clostridium* sp; (iv) no jejuno e íleo proximal, *Bacteróides* sp, *Proteus* sp and *Staphilococcus* sp, além da *Veillonella* sp ; (v) no colon, foi revelada a presença "não patogênica" da bactéria anaeróbica *Veillonella* sp numa concentração média de 10^5 unidades formadoras de colônia, indicando um meio de baixo potencial de oxido-redução e a possibilidade de se conceituar esta bactéria como um marcador biológico do trato digestivo total em sadios.
CONCLUSÃO: Estes métodos de coleta foram considerados eficientes para obtenção adequada de amostra em cada segmento do trato digestivo total para caracterizar a microbiota normal. Estes procedimentos são seguros e facilmente reprodutível para estudo microbiológico.

UNITERMOS: Bactérias Anaeróbias. Bactérias Aeróbias. Fungos. Contagem de Colônia Microbiana. Trato Gastrointestinal.

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ERRATA

CLINICS 2007;62(1):47-54

Page 52, 1º Column, Line 22
Replace: Quintanilha et al [20,21] for Quintanilha et al [21-22]

Page 52, Tables 4 e 5

Table 4 - Mean Concentration and Prevalence (%) of Microorganisms in each Site of the LDT

	ÍLEUM		CECUM		COLON								RECTUM	
					Ascending		Transverse		Descending		Sigmoid			
Microorganisms	MC	%	MC	%	MC	%	MC	%	MC	%	MC	%	MC	%
Bacillus sp	1	9.5	2	4.2	5	4.2	3	4.2			1	4.3	3	5.0
Bacteroide sp (*pig.)*	5	4.8									5	4.3	1	5.0
Bacteroides sp (npg)	3	47.6	5	29.2	4	37.5	5	12.5	5	12.5	5	47.8	5	65.0
Bacteroides sp	2	4.8					2	4.2					3	5.0
Bifidobacterium sp	2	9.5			3	4.2	4	4.2	4	8.3	4	8.7	3	5.0
Candida sp	3.5	9.5	2	20.8	3	20.8	2	29.2	4	16.7	3	34.8	3	35.0
Clostridium rammosum	1	4.8							3	4.2				
Clostridium sp (gel -)	4	66.7	4	45.8	5	50.0	4.5	58.3	5	54.2	5	69.6	7	60.0
Clostridium sp (gel+)			2	4.2	4	4.2							3	5.0
Clostridium sp	1	4.8	4	45.8	7	4.2							5	5.0
Corynebacterium sp	3	57.1	3	54.2	3.5	50.0	4	41.7	4	45.8	5	60.9	5	65.0
E.coli	5	47.6	5	37.5	5	45.8	4.5	50.0	5	37.5	5	69.6	7	80.0
Enterobacter cloacae			4	4.2	1	4.2								
Enterobacter sp	4	28.6	4	20.8	7	37.5	4	29.2	5	45.8	5	52.2	7	35.0
Enterococcus faecalis	4.5	9.5	3	4.2	4	4.2	3	4.2	1	4.2	4	8.7		
Enterococcus faecium							5	4.2						
Enterococcus sp	4	38.1	2.5	33.3	3.5	33.3	3	58.3	5	58.3	5	34.8	5	60.0
Eubacterium lentum	4	4.8												
Eubacterium sp	3.5	9.5												
Fusobacterium fusiformes	3	4.8												
Fusobacterium sp	3.5	19.0	2.5	25.0	3	20.8	4	16.7	4	8.3	3	21.7	4	55.0
Klebsiella pneumoniae									5	4.2				
Klebsiella sp	5	76.2	4	54.2	6	62.5	5	54.2	5	41.7	7	69.6	7	65.0
Lactobacillus acidophillus	4	4.8	3	4.2										
Lactobacillus sp	4	33.3	2	29.2	2.5	33.3	3	37.5	3	25.0	2	43.5	4	70.0
Leptotrichia sp					1	4.2					1	4.3		
Peptococcus anaerobius	2	4.8	4	4.2	2	4.2					1	4.3		
Peptococcus assachalyticus	2	4.8												
Peptococcus sp	2	28.6	2.5	33.3	3	25.0	3	33.3	3	25.0	3	56.5	3	35.0
Peptostreptococcus sp	3	9.5			2	8.3			1	4.2	5	13.0		
Propionibacterium sp	3	52.4	3	20.8	4	33.3	3	50.0	3	29.2	5	26.1	5	30.0
Proteus sp	3.5	19.0	4	12.5	5	33.3	5	37.5	5	25.0	7	34.8	7	30.0
Pseudomonas sp	3	9.5					1	4.2						
Rodothorula sp	4	14.3	2	20.8	1	4.2	1	12.5	3	16.7	1	4.3	3.5	20.0
Selenomonas sp													2	5.0
Staphylococcus sp	5	4.8	2	4.2										
Staphylococcus sp coag -	2	33.3	2	20.8	1.5	8.3	2	20.8	2	8.3	3	21.7	3	45.0
Staphylococcus sp (coag+													3	5.0
Streptococcus/ gama hem									2	8.3				
Streptococcussp/alfa hemolítico grupo Viridans	4	14.3	4	16.7	2	8.3	3.5	8.3			2	13.0	3	5.0
Veillonella sp (Gel -)							3	8.3						
Veillonella sp	4	90.5	2.5	83.3	4	70.8	4	62.5	5	75.0	5	95.7	5	90.0

MC = Mean concentration (UFC / Log_{10}), % = prevalence

Table 5 - Association between microorganisms/sites of the lower digestive tract

Microbiota	Lower Digestive Tract site
Klebisiella sp, *Clostridium* sp (gel-), *Veillonella* sp	All sites
Enterobacter sp	Sigmoid*
Cândida sp	Sigmoid and Rectum*
Enterococcus sp e *Lactobacillus sp*	Rectum*

sp = species, *$p < 0,05$ or $< 5\%$

This article has received corrections in agreement with the ERRATUM published in Volume 62 Number 2.