Hiroshima J. Med. Sci.
Vol. 66, No. 3, 71~76, September, 2017
HIJM 66−13

Use of the Hydrogen Breath Test to Determine the Influence of Antibiotic Prophylaxis on Intestinal Flora

Tomoko TANAKA[1)], Hiroki OHGE[2*)], Mitsuru SAKASHITA[2)], Akiko NAGANO[2)], Yusuke WATADANI[2)], Norimitsu SHIMADA[2)], Raita YANO[2)], Shinya TAKAHASHI[2)], Kenichiro UEMURA[2)], Yoshiaki MURAKAMI[2)], and Taijiro SUEDA[2)].

1) Graduate School of Biomedical Sciences Programs for Applied Biomedicine
2) Department of Surgery, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

ABSTRACT

Purpose: This experimental study was designed to use the hydrogen (H_2) breath test to investigate changes in the intestinal flora of patients that were administered prophylactic antibiotics for 48 hours after surgery.

Methods: Altogether, 22 patients were divided into two groups and the antimicrobial prophylactics, cefazolin (3.0 g/day) or sulbactam/ampicillin (4.5 g/day), were administered on induction of anaesthesia for 48 hours after surgery. End expiratory breath samples were collected on the morning of the day of surgery and every morning for 1-6 days after surgery.

Results: H_2 breath concentration significantly decreased in each group on day 1 (cefazolin: 1.20 ± 0.39 ppm vs. sulbactam/ampicillin: 1.17 ± 0.34 ppm). On day 2, the H_2 concentration in the sulbactam/ampicillin group was significantly lower than the cefazolin group (cefazolin: 6.4 ± 2.2 ppm vs. sulbactam/ampicillin: 1.0 ± 0.4 ppm, $p < 0.05$). H_2 concentration was still lower in the sulbactam/ampicillin group (1.3 ± 0.3 ppm vs. 3.3 ± 1.0 ppm, $p = 0.10$) on day 3. On days 4-6, H_2 concentration was essentially the same for both groups.

Discussion: Colonic anaerobes are thought to be a reservoir of resistant organisms and prolonged antimicrobial treatment is a major cause for the development of resistance. Surgical prophylaxis is basically recommended for use within 24 hours after surgery. The breath H_2 concentration in both groups significantly decreased 24 hours after administration. These results suggest that both antibiotics influence the activity of colonic anaerobes and the duration of surgical antibiotic prophylaxis should be as short as possible.

Key words: *Intestinal flora, antibiotics, prophylaxis, breath hydrogen*

Anaerobic bacteria comprise a large proportion of the normal human commensal gut flora and evidence continues to mount that normal intestinal flora provides protection against a broad range of enteric pathogens, including bacteria such as *Escherichia coli* and *Pseudomonas* spp., anaerobic *Clostridium* spp., and yeasts like *Candida albicans*.[21,22] The ability to prevent or limit colonization of the intestinal tract by pathogenic microorganisms has been termed as colonization resistance.[53] Antimicrobial therapy induces rapid and profound changes in the intestinal flora, thereby disrupting colonization resistance.[14,37,52] Imbalances in the microbial ecosystem caused by the administration of antimicrobial agents may allow colonization or overgrowth of resistant organisms that might spread within the body and cause infection.[12]

Microorganisms in the lower gut also ferment dietary fibre and produce hydrogen (H_2), methane, and carbon dioxide gases. A portion of these gases enters the blood stream and is excreted via the lungs.[30,31,39] The hydrogen breath test, which is based on the premise that H_2 gas in humans is produced exclusively by colonic fermentation, uses levels of expired H_2 as an indirect measure of disturbances in the intestinal flora.[20,29,30] The test is widely used to detect a battery of non-structural gastrointestinal disorders, particularly carbohydrate malabsorption, small intestinal bacterial overgrowth, and irritable bowel syndrome. The breath test is also used in studies of food metabolism and as various indicators of intestinal flora.[5-8,17,25,32,34,39,42-45,47]

The prolonged use of antibiotics is associated with an increased risk of drug toxicity, a change

* Corresponding author: Hiroki OHGE ohge@hiroshima-u.ac.jp 1-2-3 Kasumi, Minami-Ku, Hiroshima, 734-8551 JAPAN
Telephone: 81-82-257-5216, Facsimile: 81-82-257-5219

in the antimicrobial susceptibility pattern of pathogens, and an alteration in intestinal flora.[15] Therefore, despite the accepted use of antimicrobial prophylaxis in surgery, its use remains controversial, especially regarding the duration of postoperative administration. In general, antimicrobial prophylaxis should last less than 24 hours, and under some circumstances, may consist of a single dose.[13]

Since the intestinal tract may be a major site for the emergence of drug-resistant organisms due to its frequent exposure to antimicrobial agents, the ecological effects of antibacterial agents on human microflora are of clinical importance.[23,48,50] This study enrolled surgical patients who were administered antibiotics at the induction of anaesthesia up to 48 hours following surgery. The hydrogen breath test was used to assess changes in their intestinal flora to determine whether a 48-hour administration was an appropriate duration for postsurgical antibiotic prophylaxis.

PATIENTS AND METHODS

Basal analysis: fasting breath hydrogen data in healthy Japanese subjects

Thirty-five healthy volunteers (21 men and 14 women, aged 21-65 years) fasted after their usual dinner until the following morning (~08:00) when hydrogen breath tests were conducted at the Hiroshima University School of Medicine. End-alveolar breath samples were obtained by having the subjects exhale end-expiratory samples into 500 ml plastic bags fitted with stopcocks. Samples were analysed for H_2 concentration with a HCMA-T1™ Gas Chromatograph (Abilit Corporation, Osaka, Japan). The data was presented as normalized breath-H_2 concentrations in parts per million (ppm).

Effect of antimicrobial agents on breath hydrogen

Subjects

Twenty-two patients (12 males and 10 females; median age 53 years; range 38-71 years) were recruited to this study. All the patients were treated surgically at the Hiroshima University Hospital. The patients had undergone laparoscopic cholecystectomy (n = 12) and inguinal hernia repair (n = 10). The following exclusion criteria were applied: previous history of bowel resection, antibiotic treatment the month before the study, allergy to beta-lactams, and evidence of diabetes mellitus.

Experimental protocol

The patients were divided into 2 groups, each of which was given an antimicrobial prophylaxis treatment (**Fig. 1**). One group (n = 11) was given cefazolin at a daily dose of 3.0 g (1.0 g × 3). Members of the second group (n = 11) took sulbactam/ampicillin at a daily dose of 4.5 g (1.5 g × 3). The antibiotics were administered upon induction of anaesthesia and continued up to 48 hours after surgery.

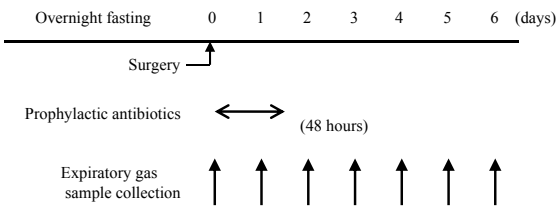

Fig. 1 . Experimental protocol

Hydrogen breath test

End expiratory breath samples were collected the morning of the day of surgery, and then every morning for 1-6 days following surgery. Patients fasted until the following morning (more than 12 hours) after their evening meal. Samples were obtained using the GaSampler System (QuinTron Instruments, Milwaukee, WI) as described previously.[46] The patients were instructed to exhale as deeply as possible to obtain alveolar air directly into the apparatus via a mouthpiece. A 5 ml aliquot of each breath sample was transferred to a silicone-greased plastic syringe fitted with a 3-way plastic stopcock. Hydrogen concentrations were measured using a gas chromatograph (HCMA-T1®, Abilit Corporation, Osaka, Japan). The data was presented as normalized breath hydrogen concentrations in ppm.

Statistical analysis

All measured results were expressed as mean concentrations. The data was analysed using the Student's *t*-test and $p < 0.05$ indicated a significant difference.

Ethical considerations

This study was approved by the Medical Ethics Committee of the Hiroshima University School of Medicine and signed informed consent was obtained from all participants. The study was carried out in accordance with the Declaration of Helsinki.

RESULTS

Basal analysis: fasting breath hydrogen data in healthy Japanese subjects

The breath H_2 concentrations of 35 healthy subjects were determined after overnight fasting (**Fig. 2**). The 5 subjects with an increase in H_2 concentration of more than 25 ppm were classified as having diabetes. The 23 subjects with an increase of less than 10 ppm were classified as normal metabolizers.

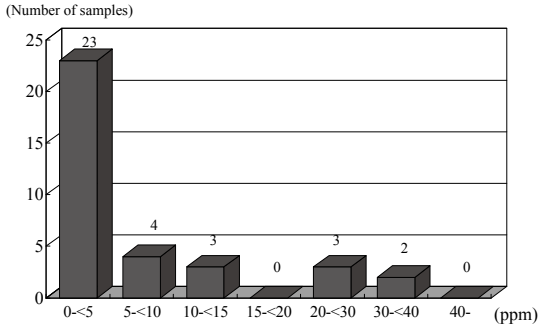

Fig. 2. Fasting breath hydrogen data in healthy Japanese subjects. The number of healthy subjects (y-axis) grouped against the breath hydrogen concentration in ppm (x-axis).

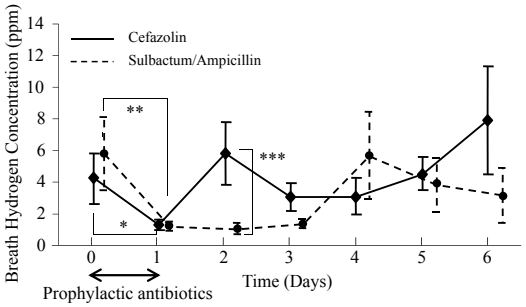

Fig. 3. Mean ± SEM breath hydrogen concentrations (ppm) from 22 patients that received cefazolin (◆) or sulbactam/ampicillin (●) for prophylaxis during and following surgical procedures. *: day 0 vs. day 1 in the cefazolin group ($p = 0.05$), **: day 0 vs. day 1 in the sulbactam/ampicillin group ($p < 0.05$), ***: cefazolin vs. sulbactam/ampicillin on day 2 ($p < 0.05$).

The effect of antimicrobial agents on breath hydrogen

The subjects were matched according to weight (cefazolin, 56 ± 5 kg; sulbactam/ampicillin, 51 ± 7 kg) and age (cefazolin, 54 ± 3 years; sulbactam/ampicillin, 49 ± 6 years) (**Table 1**). None of the patients classified as normal metabolizers were excluded. As no adverse effects due to antibiotic use were observed, all patients completed the study.

Changes in the daily breath H_2 concentrations that were observed after receiving the 2 antibiotics are shown in **Fig. 3**. H_2 concentration before antibiotic administration was not significantly different from that on the day of surgery (day 0; cefazolin: 4.6 ± 1.8 ppm vs. sulbactam/ampicillin: 6.0 ± 2.4 ppm). Breath H_2 concentration significantly decreased in each group on day 1 (cefazolin: 1.20 ± 0.39 ppm vs. sulbactam/ampicillin: 1.17 ± 0.34 ppm). On day 2, the sulbactam/ampicillin group had a significantly lower H_2 concentration (cefazolin: 6.4 ± 2.2 ppm vs. sulbactam/ampicillin: 1.0 ± 0.4 ppm, $p < 0.05$). There was no significant difference in the breath H_2 concentration of the cefazolin group between day 0 and day 2. H_2 concentration was still lower in the sulbactam/ampicillin group (1.3 ± 0.3 ppm) when compared with the cefazolin group (3.3 ± 1.0 ppm, $p = 0.10$) on day 3. On days 4-6, there was no significant difference in the breath H_2 concentrations of the two groups.

Table 1. Comparison of the cefazolin and sulbactam/ampicillin groups

	Administrated antimicrobial agents	
	Cefazolin	Sulbactum/Ampicillin
No. of patients	11	11
Median age, range (years)	54(38-71)	49(22-72)
Sex ratio, M/F	6/5	6/5
No. of patients consisted of surgery		
Inguinal hernia repair	3	0
Laparoscopy assisted cholecystectmy	8	3
Hemorrhoids	0	1
Resection of small intestine (Crohn's disease)	0	7

DISCUSSION

Hydrogen is not a by-product of the mammalian cell metabolism. It is only formed in the body due to bacterial fermentation of carbohydrates in the colon. The anaerobic fermentation of carbohydrates results in the production of carbon dioxide, methane, and hydrogen. These gases are consumed by bacteria or are quickly absorbed into the blood stream.[20,29-31,39] Therefore, H_2 production in individuals can be studied by means of a breath test using lactulose (4-*O*-b-D-galactopyranosyl-D-fructose) as a substrate.[16-18,38] This synthetic carbohydrate is not absorbed in the small intestine and is thereby, fermented in the colon. The fermentation and subsequent metabolic processes result in the production of gasses that are absorbed by the colonic mucosa and eventually exhaled. Therefore, breath H_2 measurements provide a semi-quantitative assessment of the quantity of soluble carbohydrate reaching the colon.[4,40]

Levine et al.[34] measured breath H_2 concentration to determine the association between individual faecal microflora and the fermentation of dietary fibres. They were able to associate anaerobic species with H_2 production, suggesting that breath H_2 concentration reflected the activity of anaerobes in the large intestine. Previous measurements regarding the activity of colonic anaerobes were based on the bacterial counts in faeces or mucosal tissues.[48,50] However, a faecal sample from a patient is not easy to collect and the costs of the counts are high. Moreover, bacterial counts do not always reflect the activity of the flora.

The healthy volunteers in this study had breath H_2 concentrations of 0-40 ppm. As most cases were within 10 ppm, the baseline concentration was stable in many patients. However, high H_2 concentrations (more than 25 ppm) were observed in

some cases. These patients were classified as diabetic, since glucose metabolic abnormalities exert a great influence on the concentration of fasting breath H_2.[40]

The H_2 breath test was then used to evaluate the influence of antibiotics on colonic flora during surgical prophylaxis. Many antibiotics can affect the colonic flora,[9,33,36,41,48,50] sometimes leading to adverse outcomes such as *Clostridium difficile* colitis.[1,9,11,19,26,28,35,37] The elimination of anaerobes should be avoided to prevent such side effects. Another issue is resistant organisms. It has been suggested that colonic anaerobes are a reservoir of resistant organisms[12] and that prolonged antimicrobial treatment is a major cause for the development of resistance.[2,14,15,23,27,37,49,52,53] Supporting this view is the finding by Harbarth et al. that associated an increased risk of antibiotic resistance with more than 48 hours of antibiotic prophylaxis.[24]

Guidelines for the use of surgical prophylaxis recommend that antibiotics should be used within 24 hours of surgery. However, a recent analysis in the U.S. demonstrated that only 40.7% of the surgeons stopped prophylactic antimicrobials within 24 hours after surgery and that 26.7% used prophylaxis beyond 48 hours after surgery.[10] Many surgeons tend to use antibiotics longer than the period recommended by several published guidelines.

In this study, we chose cefazolin and sulbactam/ampicillin for surgical prophylaxis. Cefazolin is a first generation cephalosporin that acts against aerobes,[37,51] while sulbactam/ampicillin covers both aerobes and anaerobes.[26] After only 24 hours of administration, both antibiotics had significantly decreased breath H_2 concentrations, suggesting that both antibiotics could influence the activity of colonic anaerobes.

The reason behind cefazolin's ability to affect anaerobes is unknown. While Akagi et al.[3] reported that a 3-day administration of cefazolin did not influence total counts of colonic anaerobes, some anaerobic species such as *Bacteroides*, *Eubacterium*, *Lactobacillus*, *Veillonella*, and *Bifidobacterium* were significantly decreased. These results suggest that the first generation cephalosporin might affect colonic anaerobes.

The difference in the effects of cefazolin and sulbactam/ampicillin was observed for 48 hours after surgery. Patients in the cefazolin group recovered from the decrease in H_2 concentration 48 hours after surgery. In contrast, suppression of H_2 concentration in the sulbactam/ampicillin group was not relieved until 4 days after surgery, finally recovering after an additional 72 hours. The anaerobic coverage by this antibiotic is thought to be the reason for the delayed recovery. It is possible that the prolonged suppression of colonic anaerobes by sulbactam/ampicillin might lead to the development of drug-induced colitis or resistant organisms.

The use of H_2 measurements to assess absorption is painless and the closed system is well tolerated by patients. The measurement of H_2 concentration using gas chromatography is simple and takes only about 3 min. The drawbacks to the widespread use of this technique include the need for relatively expensive equipment and the time required for each study, which consists of sampling every morning (after overnight fasting) to measure the H_2 response to antimicrobial load. In addition, the results of the test can be difficult to interpret. Understanding the factors that influence H_2 production and excretion could have important clinical implications and provide basic information on the regulation of the colonic ecosystem. A better understanding of H_2 physiology would also facilitate a more accurate interpretation of the H_2 breath tests that are widely used for the study of carbohydrate malabsorption, small-bowel transit time, and bacterial overgrowth.[5-8,25,32,42-45,47]

In this study, breath hydrogen was used as a relative evidence of the bacteriological condition of the colon. As the test is simple, this study could indicate new therapeutic approaches to surgical antibiotic prophylaxis. However, more experiments that assess intestinal microflora using the H_2 breath test are needed because the data obtained in this study was based on a small sample size. Ultimately, further studies will be required to determine the selection and duration of prophylactic antimicrobial agents to prevent postoperative infection and the emergence of resistant organisms.

In summary, we evaluated the influence of cefazolin and sulbactam/ampicillin on the intestinal microflora, and found that the concentration of breath hydrogen was only slightly suppressed under cefazolin treatment when compared to the sulbactam/ampicillin treatment. As these results suggest that both antibiotics influence the activity of colonic anaerobes, the duration of surgical antibiotic prophylaxis should be kept as short as possible.

REFERENCES

1. **Alestig, K., Carlberg, H., Nord, C.E. and Trollfors, B.** Effect of cefoperazone on faecal flora. J Antimicrob Chemother 1983; **12**: 163-7.
2. **Archer, G.L. and Armstrong, B.C.** Alteration of staphylococcal flora in cardiac surgery patients receiving antibiotic prophylaxis. J Infect Dis 1983; **147**: 642–9.
3. **Akagi, S., Takesue, Y., Yokoyama, T., Murakami, Y., Imamura, Y. and Yokoyama, Y. et al.** Impact of Cefazolin on the gastrointestinal microflora at the gastrointestinal surgery (in Japanese with English abstract). Jpn J Gastroenterol Surg 2000; **33**: 255-9.
4. **Bond, J.H., and Levitt, M.D.** Use of pulmonary hydrogen (H_2) measurements to quantitate carbohy-

drate absorption. Study of partially gastrectomized patients. J Clin Invest 1972; **51**: 1219-25.
5. **Bond, J.H., Levitt, M.D.** and Prentiss, R. Investigation of small bowel transit time in man utilizing pulmonary hydrogen (H_2) measurements. J Lab Clin Med 1975; **85**: 546-55.
6. **Bond, J.H and Levitt, M.D.** Use of breath hydrogen (H_2) in the study of carbohydrate absorption. Am J Dig Dis 1977; **22**: 379-82.
7. **Bond, J.H. and Levitt, M.D.** Use of breath hydrogen (H_2) to quantitate small bowel transit time following partial gastrectomy. J Lab Clin Med 1977; **90**: 30-6.
8. **Bond, J.H. and Levitt, M.D.** Effect of dietary fiber on intestinal gas production and small bowel transit time in man. Am J Clin Nutr 1978; **31**(10 Suppl): S169-S174.
9. **Berg, R.D.** Bacterial translocation from the gastrointestinal tract. J Med 1992; **23**: 217-43.
10. **Bratzler, D.W. and Houck, P.M.** Surgical Infection Prevention Guidline Writers Workgroup. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. Am J Surg 2005; **189**: 395-404.
11. **Carlberg, H., Alestig, K. and Nord, C.E. and Trollfors B.** Intestinal side effects of cefoperazone. J Antimicrob Chemother 1982; **10**: 483-7.
12. **Chesener, H.A.L., Vollaard, E.J. and van Saene, H.K.F.** Long-term prophylaxis of infection by selective decontamination in leukopenia and in mechanical ventilation. Rev Infect Dis 1987; **9**: 295-328.
13. **DiPiro, J.T., Cheung, R.P.F., Bowden, T.A. Jr. and Mansberger, J.A.** Single dose systemic antibiotic prophylaxis of surgical wound infections. Am J Surg 1986; **152**: 552-9.
14. **Edlund, C. and Nord, CE.** Effect on the human normal microflora of oral antibiotics for treatment of urinary tract infections. J Antmicrob Chemother 2000; **26** (Suppl S1): 41-8.
15. **Fry, D.E., Harbrecht, P.J. and Polk, H.C. Jr.** Antibiotic prophylaxis: need the cost be so high? Arch Surg 1981; **116**: 466-9.
16. **Florent, C., Flourie, B., Leblond, A., Rautureau, M., Bernier, J.J. and Rambaud, J.C.** Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an in vivo study). J Clin Invest 1985; **75**: 608-13.
17. **Flourie, B., Florent, C., Etanchaud, F., Evard, D., Franchisseur, C. and Rambaud, J.C.** Starch absorption by healthy man evaluated by lactulose hydrogen breath test. Am J Clin Nutr 1988; **47**: 61-6.
18. **Flourie, B., Briet, F., Florent, C., Pellier, P., Maurel, M. and Rambaud, J.C.** Can diarrhea induced by lactulose be reduced by prolonged ingestion of lactulose? Am J Clin Nutr 1993; **58**: 369-75.
19. **Giuliano, M., Barza, M., Jacobus, N.V. and Gorbach, S.L.** Effect of broad-spectrum parenteral antibiotics on composition of intestinal microflora of humans. Antimicrob Agents Chemother 1987; **31**: 202-6.
20. **Gibson, G.R., Cummings, J.H., Macfarlane, G.T., Allison, C., Segal, I., Vorster, H.H., et al.** Alternative pathways for hydrogen disposal during fermentation in the human colon. Gut 1990; **31**: 679-83.
21. **Hentges, D. J., Stein, A.J., Casey, S.W. and Que, J.U.** Protective role of intestinal flora against infection with *Pseudomonas aeruginosa* in mice: influence of antibiotics on colonization resistance. Infect Immun 1985; **47**: 118-22.
22. **Hentges, D.J., Pongpech, P. and Que, J.U.** How streptomycin treatment compromises colonization resistance against enteric pathogens in mice. Microb Ecol Health Dis 1990; **3**: 105-11.
23. **Hall, J.C., Christistiansen, K.J., Goodman, M., Lawrence-Brown, M., Prendergast, F.J., Rosenberg, P., et al.** Duration of antimicrobial prophylaxis in vascular surgery. Am J Surg 1998; **175**:87-90.
24. **Harbarth, S., Samore, M.H., Lichtenberg, D. and Carmeli, Y.** Prolonged antibiotic prophylaxis after cardiovascular surgery and its effect on surgical site infections and antimicrobial resistance. Circulation 2000; **101**: 2916-21.
25. **Justino, S.R., Goncalves, Dias. M.C., Maculevicius, J., Batista, de Morais. M., Sing, T.C. and Halpern, A. et al.** Fasting breath hydrogen concentration in short bowel syndrome patients with colon incontinuity before and after antibiotic therapy. Nutrition 2004; **20**: 187-91.
26. **Kager, L., Liljeqvist, L., Malmborg, A.S., Nord, C.E. and Pieper, R.** Effects of ampicillin plus sulbactam on bowel flora in patients undergoing colorectal surgery. Antimicrob Agents Chemother 1982; **22**: 208-12.
27. **Kernodle, D.S., Barg, N.L. and Kaiser, A.B.** Low-level colonization of hospitalized patients with methicillin-resistant coagulase-negative staphylococci and emergence of the organisms during surgical antimicrobial prophylaxis. Antimicrob Agents Chemother 1988; **32**:202–8.
28. **Kodama, T., Santo, T., Yokoyama, T., Takesue, Y., Hiyama, E. and Imamura, Y. et al.** Postoperative enteritis caused by methicillin-resistant *Staphylococcus aureus*. Surg Today 1997; **27**: 816-25.
29. **Levitt, M.D. and Ingelfinger, F.J.** Hydrogen and methane production in man. Ann N Y Acad Sci 1968; **150**: 75-81.
30. **Levitt, MD.,** Production and excretion of hydrogen gas in man. N Engl J Med 1969; **281**: 122-7.
31. **Levitt, M.D. and Bond, J.H. Jr.** Volume, composition and source of intestinal gas. Gastroenterology 1970; **59**: 921-9.
32. **Levitt, M.D. and Donaldson, R.M.** Use of respiratory hydrogen(H_2) excretion to detect carbohydrate malabsorption. J Lab Clin Med 1970; **75**: 937-45.
33. **Levy. S.B., Marshall, B., Schuluederberg, S., Rowse, D. and Davis, J.** High frequency of antimicrobial resistance in human fecal flora. Antimicrob Agents Chemother 1988; **32**: 1801-6.
34. **Levine, A.S., Tallman, J.R., Grace, M.K., Parker, S.A., Billington, C.J. and Levitt, M.D.** Effect of breakfast cereals on short term food intake. Am J Clin Nutr 1989; **50**:1303-7.
35. **Mulligan, M.E., Citron, D.M., McNamara, B.T. and Finegold, S.M.** Impact of cefoperazone therapy on fecal flora. Antimicrob Agents Chemother 1982; **22**: 226-30.
36. **Nord, C.E., Kager, L. and Heimdahl, A.** Impact of antimicrobial agents on the gastrointestinal microflora and the risk of infections. Am J Med 1984; **76**: 99-106.

37. **Nord, C.E. and Edlund, C.** Ecological effects of antimicrobial agents on the human intestinal microflora. Microb Ecol Health Dis 1991; **4**: 193-207.
38. **Perman, J.A., Modler, S. and Olson, A.C.** Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora: studies in vivo and in vitro. J Clin Invest 1981; **67**: 643-50.
39. **Perman, J.A. and Modler, S.** Glycoproteins as substrates for production of hydrogen and methane by colonic bacterial flora. Gastroenterology 1982; **83**: 388-93.
40. **Perman, J.A., Modler, S., Barr, R.G. and Rosenthal, P.** Fasting breath hydrogen concentration: normal values and clinical application. Gastroenterology 1984; **87**: 1358-63.
41. **Rosenblatt, J.E. and Brook, I.** Clinical relevance of susceptibility testing of anaerobic bacteria. Clin infect Dis 1993; **16** (Supple 4): S446-8.
42. **Rumessen, J.J., Nordgaard-Andersen, I. and Gudmand-Hoyer, E.** Carbohydrate malabsorption: quantification by methane and hydrogen breath tests. Scand J Gastroenterol 1994; **29**: 826-832.
43. **Riordan, S.M., McIver, C.J., Duncombe, V.M., Thomas, M.C. and Bolin, T.D.** Evaluation of the rice breath hydrogen test for small intestinal bacterial overgrowth. Am J Gastroenterol 2000; **95**: 2858-64.
44. **Savaiano, D.A., AbouElAnouar, A., Smith, D.E and Levitt, M.D.** Lactose malabsorption from yogurt, pasteurized yogurt, sweet acidophilus milk, and cultured milk in lactose-deficient individuals. Am J Clin Nutr 1984; **40**: 1219-23.
45. **Strocchi, A., Corazza, G., Ellis, C.J., Gasbarrini, G. and Levitt, M.D.** Detection of malabsorption of low doses of carbohydrate: accuracy of various breath H_2 criteria. Gastroenterology 1993; **105**: 1404-10.
46. **Suarez, F.L., Savaiano, D.A. and Levitt, M.D.** A comparison of symptoms with milk or lactose-hydrolyzed milk in people with self-reported severe lactose intolerance. N Engl J Med 1995; **333**:1-4.
47. **Suarez, F.L., Savaiano, D., Arbisi, P. and Levitt, M.D.** Tolerance to the daily ingestion of two cups of milk by individuals claiming lactose intolerance. Am J Clin Nutr 1997; **65**: 1502-6.
48. **Takesue, Y., Yokoyama, T., Akagi, S., Ohge, H., Murakami, Y., Sakashita, Y., et al.** Changes in intestinal flora after administration of panipenem/betamipron or sulbactum/cefoperazone for treatment of postoperative infections in gastrectomy patients. J Infect Chemother 1999; **5**: 52-7.
49. **Terpstra, S., Noordhoek, G.T., Voesten, H.G., Hendriks, B. and Degener, J.E.** Rapid emergence of resistant coagulase-negative staphylococci on the skin after antibiotic prophylaxis. J Hosp Infect 1999; **43**: 195–202.
50. **Takesue, Y., Yokoyama, T., Akagi, S., Ohge, H., Imamura, Y., Murakami, Y., et al.** Changes in the intestinal flora after the administration of prophylactic antibiotics to patients undergoing a gastrectomy. Surg Today 2002; **32**: 581-6.
51. **Vogel, F. and Knothe, H.** Changes in aerobic faecal bacterial flora of severely ill patients during antibiotic treatment. Klin Wochenschr 1985; **63**: 1174-9.
52. **Vollaard, E.J., Clasener, H.A.L., van Saene, H.K.F. and Muller, NF.** Effect on colonization resistance: an important criterion in selecting antibiotics. DICP Ann Parmacother 1990; **24**: 60-6.
53. **van der. Waaiji.D.** Colonization resistance of the digestive tract: clinical consequence and implications. J Antimicrob Chemother 1982; **10**: 270-93.