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Mini-review

Human microbial ecology: Lactobacilli, probiotics, selective decontamination

Marika Mikelsaar*

Department of Microbiology, Medical Faculty, University of Tartu, Tartu 50411, Estonia

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ABSTRACT

Health care-associated infections are closely associated with different medical interventions which interrupt the balance of human microbiota. The occasional predominance of opportunistic pathogens may lead to their translocation into the lymph nodes and bloodstream, causing endogenous (primary or secondary) hospital infections. The question is raised as to if there is a possibility for prevention of the imbalance of GI microbiota during medical interventions in critically ill patients. Prophylactic selective decontamination of the digestive tract (SDD) simultaneously applies three to four different antimicrobials for the suppression of enteric aerobic microbes, which are potentially pathogenic microorganisms. However, there is no convincing evidence that the indigenous beneficial intestinal microbiota are preserved, resulting in reduced mortality of high-risk patients. In this overview, we have evaluated the antimicrobial treatment guidelines of the Infectious Diseases Society of America (IDSA) for intra-abdominal infections in adults and seniors according to their safety for different *Lactobacillus* spp. The data from our group and in the literature have shown that all tested lactobacilli strains (nearly one hundred) were insusceptible to metronidazole while different species of lactobacilli of the three fermentation groups expressed particular antibiotic susceptibility to vancomycin, cefoxitin, ciprofloxacin and some new tetracyclines. We have relied on microbial ecology data showing that the GI tracts of adults and the elderly are simultaneously colonised at least with several (four to a maximum of 12) *Lactobacillus* species expressing variable intrinsic insusceptibility to the aforementioned antimicrobials, according to the provided data in table. This finding offers the possibility of preserving the colonisation of the intestine with some beneficial lactobacilli during antimicrobial treatment in critically ill patients with health care-associated infections. Several probiotic *Lactobacillus* spp. strains are intrinsically resistant to antimicrobials and can be used during antibacterial therapy, however, their application as an additive to antimicrobial treatment in critically ill patients needs to be investigated in well-designed clinical trials.

1. Introduction

The intestinal microbiota is a dynamic and complex mixture which is consisting of bacteria, archaea, some protozoa, anaerobic fungi and different bacteriophages and viruses with more than 1000 species of microbes inhabiting the human intestine [1–3]. There are a wide variety of host, microbiological and dietary factors that affect bacterial colonisation of the intestinal tract. Diverse functions, including the digestion of essential nutrients, maturation of intestinal epithelial cells and baseline physiologic parameters, including systemic effects on blood lipids, inhibition of harmful bacteria and stimulation of the immune system are carried out by microbiota [4,5]. Generally, these functions may be divided into three categories: structural, metabolic and protective [6]. The latter function has been widely used in the management of infections.

2. Prevalence of health care-associated infections

Variable environmental factors like food contamination with some pathogens and the high load of antibiotics in soil and animal feed simultaneously damage the microbial ecology of humans. From the standpoint of health care-associated infections, the wide shifts in human microbial ecology that are caused by medical issues have drawn a significant amount of attention by researchers. The application of broad-spectrum antimicrobial preparations for the treatment of infections and some inflammatory complications may cause a profound imbalance within the gastrointestinal (GI) microbiota [7–9]. The high carriage of opportunistic pathogens by different groups of individuals in the community, particularly the elderly and immuno-compromised persons, has been described [10,11]. Namely, during medical interventions which interrupt the balance of human microbiota, the occasional predominance of opportunistic pathogens may lead to translocation through the skin and mucosal membranes of GI tract but also from the respiratory

* Tel.: +372 7 374 179; fax: +372 7 374 172.
E-mail address: marika.mikelsaar@ut.ee.

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and urinary-genital tracts into the lymph nodes and bloodstream [12,13]. Furthermore, the possibility of the development of endogenous (primary or secondary) hospital infections is high [14].

A 2009 report from the CDC updated previous estimates of health care-associated infections showing that in American hospitals alone, health care-associated infections account for an estimated 1.7 million infections and 99,000 associated deaths each year. Of these infections, 32 percent of all health care-associated infections are urinary tract infections, 22 percent are surgical site infections, 15 percent are pneumonia (lung infections) and 14 percent are bloodstream infections (http://www.cdc.gov/ncidod/dhqp/hai.html). In Estonia, the most frequent bacterial agents responsible for mortality from nosocomial bacteraemia are enterococci (57%), *Candida* spp. (51%), *Pseudomonas* spp.(44%) and enterobacteria (40%), which are all host-derived opportunistic pathogens, mainly residing in the GI tract [15]. Enterococci have been listed as the third most common cause of nosocomial bacteraemias in the past two decades, implying their origin from disturbed intestinal microbial ecology of gut [16]. The question is therefore raised as to whether there is a possibility for the prevention of this imbalance of GI microbiota during medical interventions of critically ill patients.

3. Selective decontamination of GI tract

Prophylactic selective decontamination of the digestive tract (SDD) is a strategy for the prevention of colonisation and infection in high-risk patients. This is achieved by suppression of aerobic, potentially pathogenic microorganisms mainly from intestine while preserving anaerobic intestinal flora which serve to protect against secondary colonisation with Gram-negative aerobic bacteria [14]. SDD has been suggested for increased survival of critically ill patients [14,17]. Classically, the local application of polymyxin E, tobramycin and amphotericin B plus systemically-applied cefotaxim have been suggested [17]. During following years, the use of gentamicin, neomycin, ceftazidime, ceftriaxone, ciprofloxacin has been introduced. The meta-analyses of numerous randomised controlled trials has shown that SDD with enteral non-absorbable antimicrobials and short-course intravenous antibiotics reduces infections and mortality in the general population of critically ill and trauma patients without promoting the emergence of resistant gram-negative bacteria [14]. However, the composition of intestinal microbiota is importantly affected by SDD [18]. Moreover, concerning the mortality rate, there have been no studies which have specifically focussed on severe sepsis. As a result, SDD is not recommended by the International Surviving Sepsis Guidelines Committee. Further investigations are required to determine the comparative efficacy of SDD either with oral or intravenous application [19]. From the standpoint of the protective role of *Lactobacillus* spp., there are also no particular studies on their preservation under SDD regimens. Thus, there is a necessity for alternative approaches to combat endogenous hospital infections, e.g. health care-associated infections, by maintaining host microbiota.

4. Treatment of intra-abdominal infections

Recently, the Infectious Diseases Society of America (IDSA) has composed guidelines for the diagnosis and management, including antimicrobial treatment, of intra-abdominal infections in adults and seniors [20]. In adults, for community-acquired infections of mild to moderate severity, ticarcillin combined with clavulanate, cefoxitin, the newer carbapenems, fluoroquinolones and tetracyclines (e.g. ertapenem, moxifloxacin or tigecycline) as single-agent therapy against enteric Gram-negative aerobic and facultative bacilli and enteric Gram-positive streptococci have been suggested. In the case of the involvement of colonic obligate anaerobes, the combination of metronidazole with cefazolin, or some third generation cephalosporins, or fluoroquinolones such as levofloxacin and ciprofloxacin have been recommended. In adults, the community-acquired infection of high-risk patients can be treated with imipenem or ciprofloxacin, levofloxacin with metronidazole or aztreonam together with metronidazole. In the treatment tactics of critically ill patients, a promising approach such as keeping the selection of antimicrobials safe for the beneficial microbiota of humans, e.g. lactobacilli and bifidobacteria [21,22], could be challenging.

5. *Lactobacillus* spp. of GI tract

Lactobacillus spp. can be subdivided on metabolic grounds into three groups according to the type of sugar fermentation: the homofermenters (OHOL) *Thermobacterium*, and two genera of heterofermenters: facultative (FHEL) *Streptobacterium* and obligate heterofermenters (OHEL) of the *Betabacterium* groups [23–25]. During fermentation of polysaccharides in the colon, the lactobacilli strains of a particular species may use different pathways, differentially providing the host with energy. To date, however, this division of lactobacilli is not in accordance with their phylogenetic classification as revealed by analysis of 16S rDNA sequences [25], and therefore not much applied. Based on DNA–DNA hybridisation and other phylogenetic methods eight major groups have been distinguished: the *Lactobacillus buchneri*, *Lactobacillus delbrueckii*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus sakei*, *Lactobacillus salivarius* and *Lactobacillus brevis* group [26,27]. In the intestinal tract the most frequently observed species are *Lactobacillus acidophilus, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus johnsonii, Lactobacillus jensenii, Lactobacillus amylovorus, L. delbrueckii, Lactobacillus helveticus, L. salivarius, Lactobacillus ruminis, L. casei, Lactobacillus paracasei, L. sakei*, *Lactobacillus curvatus*, *Lactobacillus mucosae, Lactobacillus rhamnosus, L. plantarum, L. reuteri*, *Lactobacillus fermentum, L. brevis, L. buchneri* ([28–37]. In our laboratory, the species-specific PCR analysis of *Lactobacillus* spp. combined with viable plating data have indicated substantial age-related structural differences in intestinal lactobacilli communities [38–40]. All the above-mentioned species have also been detected in healthy adult and elderly persons; however, we have not found *L. jensenii, L. amylovorus* and *L. mucosae.* At the same time, *L. casei* and *L. ruminis* of the FHEL group were the most prevalent ($\geq$70%) *Lactobacillus* species detected in both adults and the elderly. This coincides with Ahrne et al. [34] who have reported the FHEL group lactobacilli (*L. casei, L. plantarum* and *L. paracasei)* as the most prevalent species in the mucosa of GI tract. Concerning differences in the elderly, the total counts of lactobacilli and the number of different *Lactobacillus* spp. were significantly higher than in adults. Besides, the high prevalence of *L. acidophilus* and *L. helveticus* (79%, 66%, respectively) was characteristic for adults, but in the elderly the prevalence of *L. paracasei* (97%), *L. plantarum* (57%) and *L. reuteri* (51%) exceeded that of adults. The individual specificity of human microbiota has been clearly demonstrated by the high number of different *Lactobacillus* spp. simultaneously inhabiting the GI tract of the person under investigation: in adults, the median number of species was lower than in the elderly, respectively four to 12 ($p = 0.042$) [40].

6. *Lactobacillus* spp. susceptibility to antimicrobial agents

Lactobacillus species susceptibility to antimicrobials [41–43] has been previously described by several researchers. Due to the possibility of studying the taxonomically well-characterised isolates of *Lactobacillus* species and applying the E-test technique

(AB Biodisk, Solna, Sweden), we have presented an overview of the level of antibiotic susceptibility of the more prevalent intestinal *Lactobacillus* spp. fermentative groups and separate species to antimicrobials used in the treatment of critically ill patients (Table 1). In our study in 2001 [42], we tested 65 *Lactobacillus* spp. strains, whereas 93 strains were included in our paper in 2010 [44,45]. The Clinical and Laboratory Standards Institute (CLSI) guidelines of breakpoints of Gram-positive bacteria were used [45]. All tested lactobacilli of the three groups (OHOL, FHEL and OHEL) were susceptible to ampicillin, gentamicin and erythromycin (data not included in Table 1). Out of the selected set of antimicrobials suggested by IDSA for the treatment of critically ill patients with GI involvement, the *Lactobacillus* spp. were uniformly susceptible to the betalactams, second generation cephalosporins and carbapenems. Conversely, all the tested lactobacilli were resistant to metronidazole and its combination with aztreonam (aimed at Gram-negative bacteria). Concerning cefoxitin, ciprofloxacin and vancomycin, the fermentative group and species-dependent antimicrobial susceptibility of different isolates of *Lactobacillus* spp. has emerged, whereas in the case of vancomycin, the entire FHEL and OHEL groups were found to be intrinsically insensitive.

Further, using the microecological approach, we suggest that antimicrobials such as cefoxitin, ciprofloxacin, some new tetracyclines, metronidazole and vancomycin seem to be suitable for the preservation of lactobacilli. Here, we have relied on the understanding that the GI tract of individuals is colonised at least with several (four to a maximum of 12) species of lactobacilli of different fermentation groups which survive differentially due to various antibiotic susceptibilities, as demonstrated in Table 1. For instance, in adult humans, with the use of cefoxitin therapy, a majority of *L. fermentum* strains and approximately half of the prevalent enteric strains of *L. acidophilus* and *L. gasseri* might survive due to their high insusceptibility to the aforementioned antimicrobial. In case of ciprofloxacin, some 83% of *L. acidophilus* and 86% of *L. plantarum* strains have the potential to survive, while the majority of the *L. paracasei* strains are susceptible. Concerning vancomycin, strains other than those of the OHOL group may survive under therapy with this antibacterial agent. This is an important consideration for the elderly with a high prevalence of indigenous FHEL strains in the case of vancomycin treatment for *Clostridium difficile*-derived infections [46]. The protection against intestinal colonization by *C. difficile* due to high counts of lactobacilli has been shown previously [47]. However, it can not be excluded that, due to the individual diversity of intestinal *Lactobacillus* spp. composition [38,40] the particular insusceptible species of lactobacilli could be absent and no defence is provided in that particular individual.

Our data for suitable antimicrobials coincided with those reported in the literature [48–51]. We were not concerned with the acquired antibiotic resistance of *Lactobacillus* spp. that until now has only been convincingly demonstrated towards tetracyclines in multiple species of lactobacilli [50] and particularly in *L. buchneri* [44]. Still, Egervärn et al. [51] have reported on the correlation between the genotype and clustered high MIC values for both ampicillin and tetracycline in some strains of *L. reuteri*, but not *L. fermentum*.

Thus, there seems to be the possibility for application of some antimicrobials suggested by IDSA which still preserve the intestinal indigenous lactobacilli due to their wide individual composition.

7. Additive treatment values with probiotics in critically ill patients

Gut microbiota modifiers include probiotics, prebiotics and synbiotics. Probiotics are nonpathogenic live microorganisms(e.g. bacteria, yeast) originating from the human GI tract. When given in adequate amounts as dietary supplements and foods, they may provide a health benefit for the host due to their particular functional properties. To correct the imbalance of microbiota composition that is closely associated with host health markers, the application of functional food, including probiotics has been suggested in patients treated with antimicrobials following surgery [52]. Several probiotic strains are intrinsically resistant to antimicrobials and can be used during specific antibacterial therapy. For instance, *L. rhamnosus* GG (LGG) is intrinsically resistant to metronidazole and vancomycin (both MIC >256) applied in the treatment of antibiotic-associated diarrhoea and pseudomembranous colitis [4,53]. Besides, LGG is also highly resistant (MIC >256) to cefoxitin and fusidic acid (unpublished data). *L. fermentum* ME-3 (DSM14241) is suggested as probiotic additive to ofloxacin

Table 1
Antibiotic susceptibility (S) of *Lactobacillus* spp. (MIC μg/ml) for antimicrobials suggested by IDSA [20] for treatment of intra-abdominal infections in critically ill patients (compiled according Mändar et al. [42]; Kõll et al. [44]).

Species of lactobacilli	MIC (μg/ml) by E-test; S%							
	AM BP16	CFX BP32	CXM BP32	IP BP 1	CIP BP 4	TET BP16	METR BP 32	VAN BP 32
OHOL								
L. acidophilus	0.19	6->256; *S* 53%	0.5	1	>32; S 13%	4; S 93%	>256	0.5; S 93%
L. gasseri	0.25	1.5->254; *S* 50%	0.5	1.5	>32	0.75	>256	1
L. delbrueckii	0.125	24	0.25	0.047	>32	0.5	>256	1
L. salivarius	0.125	3	0.125	0.032	>32	0.25	>256	>256
FHEL								
L. paracasei	0.75	>256	1–12; S 94%	1.5	1->32; S 94%	0.75	>256	>256
L. plantarum	0.016	>256	0.125	0.047	2; S 14%	8; S 43%	>256	>256
OHEL								
L. fermentum	0.125	>256	0.75, 1	0.047	0.5	2	>256	>256
L.buchneri	0.5	8	0.125	0.064	1.2; S 50%	6–24; S 67%	>256	>256
L. brevis	ND	S 14%	S 43%	ND	1; S 0%	S 29%	>256	>256
Altogether	S	VS	S	S	VS	VS	IS	IS FHEL and OHEL

AM – ampicillin; CFX – cefoxitin; IP – imipenem; CXM – cefuroxime; METR – metronidazole; VAN – vancomycin; MFL – moxifloxacin vs. CIP – ciprofloxacin, TET – tetracycline, BP – breakpoint, ND- not determined; S – susceptible, IS – insusceptible, VS – various susceptibility of particular spp. The susceptibility (S %) and MIC values of particular species of lactobacilli have been composed according to the data of Mändar et al. [42]; Kõll et al. [44]) of intestinal lactobacilli in 1- to 2-year-old Swedish and Estonian children. The suggested by IDSA [20] antimicrobials like cefazolin were substituted for cefuroxime, ertapenem for imipenem, moxifloxacin for ciprofloxacin and tigecycline for tetracycline. Susceptibility of the reference strains did not differ from strains under investigation; no differences were also connected with the origin of strains. The MIC values of lactobacilli to previously reported values of moxifloxacin [50] are somewhat lower than these of ciprofloxacin indicating more efficient preservation of lactobacilli.In both studies the minimum inhibitory concentrations (MICs) to 13 antibiotics were determined using Wilkins-Chalgren (Oxoid) agar plates with 5% horse blood and E-test antibiotic strips (AB Biodisk, Solna, Sweden) at 48 h of incubation at 37 °C in an anaerobic glove chamber. The breakpoints were determined in accordance with the CLSI guidelines for gram-positive microorganisms [45].

(MIC 8 μg/ml) in the treatment of *Salmonella enterica* Typhimurium experimental infections [54].

However, there is a need for evidence based on the efficacy of probiotic treatments using properly designed clinical trials. In the case of critically ill patients, the first studies investigating the use of probiotic application have not yet been successful [55]. In a DBPCR (Double Blind Placebo-Controlled Randomised) registered multicentre trial of patients with severe pancreatitis ($n = 298$ patients) whose ordinary treatment was supported by a multistrain probiotic, administered at a high dose (10^{10} cfu/dose), the mortality for the probiotic group was 16% in comparison with the placebo group of 6% ($p < 0.04$). Endotoxaemia due to the high prevalence of *Escherichia coli* in the small intestine, multi-organ failure with bowel ischemia and damage to the gut mucosa were presented as reasons for failure. Thus, currently there are limitations and concerns about the optimal doses and strains used in critically ill patients and therefore the gut microbiota modifiers should be avoided [14].

Moreover, in the selection of probiotic *Lactobacillus* species for different age groups, the interconnection of different species with shifts of some health indices such as blood glucose content and white blood cell count (WBC) of the host have not been properly considered [39,40]. For instance, in the elderly, the colonisation of the gut with indigenous *L. reuteri* was significantly associated with increased WBC adjusted for age, sex, body mass index and consumption of probiotics [39]. It is possible that in individuals with inflammation, the introduction of the probiotic of species *L. reuteri* can promote the inflammatory state and increase the complication risk for critically ill patients. Recently, the leukocyte count has been used as a predictor of cardiovascular events and mortality [56]. Moreover, we have found that intestinal *Lactobacillus* spp. diversity in adults and the elderly was closely connected to metabolic shifts in blood sera. We have demonstrated that some species in the OHOL group, namely *L. acidophilus*, were associated with increased blood glucose levels in adults, while *L. paracasei* was found to decrease this level in the elderly.

8. Conclusion

The correct antibiotic policy should help to sustain beneficial *Lactobacillus* spp. and prevent the imbalance of GI microbiota in critically ill patients. Properly designed clinical trials must be performed to determine the efficacy of probiotic treatments. These will both be the first steps in the prevention of secondary infections in critically ill patients.

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References

[1] Mackie R. Mutualistic fermentative digestion in the gastrointestinal tract: diversity and evolution. Integ Comp Biol 2002;42:319–26.
[2] Lay C, Rigottier-Gois L, Holmstrom K, Rajilic M, Vaughan EE, de Vos WM, et al. Colonic microbiota signatures across five northern European countries. Appl Environ Microbiol 2005;71:4153–5.
[3] Dethlefsen L, Eckburg PB, Bik EM, Relman DA. Assembly of the human intestinal microbiota. Trends Ecol Evol 2006;21:517–23.
[4] McFarland L. Renewed interest in a difficult disease: *Clostridium difficile* infections - epidemiology and current treatment strategies. Curr Op Gastroenterol 2008;25:24–35.
[5] Canny GO, McCormick BA. Bacteria in the intestine, helpful residents or enemies from within? Infect Immun 2008;76:3360–73.
[6] Guarner F. Enteric flora in health and disease. Digestion 2006;73(Suppl. 1):5–12.
[7] Andremont A. Consequences of antibiotic therapy to the intestinal ecosystem. Ann Fr Anesth Reanim 2000;19:395–402.
[8] Woodmansey EJ, McMurdo ME, Macfarlane GT, Macfarlane S. Comparison of compositions and metabolic activities of fecal microbiotas in young adults and in antibiotic-treated and non-antibiotic-treated elderly subjects. Appl Environ Microbiol 2004;70:6113–22.
[9] Zuccotti GV, Meneghin F, Raimondi C, Dilillo D, Agostoni C, Riva E, et al. Probiotics in clinical practice: an overview. J Int Med Res 2008;36(Suppl. 1): 1A–53A.
[10] Bartosch S, Fite A, Macfarlane GT, McMurdo ME. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. Appl Environ Microbiol 2004;70:3575–81.
[11] Burlaud A, Mathieu D, Falissard B, Trivalle C. Mortality and bloodstream infections in geriatrics units. Arch Gerontol Geriatr 2010;51:e106–9.
[12] Leibovici L, Pitlik SD, Konisberger H, Drucker M. Bloodstream infections in patients older than eighty years. Age Ageing 1993;22:431–42.
[13] Cretel E, Veen I, Pierres A, Bongrand P, Gavazzi G. Immunosenescence and infections, myth or reality? Med Mal Infect 2010;40:307–18.
[14] Haas LE, Schultz MJ. Selective decontamination of the digestive tract reduces pneumonia and mortality. Crit Care Res Pract 2010;2010:501031.
[15] Mitt P, Adamson V, Loivukene K, Lang K, Telling K, Paro K, et al. Epidemiology of nosocomial bloodstream infections in Estonia. J Hosp Infect 2009;71:365–70.
[16] Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004;39: 309–17.
[17] Stoutenbeek CP, van Saene HK, Zandstra DF. The effect of oral non-absorbable antibiotics on the emergence of resistant bacteria in patients in an intensive care unit. J Antimicrob Chemother 1987;19:513–20.
[18] Benus RF, Harmsen HJ, Welling GW, Spanjersberg R, Zijlstra JG, Degener JE, et al. Impact of digestive and oropharyngeal decontamination on the intestinal microbiota in ICU patients. Intensive Care Med 2010;36:1394–402.
[19] Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Intensive Care Med 2008;34:17–60.
[20] Solomkin JS, Mazuski JE, Bradley JS, Rodvold KA, Goldstein EJ, Baron EJ, et al. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. Clin Infect Dis 2010;50:133–64.
[21] Vaughan EE, Heilig HG, Ben-Amor K, de Vos WM. Diversity, vitality and activities of intestinal lactic acid bacteria and bifidobacteria assessed by molecular approaches. FEMS Microbiol Rev 2005;29:477–90.
[22] Tannock GW. A special fondness for lactobacilli. Appl Environ Microbiol 2004; 70:3189–94.
[23] Kandler O, Weiss N. Regular Non-sporing Gram-positive Rods. 1986:1209–1234.
[24] Hammes W, Vogel RF. The Genus Lactobacillus. 1995:19–54.
[25] Axelsson L. Lactic acid bacteria: classification physiology. 1998:1–72.
[26] Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, et al. Demonstration of safety of probiotics – a review. Int J Food Microbiol 1998; 44:93–106.
[27] Felis GE, Dellaglio F. Taxonomy of lactobacilli and bifidobacteria. Curr Issues Intest Microbiol 2007;8:44–61.
[28] Rettger L. *Lactobacillus acidophilus*. Its Therapeutic Application. New Haven: Yale University Press; 1935.
[29] Lerche M, Reuter G. A contribution to the method of isolation and differentiation of aerobic "lactobacilli" (Genus "*Lactobacillus* Beijerinck"). Zentralbl Bakteriol 1960;179:354–70.
[30] Finegold SM, Sutter VL, Sugihara PT, Elder HA, Lehmann SM, Phillips RL. Fecal microbial flora in seventh day adventist populations and control subjects. Am J Clin Nutr 1977;30:1781–92.
[31] Moore WE, Cato EP, Holdeman LV. Some current concepts in intestinal bacteriology. Am J Clin Nutr 1978;31:S33–42.
[32] Molin G, Jeppsson B, Johansson ML, Ahrne S, Nobaek S, Stahl M, et al. Numerical taxonomy of *Lactobacillus* spp. associated with healthy and diseased mucosa of the human intestines. J Appl Bacteriol 1993;74:314–23.
[33] Tannock GW. The microecology of lactobacilli inhabiting the gastrointestinal tract. 1990:147–171.
[34] Ahrne S, Nobaek S, Jeppsson B, Adlerberth I, Wold AE, Molin G. The normal *Lactobacillus* flora of healthy human rectal and oral mucosa. J Appl Microbiol 1998;85:88–94.
[35] Heilig HG, Zoetendal EG, Vaughan EE, Marteau P, Akkermans AD, de Vos WM. Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA. Appl Environ Microbiol 2002;68:114–23.
[36] Dal Bello F, Walter J, Hammes WP, Hertel C. Increased complexity of the species composition of lactic acid bacteria in human feces revealed by alternative incubation condition. Microb Ecol 2003;45:455–63.
[37] Walter J. Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. Appl Environ Microbiol 2008; 74:4985–96.

[38] Mikelsaar M, Annuk H, Stsepetova J. Intestinal lactobacilli of Estonian and Swedish children. Microb Ecol Health Dis 2002;14:75–80.
[39] Mikelsaar M, Stsepetova J, Hutt P, Kolk H, Sepp E, Loivukene K, et al. Intestinal *Lactobacillus* spp. is associated with some cellular and metabolic characteristics of blood in elderly people. Anaerobe 2010;16:240–6.
[40] Stsepetova J, Sepp E, Kolk H, Lõivukene K, Songisepp E, Mikelsaar M. Diversity and metabolic impact of intestinal *Lactobacillus* spp. in healthy adults and the elderly. Brit J Nutr 2011;105:1235–44.
[41] Charteris WP, Kelly PM, Morelli L, Collins JK. Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. J Food Prot 1998;61:1636–43.
[42] Mändar R, Lõivukene K, Hütt P, Karki T, Mikelsaar M. Antibacterial susceptibility of intestinal lactobacilli of healthy children. Scand J Infect Dis 2001;33:344–9.
[43] Danielsen M, Wind A. Susceptibility of *Lactobacillus* spp. to antimicrobial agents. Int J Food Microbiol 2003;82:1–11.
[44] Koll P, Mandar R, Smidt I, Hutt P, Truusalu K, Mikelsaar RH, et al. Screening and Evaluation of human intestinal lactobacilli for the development of Novel gastrointestinal probiotics. Curr Microbiol 2010;61:560–6.
[45] Jorgensen J, Turnidge JD. Susceptibility test methods; general conditions; 2003.
[46] Bauer MP, Kuijper EJ, van Dissel JT. European Society of clinical Microbiology and Infectious Diseases (ESCMID): treatment guidance document for *Clostridium difficile* infection (CDI). Clin Microbiol Infect 2009;15:1067–79.
[47] Naaber P, Klaus K, Sepp E, Bjorksten B, Mikelsaar M. Colonization of infants and hospitalized patients with *Clostridium difficile* and lactobacilli. Clin Infect Dis 1997;25(Suppl. 2):S189–90.
[48] Delgado S, Suarez A, Mayo B. Dominant cultivable *Lactobacillus* species from the feces of healthy adults in northern Spain. Int Microbiol 2007;10:141–5.
[49] Klare I, Konstabel C, Muller-Bertling S, Reissbrodt R, Huys G, Vancanneyt M, et al. Evaluation of new broth media for microdilution antibiotic susceptibility testing of Lactobacilli, Pediococci, Lactococci, and Bifidobacteria. Appl Environ Microbiol 2005;71:8982–6.
[50] Klare I, Konstabel C, Werner G, Huys G, Vankerckhoven V, Kahlmeter G, et al. Antimicrobial susceptibilities of *Lactobacillus*, Pediococcus and Lactococcus human isolates and cultures intended for probiotic or nutritional use. J Antimicrob Chemother 2007;59:900–12.
[51] Egervärn M, Danielsen M, Roos S, Lindmark H, Lindgren S. Antibiotic susceptibility profiles of *Lactobacillus reuteri* and *Lactobacillus fermentum*. J Food Prot 2007;70:412–8.
[52] Bengmark S. Ecoimmunonutrition: a challenge for the third millennium. Nutrition 1998;14:563–72.
[53] Salminen MK, Rautelin H, Tynkkynen S, Poussa T, Saxelin M, Valtonen V, et al. *Lactobacillus bacteremia*, species identification, and antimicrobial susceptibility of 85 blood isolates. Clin Infect Dis 2006;42:e35–44.
[54] Truusalu K, Mikelsaar RH, Naaber P, Karki T, Kullisaar T, Zilmer M, et al. Eradication of Salmonella Typhimurium infection in a murine model of typhoid fever with the combination of probiotic *Lactobacillus fermentum* ME-3 and ofloxacin. BMC Microbiol 2008;8:132.
[55] Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008;371:651–9.
[56] Margolis KL, Manson JE, Greenland P, Rodabough RJ, Bray PF, Safford M, et al. Leukocyte count as a predictor of cardiovascular events and mortality in postmenopausal women: the Women's Health Initiative Observational Study. Arch Intern Med 2005;165:500–8.