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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**A B S T R A C T**

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...
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/dhp/hai.html). In Estonia, the most frequent bacterial agents responsible for mortality from nosocomial bacteremia 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 bacteremia 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, cefazidime, 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 patients [14,17,18]. 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. ceftepime, 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 rRNA 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 (≥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 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

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### Table 1

Antibiotic susceptibility (%) 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]; Köll et al. [44]).

<table>
<thead>
<tr>
<th>Species of lactobacilli</th>
<th>AM BP16</th>
<th>CFX BP32</th>
<th>CXM BP32</th>
<th>IP BP1</th>
<th>CIP BP4</th>
<th>TET BP16</th>
<th>METR BP32</th>
<th>VAN BP32</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OHOL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>0.19</td>
<td>&gt;256; 53</td>
<td>0.5</td>
<td>1</td>
<td>&gt;32; 13</td>
<td>4; 93</td>
<td>&gt;256</td>
<td>0.5; 93</td>
</tr>
<tr>
<td>L. gasseri</td>
<td>0.25</td>
<td>1.5–254; 50</td>
<td>0.5</td>
<td>1.5</td>
<td>&gt;32</td>
<td>0.75</td>
<td>&gt;256</td>
<td>1</td>
</tr>
<tr>
<td>L. delbrueckii</td>
<td>0.125</td>
<td>24</td>
<td>0.25</td>
<td>0.047</td>
<td>&gt;32</td>
<td>0.5</td>
<td>&gt;256</td>
<td>1</td>
</tr>
<tr>
<td>L. salivarius</td>
<td>0.125</td>
<td>3</td>
<td>0.125</td>
<td>0.032</td>
<td>&gt;32</td>
<td>0.25</td>
<td>&gt;256</td>
<td>&gt;256</td>
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<tr>
<td><strong>FHEL</strong></td>
<td></td>
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</tr>
<tr>
<td>L. paracasei</td>
<td>0.75</td>
<td>&gt;256</td>
<td>1–12; 94</td>
<td>1.5</td>
<td>&gt;32; 94</td>
<td>0.75</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>0.016</td>
<td>&gt;256</td>
<td>0.125</td>
<td>0.047</td>
<td>2; 14</td>
<td>0.4</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td><strong>OHEL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. fermentum</td>
<td>0.125</td>
<td>&gt;256</td>
<td>0.75, 1</td>
<td>0.047</td>
<td>0.5</td>
<td>2</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>L. buchneri</td>
<td>0.5</td>
<td>8</td>
<td>0.125</td>
<td>0.064</td>
<td>12; 50</td>
<td>6–24; 67</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>L. brevis</td>
<td>ND</td>
<td>S 14%</td>
<td>S 43%</td>
<td>ND</td>
<td>S 0%</td>
<td>S 29%</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Altogether</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

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 (%) and MIC values of particular species of lactobacilli have been composed according to the data of Mändar et al. [42]; Köll 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].
with in the gut with indigenous increased WBC adjusted for age, sex, body mass index and formed to determine the ef
spp. and prevent the imbalance of GI microbiota in Lactobacillus 8. Conclusion with increased blood glucose levels in adults, while to metabolic shifts in blood sera. We have demonstrated that some spp. diversity in adults and the elderly was closely connected 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 Lactoba-
cillus 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
