Prevention of febrile neutropenia in cancer patients by probiotic strain *Enterococcus faecium* M-74. Pilot study phase I*

M. MEGO[1], L. EBRINGER[2], L. DRGOŇA[1], J. MARDIAK[1], J. TRUPL[1], R. GREKSAK[1], I. NEMOVA[1], E. ORAVCOVA[1], V. ZAJAC[3], I. KOZA[1]

[1]Department of Medical Oncology, e-mail: michal.mego@nou.sk, National Cancer Institute, 833 10 Bratislava, [2]Department of Cell Biology, Faculty of Natural Sciences, Comenius University, 811 07 Bratislava, and [3]Cancer Research Institute, Slovak Academy of Sciences, 833 91 Bratislava, Slovak Republic

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Febrile neutropenia (FN) remains a potentially life-threatening complication of anticancer chemotherapy. Bacterial translocation via intestinal mucosa is a significant mechanism of FN development. Competitive inhibition of bowel colonization by pathogenic microorganisms by lactic acid bacteria could be a useful prevention of FN. The aim of the study was the evaluation of dose and safety of probiotic strain *Enterococcus faecium* M-74 enriched with organic selenium in patients with solid and hematological malignancies. Eleven (9M/2F) patients were included in the study. In the first phase six patients with germ cell tumors treated by chemotherapy were included. They received prophylaxis by nonpathogenic strain *E. faecium* M-74 during 2 cycles of chemotherapy. The planned daily dose was $6x10^9$ bacteria. Regarding the insufficient colonization of the gut, the dose was further increased up to $18x10^9$ tid. After safety evaluation, five patients were included with relapse of acute leukemia. In patients with germ cell cancer, severe neutropenia G3/4 was noted in 10 of 12 cycles of chemotherapy. The febrile episode was not observed in any of the patients. The gut colonization by enterococci reaches 10^6 CFU/g stool. In 5 patients with acute leukemia during 127 days of severe neutropenia 12 febrile episodes occurred. There was not noted any febrile episode or infection provoked by the tested strain. Tolerance of therapy was excellent without significant undesirable effects. Optimal dose was assessed and safety of probiotic strain was evaluated in neutropenic patients with solid, or hematological malignancies. Based on these results we plan phase II study to evaluate the effectiveness of this strain in FN prophylaxis.

Key words: febrile neutropenia, probiotics, infection prevention, safety

Febrile neutropenia (FN) remains a potentially life-threatening complication of anticancer chemotherapy. Main source of infection in neutropenic patients is endogenous intestinal flora [6, 22]. Infection is preceded by bowel colonization by pathogenic bacteria followed by translocation across the gut mucosa and systemic dissemination [25, 39].

Alteration in bowel flora is the result of chemotherapy and particularly of the use of broad-spectrum antibiotics supressing the growths of normal anaerobic bowel flora leading to diminishing of colonization resistance. Maintenance of the natural commensal flora provides a potent barrier to acquisition of pathogenic aerobic gram-negative rods [4].

Prevention of febrile neutropenia by selective decontamination of bowel by quinolones and trimethoprim-sulphamethoxazole was not successful leading to development of multiresistant strains. In addition, the treatment does not affect the incidence of gram-positive infections and is expensive [11]. The measures of protective isolation also failed to decrease incidence of infections [29].

Lactic acid bacteria were used in prophylaxis and therapy of some infectious diseases. Their beneficial effect consists in stimulation of the immune system [24, 30, 31], competition for nutrition with pathogenic bacteria, in production of bacteriocins, competitive inhibition of bacterial adhesion sites and increase transepithelial resistance [33, 41].

The experience with the use of probiotic bacteria in neutropenic patients with oncological disease is very limited with regard to evoking the iatrogenic infection in immunocompromised patient. However, in two published clinical

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studies with a limited number of participants, the treatment with lactobacilli during the neutropenic period did not lead to bacteriemia caused by the probiotic strain [9, 22].

Competitive inhibition of bowel colonization by pathogenic microorganism by lactic acid bacteria could be one of the possibilities how to prevent febrile neutropenia in oncological patients. In comparison with the existing selective bowel decontamination by quinolones and/or trimethoprim-sulfamethoxazole, we can expect a decreased incidence of mycotic and gram-positive infections based on the effect of bowel microflora. Based on the results of animal studies, probiotics could lead to the reduced time of neutropenia and immunity increase.

Enterococci are a part of natural intestinal flora and their concentration in large bowel reaches up to 10^8 CFU/g of stool [20]. Probiotic strain *Enterococcus faecium* M-74 enriched with selenium has important immunostimulatory, antimutagenic, and hypocholesterolemic properties after oral administration of the lyophilized form in capsules [8, 27]. Immunostimulatory effects were also confirmed in randomized double-blinded, placebo-controlled study [12]. Its advantage is resistance to low pH, as well as to bile acids, facilitating a further bowel colonization.

The aim of our pilot study was to evaluate the safety of the probiotic strain *E. faecium* M 74 in neutropenic patients with solid and hematological malignancies.

Material and methods

The study protocol was reviewed and approved by the Scientific Board and Ethical Committee of the National Cancer Institute of Bratislava, Slovakia. All patients were required to provide written informed consent before enrolment. Eligible patients were men or women aged 18 years or older, afebrile, without signs of infection 24 hours before the start of prophylaxis, without any antibiotic therapy 48 hours before prophylaxis, except standard antibiotic prophylactic regimens. Patients were excluded from the study in cases when oral intake was not possible, when they suffered from intercurrent disease preventing to proceede in the study, and in patients with bacteremia caused by probiotic strain.

A pilot, non-randomized, unicentric phase I trial was induced. The aim of the study was the evaluation of safety of probiotic strain *Enterococcus faecium* M-74 enriched with selenium in cancer patients with severe neutropenia. When bacteremia caused by the test strain occurs, the study will be prematurely terminated.

The study ran in two phases. Regarding the limited experience and fear of iatrogenic infection, enterococci were first administered to patients with solid tumors, with a short duration of neutropenia after chemotherapy and low risk of development of febrile neutropenia. Therefore, in the first phase probiotic strain was administered to 5 patients treated with chemotherapy for germ cell cancer (GCC). If they did not experience febrile neutropenia during the first two cycles of chemotherapy, they received prophylaxis by enterococci during the 3 and 4 cycle, i.e. 42 days.

In the second phase, after the evaluation of safety, five patients were included with acute leukemia. These patients had relapsed disease and underwent remission induction or had disease progression with neutropenia on symptomatic therapy only. Probiotic strain was administered for 2 months.

E. faecium M-74 (produced by Ivax-CR, Czech Republic) was orally administered as a lyophilysate with organically bound selenium in gelatinous capsules. Each capsule contains $6x10^9$ CFU of enterococci plus 55 μg of organic selenium.

The use of the immunomodulants was not allowed only in cases when they were a standard part of chemotherapeutic regimens. Systemic antibacterial therapy was initiated whenever the granulocytopenic patient developed a single fever of 38.3 °C or a temperature of 38 °C on two measurements >1 h apart within 24 h period. Febrile episode was not considered as a reason for interruption of administration of the probiotic strain.

In patients with germ cell cancer, the stool was microbiologically evaluated before each cycle of chemotherapy and at the end of the treatment. Measurement of complete blood cell count for the purpose of assessment of the degree of neutropenia was done once a week. In patients with leukemia, microbiological examination of stool and complete blood cell count was done weekly. The amount of enterococci in stool was measured by standard microbiological examination. In case of febrile episode blood cultures and standard surveillance cultures were obtained.

Each febrile episode (fever of >38 °C from any cause) was categorized as due to 1) microbiologically documented infection (a febrile episode accompanied by additional signs and symptoms of infection including microbiological confirmation), 2) clinically documented infection (a febrile episode accompanied by additional signs and symptoms of infection, treated with antimicrobial agents but lacking microbiological confirmation), 3) indeterminate cause (a febrile episode not confirming to the first two categories above, but in which antimicrobial therapy was administered), 4) non-infectious cause (a febrile episode in which no antimicrobial therapy was given). A febrile episode was considered to have ended when the maximum temperature was below 38 °C for more than 2 days. Toxicity was graded according to NCI-CTC (version 2.0) criteria [28].

Results

In the first phase six patients with germ cell cancer were involved. The basic characteristics of patients and maximal degree of neutropenia are summarized in Table 1. Capsules with probiotic strain *E. faecium* M-74 enriched with selenium were administered to patients during the 3rd and 4th cycles of chemotherapy. The administration was in a total 12 cycles of chemotherapy, while neutropenia G3/4 occurred in

Table 1. Patients with solid tumors. All patients have germ cell cancer. During this period there was not noted any febrile episode or infection provoked by tested strain

Patient No.	Age	Daily dose	Neutropenia[a]		Gut colonization[b]
			3. cycle	4. cycle	
1	31	$6x10^9$	3	4	10^3
2	31	$6x10^9$	3	4	10^3
3	21	$6x10^9$	2	2	$<10^3$
4	35	$18x10^9$	4	4	10^5
5	28	$18x10^9$	3	3	$>10^6$
6	29	$18x10^9$	3	3	10^5

[a] maximal degree of neutropenia (NCI-CTC criteria, version 2.0) achieved during the cycle of chemotherapy, when probiotic strain was administered, [b] maximal degree of bowel colonization by *E. faecium* M-74 (CFU/g stool) after 2 cycles (42 days) of chemotherapy

10 of them. The first 3 patients received the daily dose of $6x10^9$ of enterococci. As the gut colonization was insufficient (less than 10^3 CFU/g stool), the dose was further increased up to $18x10^9$ tid. In total, patients received the probiotic strain during 250 days. During this period no febrile episode or infection induced by the tested strain was observed. There was no significant mucositis or diarrhea after chemotherapy. The therapy was very well tolerated.

In the second phase we administered the probiotic strain to 5 patients with relapsed acute leukaemia. The daily dose of enterococci was $18x10^9$ tid, as assessed in the first phase of the trial. Patients' characteristics and the results are summarized in Tables 2 and 3. The median of age was 54 years (51–59). Four patients underwent the reinduction chemotherapy; one patient had only the symptomatic therapy. In total, patients received the probiotic strain during 236 days (and more than one half of that time) 127 days they had severe neutropenia G3/4. Patients No. 9 and 11 had antibiotic prophylaxis by ciprofloxacine during the first or/and first and second reinduction. All patients experienced febrile neutropenia. Generally, 12 febrile episodes were noted. In 7 cases there was bacteremia, mainly caused by coagulase-negative *Staphylococcus.* Two patients had enterocolitis caused by *Pseudomonas aeruginosa*, one had candidemia and one pneumonia caused by *Klebsiela pneumoniae*. The median time from beginning of prophylaxis to the first febrile episode was 10 days (10–65). The longest time interval was ob-

Table 2. Characteristics of patients with acute leukemia. All patients have relapsed disease. Patients received *E. faecium* M-74 daily $18x10^9$ tid

Patient No.	Age	Diagnose	Administration of *E. faecium* (days)	Neutropenia (days)		Chemotherapy (cycles)
				G3/4	G4	
7.	59	AML-M2	28	28	28	–
8.	54	AML-M2	35	19	17	Etoposid+CBDCA, (1) FLAG (1)
9.	51	AML-M4/5	59	24	23	HD-ARAC+Mitoxantron (2)
10.	57	AML-M1	55	38	28	HD-ARAC+Mitoxantron (2)
11.	52	B-ALL	60	18	14	Hyper-CVAD (2)
total			236	127	110	

AML – acute myeloblastic leukemia, ALL – acute lymphoblastic leukemia

Table 3. Febrile episods and infections in patients with acute leukemia

Patient No.	ATB prophylaxis	FE[a]	Infection	Pathogen	ATB	Lenghts of th. (days)	Time to first FE (days)
7.	–	1x	bacteremia	*Klebsiela sp.*	AUG+CIP	7	21
8.	–	2x	bacteremia, pneumonia	*Staphylococcus* coagulase negat. *Klebsiela sp.*	TAZ CIP AZI	7 7 5	9
9.	CIP[b]	5x	bacteremia enterocolitis bacteremia bacteremia candidemia	*Sternotrophomonas sp.* *Pseudomonas aeruginosa* *Enterobacter sp.* *Staphylococcus coagulase negat.* *Candida non-albicans*	MAX FOR+AMI TAZ+AMI VAN, Liposom. AMPHO-B	4 6 7 7 23	10
10.	–	1x	bacteremia	*E. coli*	CEFT	11	6
11.	CIP[c]	3x	enterocolitis bacteremia	*Pseudomonas aeruginosa* *Staphylococcus* coagulase negat. Streptococcus sp.	TAZ VAN	11 7	50

[a] febrile episode, [b] during first reinduction, [c] during first and second reinduction; CIP – ciprofloxacin, AUG – amoxicillin plus clavulanic acid, TAZ – piperacillin plus tazobactam, AZI – azithromycin, VAN – vancomycin, Liposom. AMPHO-B – liposomal amphotericin B, CEFT – cephtazidim.

served in patient No. 11, who had also antibiotic prophylaxis. Colonization of the gut by enterococci was between 10^4 and 10^6 CFU/g stool, but decreased rapidly below 10^2 CFU/g stool after the beginning of the antibiotic treatment of febrile neutropenia. No infection caused by the tested probiotic strain was observed. The tolerance of the treatment was excellent, only in patient No.10 mild meteorism was noted during administration of lactic acid bacteria.

Discussion

Approximately 80 % of infection in neutropenics are caused by endogenous flora and the main entrance is intestinal mucosa [39].

Microflora of large intestine consists of approximately 500 bacterial species, and their concentration reaches up to 10^{12} cell per gram of luminal content [38]. The microflora represents an important barrier against pathogens and produces short chain fatty acids that are the main source of nutrition for colonocytes [2]. Resident bacteria are crucial in defence against colonization by exogenous microbes and in prevention of pathogens invasion to tissues. The use of chemotherapeutics and particularly the antibiotics lead to dysmicrobia and further disruption of colonization resistance [7, 16].

A probiotic is a live microbial feed supplement, which beneficially affects the host by improving its intestinal microbial balance [14, 18]. The effects of probiotics were evaluated in prevention and therapy of certain infectious diseases. Meta-analysis from 9 randomized placebo controlled studies shows that probiotic strains significantly decrease the incidence of antibiotic associated diarrhea [7]. Also in prevention of infectious diseases caused by *Clostridium difficile* administration of probiotics led to significant decrease of disease recurrence [26]. Duration of rotavirus associated diarrhea in children and in immunocompromised HIV patients significantly shortened after administration of probiotic strains of *Lactobacillus* and *Saccharomyces boulardii* [3, 21, 36]. Lactic acid bacteria are also evaluated in prophylaxis of urogenital infections [32].

Lactic acid bacteria during fermentation produce short chain fatty acids, including butyric acid and thus they provide nutrition for colonocytes and participate in restitution of colonocytes after chemotherapy.

There are only anecdotal reports in the literature concerning the use of probiotics in granulocytopenic patients. In experimental animal model, in cyclophosphamid-induced neutropenia administration of heat inactivated strain *Enterococcus faecalis* FK-23 led to shortening of duration of neutropenia and to augmentation of leukocyte reconstituting capacity [17]. Also oral or intraperitoneal prophylactic administration of FK-23 preparation to mice significantly prolonged survival periods of mice infected by *Candida albicans*, and decreased viable counts of *C. albicans* recovered from their kidneys [37].

HENGENS and KLASTERSKY administered a strain of lactobacilli to 5 granulocytopenic patients, with intestinal flora suppressed by antibiotics. Lactobacilli were not successful in spontaneous recolonization of bowel by enteric bacteria. Only in two patients significant number of lactobacilli in stool was detected [19].

In randomized study 33 children with leukemia and solid tumors received framycetin, colimycin, nystatin, and metronidazol in 35 neutropenic episodes, while 35 children received co-trimoxazol with lactobacilli in 35 episodes. There were not significant differences in incidence of infections during neutropenia nor in duration of neutropenia. Combination of co-trimoxazol with lactobacilli was considerably better tolerated [9].

The problem of these studies is the limited number of participants and the dose of the probiotic itself. For colonization resistance only massive doses of the probiotic must be used. Also in our study only the increase of the daily dose to $18x10^9$ led to a successful settlement of the intestine. Concomitant antibiotic therapy also decreases the success of colonization. However, in our set of patients prophylactic administration of ciprofloxacin did not prevent transient colonization by enterococci, despite the sensitivity of the tested strain to ciprofloxacin. The initiation of empiric therapy for febrile neutropenia led to the loss of colonization also in our patients.

Despite the fact, that the incidence of infection caused by lactic acid bacteria is extremely low, there exists certain risk, that they become pathogenic [15]. This risk naturally increases in immunocompromised patients. Therefore, this is one of the main reasons for limited experience with administration of probiotics in granulocytopenic patients. In addition, due to chemotherapy it comes not only to neutropenia but also to local affection of gut mucosa. In case reports lactic acid bacteria are mentioned as causing local infections such as chest infections, digestive tract infections, urinary tract infections, and meningitis [13, 15]. *Bacillus subtillis* bacteremia occurred in 4 of 20 oncologic patients, but was also reported in other severely sick patients [34]. The experience of our hospital confirms this fact, too. Fungemia caused by *Saccharomyces* was also reported in a neutropenic patient with acute leukemia. It was a 6 months-old child that received diarrhea prophylaxis by *Saccharomyces boulardii* [5]. A similar infection was also noted in two other immunocompromised persons receiving the prophylaxis by *Saccharomyces boulardii* [35].

In the present study, the administration of probiotic strain *E. faecium* M-74 enriched with selenium did not lead to bacteremia or infection, even in leukemic patients with long lasting, severe neutropenia. On the other hand, the tested strain was not able to prevent the development of febrile neutropenia, which was, however, not the main point of our study. For the reduction of frequency of gut colonization by virulent bacteria, the germ free environment is considered to be an important tool for reduction of infections in

neutropenics. NAUSEEF and MAKI [29] compared protective isolation with standard hospital care in neutropenic patients with acute leukemia. The two groups were similar with respect to incidence of infection and fever. Paradoxically, the rate of bacteremia was higher in patients randomized to protective isolation. Although from animal models it is known that germ-free animals have higher rates of infection and are more susceptible to infection in comparison to normal animals [1, 40]. Therefore, we assume that augmentation of colonization resistance by lactic acid bacteria could be a more effective and also less expensive way for infection prevention in granulocytopenic patients. At present, studies in which the real risk/benefit ratio in immunocompromised persons would be assesed, are missing.

A possible risk exists with respect to the development and transfer of antibiotic resistance between probiotic strain and endogenous flora. The increase in vancomycin-resistant enterococci may be associated with the possible use of antimicrobial preparations in animals and humans. Gene transfer has been reported between enterococci and lactobacilli in gastrointestinal tract of experimental rats [10, 23]. The vancomycin and tetracycline resistant isolates were examined for transferability of resistance to *Enterococcus faecium* M-74. No transconjugans were obtained from any of the isolates (personal communications with Aarestrup FM).

In conclusion, we could state that our results demonstrate the safety of tested probiotic strain *E. faecium* M-74 enriched with selenium in cancer patients with severe neutropenia. In patients with acute leukemia its administration was not effective in prevention of febrile neutropenia. Tolerance to the therapy was excellent without significant undesirable effects. Based on these results we plan the phase II study, to evaluate effectiveness of this strain in prophylaxis of febrile neutropenia in patients with solid and hematological malignancies treated with chemotherapy.

All experiments described in this paper comply with the current laws of the country in which they were performed.

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References

[1] BABA E, NAGAISHI M, FUKATA T, ARAKAWA A. The role of intestinal microflora on the prevention of *Salmonella* colonization in gnotobiotic chickens. Poultry Sci 1991; 70: 1902–1907.

[2] BATT RM, RUTGERS HC, SANCAK AA. Enteric bacteria: friend or foe? J Small Anim Pract 1996; 37: 261–267.

[3] BORN P, LERSCH C, ZIMMERHACKL B, CLASSEN M. The *Saccharomyces boulardii* therapy of HIV-associated diarrhoea (letter). Dtsch Med Wochenschr 1993; 118: 765.

[4] BUCK AC, COOKE EM. The fate of ingested *Pseudomonas aeruginosa* in normal persons. J Med Microbiol 1969; 2: 521–525.

[5] CESARO S, CHINELLO P, ROSSI L, ZANESCO L. *Saccharomyces cerevisiae* fungemia in a neutropenic patient treated with *Saccharomyces boulardii*. Support Care Cancer 2000; 8: 504–505.

[6] COLE GT, HALAWA AA, ANAISSIE EJ. The role of gastrointestinal tract in hematogenous candidiasis from the laboratory to the bedside. Clin Infect Dis 1996; 22 (Suppl): 73S–88S.

[7] D'SOUZA AL, RAJKUMAR CH, COOKE J, BULPITT CHJ. Probiotics in prevention of antibiotic associated diarrhoe: meta-analysis. BMJ 2002; 324: 1361–1366.

[8] EBRINGER L, FERENČÍK M, LAHITOVÁ N, KAČÁNI L, MICHÁLKOVÁ D. Antimutagenic and imunostimulatory properties of lactic acid bacteria. World J Microbiol Biotechnol 1995; 11: 294–298.

[9] EKERK H, JURK IH, WATERS KD, TIEDEMANN K. Prophylactic co-trimoxazole and lactobacilli preparation in neutropenic patients. Med Pediatr Oncol 1980; 8: 47–51.

[10] ELMER GW, SURAWICZ CM, McFARLAND LV. Biotherapeutic agents. A neglected modality for the treatment and prevention of selected intestinal and vaginal infections. JAMA 2002; 275: 870–876.

[11] ENGELS AE, LAU J, BARZA M. Efficacy of quinolone prophylaxis in neutropenic cancer patients: a meta-analysis. J Clin Oncol 1998; 16: 1179–1187.

[12] FERENČÍK M, EBRINGER L, MIKEŠ Z, JAHNOVÁ E, ČIZNÁR I. Beneficial modification of the human intestinal microflora using orally administered lactic acid bacteria. Bratisl Lek Listy 1999; 100: 238–245.

[13] FRUCHART C, SALAH A, GRAY C, MARTIN E, STAMATOULLAS A et al. *Lactobacillus* species as emerging pathogens in neutropenic patients. Eur Clin Microbiol Infect Dis 1997; 16: 681–684.

[14] FULLER R. Probiotics in man and animals. J Appl Bacteriol 1989; 66: 365–378.

[15] GASSER S. Safety of lactic acid bacteria and their occurence in human clinical infection. Bull Inst Pasteur 1994; 92: 45–67.

[16] GORBACH SL, BARZA M, GIULIANO M, JACOBUS NV. Colonization resistance of the human intestinal microflora: testing the hypothesis in normal volunteers. Eur J Clin Microbiol Infect Dis 1988; 7: 98–102.

[17] HASEGAWA T, KANASUGU H, HIDAKA M, YAMAMOTO T, ABE S, YAMAGUCHI H. Effect of orally administered heat-killed *Enterococcus faecalis FK-23* preparation on neutropenia in dogs treated with cyclophosphamide. Int Immunopharmacol 1996; 18: 103–112.

[18] HAVENAAR R, SPANHAAK S. Probiotics from an immunological point of view. Curr Opin Biotechnol 1994; 5: 320–325.

[19] HENGENS C, KLASTERSKY J. Intestinal colonization with lactobacilli strains in neutropenic patients. Biomedicine 1976; 25: 11–15.

[20] HUYCKE MM, SAHM DF, GILMORE MS. Multiple drug resistant enterococci: The nature of the problem and an agenda for the future. Emerging Infectious Diseases 1998; 4: 239–249.

[21] ISOLAURI E, JUNTUNEN M, RAUTANEN T, SILLANAUKEE P, KOIVULA T. A human *Lactobacillus* strain (*Lactobacillus casei sp* strain GG) promotes recovery from acute diarrhea in children. Pediatrics 1991; 88: 90–97.

[22] KLASTERSKY J. A review of chemoprophylaxis and therapy of bacterial infections in neutropenic patients. Diagn Microbiol Infect Dis 1989; 12 Suppl 4: 201S–207S

[23] LEONARD F, ANDREMONT A, LECLERQ B, LABIA R, TANCREDE C. Use of B-lactamase-producing anaerobes to prevent ceftriaxone from degrading intestinal resistance to colonization. J Infect Dis 1989; 160: 274–280.

[24] MARIN ML, TEJADA-SIMON MV, LEE JH, MURTHA J, USTUNOL Z, PESTKA JJ. Stimulation of cytokine production in clonal macrophage and T-cell models by *Streptococcus thermophilus*: comparison with *Bifidobacterium* sp. and *Lactobacillus bulgaricus*. J Food Prot 1998; 61: 859–864.

[25] MARSHALL JC. Gastrointestinal flora and its alterations in critical illness. Curr Opin Clin Nutr Metab Care 1999; 2: 405–411.

[26] McFARLAND LV, SURAWICZ CM, GREENBERG RN, FEKETY R, ELMER GW et al. A randomised placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. J Am Med Assoc 1994; 271: 1913–1918.

[27] MIKEŠ Z, FERENČÍK M, JAHNOVÁ E, EBRINGER L, ČIZNÁR I. Hypocholesterolemic and immunomodulaory effects of orally applied *Enterococcus faecium M-74* in man. Folia Microbiol 1995; 40: 639–646.

[28] Reporting results of cancer treatment. Cancer 1981; 47: 207–214.

[29] NAUSEEF WM, MAKI DG. A study of the value of simple protective isolation in patients with granulocytopenia. N Engl J Med 1981; 304: 448–453.

[30] NEUMANN E, OLIVEIRA MA, CABRAL CM, MOURA LN, NICOLI JR et al. Monoassociation with *Lactobacillus acidophilus* UFV-H2b20 stimulates the immune defense mechanisms of germ free mice. Braz J Med Biol Res 1998; 31: 1565–1573.

[31] PERDIGON G, ALVAREZ S, RACHID M, AGUERO G, GOBBATO N. Immune system stimulation by probiotics. J. Dairy Sci 1995; 78: 1597–1606.

[32] REID G, BRUCE AW. Urogenital infections in women: can probiotics help? Postgrad Med J 2003; 79: 428–432.

[33] RESTA-LENERT S, BARRETT KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherischia coli* (EIEC). Gut 2003; 52: 988–997.

[34] RICHARD V, VAN DER AUWERA P, SNOECK R, DANEAU D, MEUNIER F. Nosocomial bacteriemia caused by *Bacillus* species. Eur J Microbiol Infect Dis 1998; 7: 783–785.

[35] RIQUELME AJ, CALVO MA, GUZMAN AM, DEPIX MS, GARCIA P et al. *Saccharomyces cerevisiae* fungemia after *Saccharomyces boulardii* treatment in immunocompromised patients. J Clin Gastroenterol 2003; 36: 41–43.

[36] SAINT-MARC T, ROSSELLO-PRATS L, TOURAINE JL. Efficacy of *Saccharomyces boulardii* in the treatment of diarrhea in AIDS (letter). Ann Med Intern 1991; 142: 64–65.

[37] SATONAKA K, OHASHI K, NOHMI T, YAMAMOTO T, ABE S et al. Prophylactic effect of *Enterococcus faecalis FK-23* preparation on experimental candidiasis in mice. Microbiol Immunol 1996; 40: 217–222.

[38] SIMON GL, GORBACH SL. Intestinal microflora in health and disease. Gastroenterology 1984; 86: 174–193.

[39] SCHIMPFF SC, YOUNG VM, GREEN WH. Origin of infection in acute lymphocytic leukemia: significance of hospital aquisition of potential pathogens. Ann Intern Med 1972; 77: 707–714.

[40] TAGUCHI H, TAKAHASHI M, YAMAGUCHI H et al. Experimental infection of germ-free mice with hyper-toxigenic enterohemorrhagic *Escherischia coli* O157:H7, strain 6. J Med Microbiol 2002; 51: 336–343.

[41] VANDENPLAS Y. Bacteria and yeast in the treatment of acuta and chronic infectious diarrhea. Part I. Bacteria. Clin Microbiol Infect 1999; 5: 299–307.