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Comparative Assessment of Antibiotic Resistance in Lactic Acid Bacteria Isolated from Healthy Human Adult and Infant Feces

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Abstract

Lactic acid bacteria are normal inhabitants of the gastrointestinal tract of humans. Their occurrence in infant and adult feces is abundant. The current study assesses and compares the antibiotic resistance in lactic acid bacteria isolated from healthy human adult and healthy infant fecal samples. A total of 255 lactic acid bacteria isolates (126 from adult feces and 129 from infant feces) were isolated and characterized from 60 fecal samples. *Lactobacillus* spp., *Pediococcus* spp. and *Enterococcus* spp. were included in the study. The study was done using the WHONET software for the analysis of antibiotic susceptibility data of lactic acid bacteria. Most of the *Lactobacillus* and *Pediococcus* strains were sensitive to vancomycin. *Enterococcus* strains showed resistance against vancomycin. Ampicillin, ciprofloxacin and cefuroxime resistance were significantly ($p<0.05$) higher in *Lactobacillus* strains isolated from adult fecal samples than those isolated from infant fecal samples. A similar pattern was observed in *Enterococcus* strains with erythromycin, gentamycin and tobramycin resistance. *Pediococcal* isolates from adult feces showed significantly higher resistance against tobramycin, ciprofloxacin, gentamycin, cefotaxime and cefuroxime in comparison with infant fecal isolates. Antibiotic resistance was exhibited by lactic acid bacteria against most commonly used antibiotics and it was higher in strains isolated from adult fecal samples than in the strains isolated from infant fecal samples. The increasing trend in antibiotic resistance from infant to adult might be due to food habits and antibiotic intakes. Thus, the widespread antibiotic resistance in different lactic acid bacteriamay pose a food safety concern as well.

Keywords: Lactic acid bacteria, Antibiotic resistance, *Lactobacillus*, Feces, Fecal microbes

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Introduction

The lactic acid bacteria (LAB) originate from a taxonomically diverse group of microorganisms, which are non-sporing rods and cocci, usually non-motile that ferment carbohydrates and form lactic acid. Lactic acid bacteria contain the genera namely *Lactobacillus, Lactococcus*, *Pediococcus*, *Streptococcus*, *Enterococcus,* *Oenococcus,* *Leuconostoc*, *Carnobacterium*, *Vagococcus*, *Tetragenococcus,* and *Weissella* [1]. The microflora of humans and animal gut is complex and it is primarily dominated by lactic acid bacteria. There is high density and rich diversity of microorganisms in the gut, and the microflora complexity increases from the upper gastrointestinal tract to the colon [2]. The human gut contains more than a thousand bacterial species and some of them start to colonize the gut during infancy [3]. Soon after the birth of a newborn infant, the gut flora begins to develop and microbes start to colonize the small intestine and large intestine. Aerobic and facultative anaerobic bacteria (*Enterobacteria*, *Enterococci* and *Streptococci*) are the early colonizers in the human gut. After they colonize, they create anaerobic environment in the gut. This helps anaerobic bacteria (*Bifidobacteria*, *Bacteroides* and *Clostridia*) to start with their colonization majorly in the large intestine [4]. The development of complex, diverse and stable microflora continues from infancy to one year of age. After a year it is similar to adults and it is stable [4]. Many factors are governing the development, diversity, composition and colonization gut microflora of infants, out of which mother's gut microflora, food and environment are the deciding ones [5]. During birth, an infant is exposed to the mother's vaginal microflora and also to fecal microflora, and with this exposure colonization of the gut in infants begins [6]. Infant gut microflora is affected by colostrum and later by breast milk. After the introduction of formula and solid foods, complexity and diversity is generated in the gut microflora of infants. Microbes present in the environment and those present directly on the skin of the infant also enter the gut and create a complex niche [7]. Colonization of the gut with diverse microflora creates continuous impacts on the immune system; and in this process, it strengthens the immune system [8].

Over the past few decades, there has been a huge interest developed in LAB physiology and genetics, involving their increasing importance as starter cultures in different industrial fermentation processes and also as probiotics. Since probiotics are directly administered in humans and animals it is very necessary to determine the level of antibiotic resistance. This is a part of the assessment of the safety of the probiotic cultures which are administered as therapeutics.

In the past 60 years, approximately 10 million tons of antibiotics have been utilized and released into the environment. As presented in the reports of European Commission there is a huge probability of the spread of antibiotic resistance in the biosphere [9]. Hence,there is a very strong selective pressure in the development of antibiotic resistance in bacterial strains [10].

Lactic acid bacteria dominate the gastrointestinal tract of humans. They are present in large amounts in the gut and are also added or sometimes additionally consumed along with the regular diet. Hence, it is speculated that the presence of antibiotic resistance in lactic acid bacteria used as probiotics can be dangerous. Probiotics are generally administered to maintain microbial balance during gastrointestinal tract infections such as diarrhea. They are administered as therapeutic agents along with antibiotics. If probiotics harbor antibiotic-resistant genes, it could be beneficial in sustaining the antibiotics during the treatment; however, there is a risk of antibiotic-resistant probiotic strains to transfer the resistance genes to the pathogenic bacteria. This could complicate the treatment of a patient with an antibiotic-resistant bacterial infection or disease. Additionally, there is the possibility of the transfer of antibiotic resistance from beneficial lactic acid bacteria, in the food chain. Therapy with any antibiotic, particularly long term and especially oral administration is liable to alter the balance of antibiotic-resistant to sensitive organisms in the intestine [11].

Certain strains of these genera are more commonly used in the food and especially dairy industries or as probiotics [12]. The World Health Organization (WHO) has established a program known as the Antimicrobial Resistance Monitoring (ARM) program for monitoring antimicrobial resistance. WHO has also devised an electronic format WHONET, freely available to download. A special focus of antimicrobial susceptibility test results is available on windows-based database software, developed for the management and analysis of microbiology data [13]. This study aimed to determine the antibiotic susceptibility of lactic acid bacteria (using WHONET software) isolated from adult and infant feces to various groups of antibacterial agents that are mainly isolated from the feces of breastfed infants. Also, the comparative assessment was done to determine the isolates that are more resistant to antibiotics.

Materials and Methods

Sample collection and ethics statement

Thirty healthy adult human volunteers (from Mumbai and Suburbs, India) aged between 25 and 30, who were not suffering from any chronic disease, had not taken antibiotics, proton pump inhibitors, bismuth compounds, Histamine H2-receptor, nonsteroidal anti-inflammatory drugs within the previous 6 months, were selected for the study. Similarly, fecal samples were also collected from thirty healthy infants aged between 3 months to 9 months. Infants who were exclusively breast-fed, healthy and free from acute or chronic disease were selected in the study. The study protocol was approved by an independent ethical committee and performed in compliance with the US Code of Federal Regulations on Good Clinical Practices (21 CFR 10.90, 50, 56 and 812) and the World Medical Association Declaration of Helsinki (1996 amendment) [14]. All adult volunteers and parents of infants signed informed consent before samples were collected.

Isolation of lactic acid bacteria from the fecal sample

Fecal samples were collected in sterile polypropylene containers and processed immediately as follows. A 0.5 g portion of feces was taken from mid sample, added in 4.5 ml of sterile

Table 1. Count of LAB isolates in the adult and infant fecal samples.

Sample source	Number of samples (n = 60)	LAB isolates (n = 255)	*Lactobacillus* spp. (n=90)	*Pediococcus* spp. (n=84)	*Enterococcus* spp. (n=81)
Adult feces	30	126	46	41	39
Infant feces	30	129	44	43	42

*LAB= Lactic Acid Bacteria

saline solution, and completely homogenized. A dilution series (10^{-1} to 10^{-7}) was made and 100 µl aliquots of each dilution were inoculated on the agar plates by spread plating. Rogosa SL agar (Hi-Media, Mumbai, India) was used to isolate LAB and the plates were incubated micro-aerobically for 3 days at 37°C. Kenner fecal (KF) agar was used for the isolation of *Enterococcus* and incubated aerobically at 37°C for 24 h [15].

Enumeration and selection of bacterial isolates

After incubation, the plates that showed discrete colonies were selected and the colonies were counted. The total count of Lactic acid bacteria in feces was expressed as colony-forming units/g (wet weight). From each fecal sample, 10-20 colonies of LAB were randomly selected. A provisional identification of genera was made based on Gram's staining, and catalase reaction using 3% (v/v) H_2O_2 on single colonies. Putative Lactobacilli colonies (Gram-positive, catalase test-negative, rod-shaped) were chosen and further purified using MRS agar. Similarly, putative colonies of Enterococci and Pediococci (Gram-positive, catalase test-negative, cocci, able to grow at 10°C and 45°C, and in 18% NaCl and at pH 4.4) from KF agar plates were purified by re-streaking on the MRS agar. The cultures were stored in MRS broth with 15% glycerol at –20°C [15].

Antibiotic resistance

The antibiotic resistance/susceptibility patterns of isolated strains of lactic acid bacteria were studied using the Kirby-Bauer disk diffusion method (according to the CLSI document M2-A9 suggestions) [16]. The antibiotics used in this study were penicillin (10 µg), ampicillin (10 µg), vancomycin (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), tobramycin (10 µg), erythromycin (15 µg) and chloramphenicol (30 µg). The culture densities were adjusted to McFarland 1.5; they were spread on MRS agar plates. Antibiotic discs (Hi-Media, Mumbai, India) were placed on the surface of the agar plates, which were incubated at 37°C for 24 h. The diameters of the clearance zones around the discs were measured and the result (the average of 2 readings) was expressed as susceptible, intermediate, or resistant according to the standard disc diffusion method [16]. The experiment was done in triplicates. Microsoft Excel (2013) was used to obtain data in the appropriate format for BacLink 2019, used to format data to be used in WHONET 2019, which automatically calculates the % resistance using a data analysis tool.

Statistical analysis

The data was analyzed to check the significant difference between groups using Student's T-test with a probability level of 0.05 ($P < 0.05$) using Microsoft Excel (2013).

Results

Isolation of lactic acid bacteria from the fecal sample

A total of 255 LAB isolates were isolated from 30 human adult and 30 human infant fecal samples. Out of the 255 isolates, 126 isolates were from the adult fecal sample, and 129 from the infant fecal sample, the results are presented in **Table 1.** The isolates were identified phenotypically and characterized. Based on the characters, the LAB isolates were characterized as mesophilic homofermentative cocci, able to grow at 10°C and 45°C as *Enterococcus* (81 isolates). Homofermentative cocci in tetrads, unable to grow in 18% NaCl, and showing growth at pH 4.4 were characterized as *Pediococcus* (84 isolates). Lactobacilli (90 isolates) were represented as catalase-negative, slender gram-positive rods. All strains grew at 4°C and 6.5% NaCl concentration.

Antibiotic resistance of lactic acid bacteria

Data of diameter of zone of clearance in mm of LAB isolated from adult and infant feces was entered in Microsoft Excel and via BacLink software incorporated into WHONET software (**Table 2**).

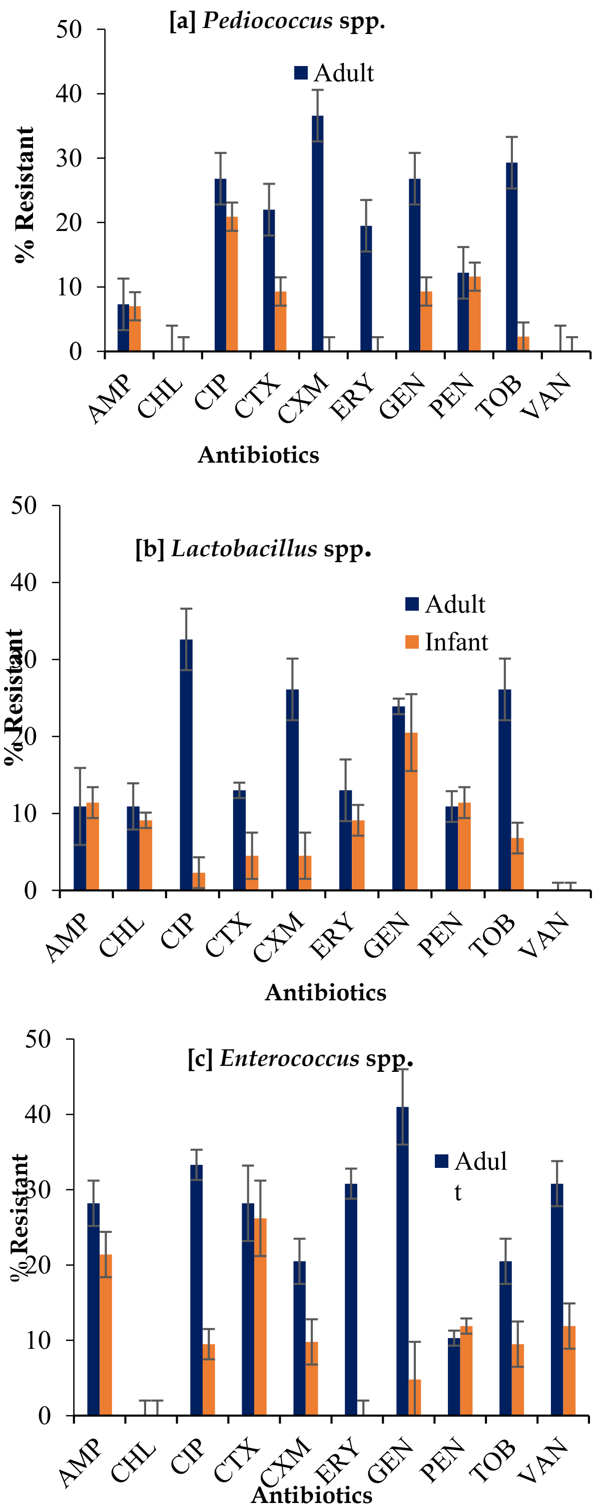

Figure 1. Antibiotic resistance pattern of *Pediococcus* spp. (a), *Lactobacillus* spp. (b) and *Enterococcus* spp. (c) isolated from adults and infant feces, respectively [AMP-Ampicillin, CHL-Chloramphenicol, CIP-Ciprofloxacin, CTX-Cefotaxime, CXM-Cefuroxime, ERY-Erythromycin, GEN-Gentamicin, PEN-Penicillin G, TOB-Tobramycin, VAN-Vancomycin]. All experiments were performed in triplicates and the error bar represents the standard deviation of independent performs experiments (n=3).

Pediococcus spp. isolated from adult feces was comparatively more resistant to antibiotics than those isolated from infant feces. Significantly higher resistances (P < 0.05) were found against ampicillin (7.3%), cefotaxime (22.0%), cefuroxime (36.6%), penicillin (12.2%) gentamycin (26.8%), erythromycin (19.5%), tobramycin (29.3%) and ciprofloxacin (26.8%) from isolates from adult feces than those isolated from infant feces, 7.0%, 9.3%, 0.0%, 11.6%, 9.3%, 0.0%, 2.3% and 20.9% respectively (**Figure 1a**). All the isolates from both adult and infant samples were sensitive to vancomycin and chloramphenicol. *Pediococcus* spp. were intrinsically resistant to high levels of glycopeptides and penicillin. Resistance to erythromycin was also reported and was due to a plasmid with an erythromycin resistance methylase B [*erm*(B)] gene [17].

Discussion

To develop probiotics for human or animal consumption, it is necessary to distinguish strains harboring antibiotic resistance genes from other strains because of potential risk for the dissemination of resistance genes. In this study, it was demonstrated that strains isolated from infants were more sensitive than those isolated from adult feces. Lactobacilli and Pediococciare widely used as probiotics and promoters for biological growth. Lactobacilli are reported to be resistant to several antibiotics [18]. In the present study, *Lactobacillus* spp. isolated from adult feces were more resistant to antibiotics than those isolated from infant feces. Significantly higher resistance was found against cefuroxime (26.1%) and ciprofloxacin (32.6%) from isolates from adult feces than those isolated from infant feces, 4.5%, and 2.3% respectively. *Lactobacillus* spp. isolated from feces also showed moderate resistance to cefotaxime (13.0%), penicillin (10.9%), chloramphenicol, (10.9%), gentamycin (23.9%), erythromycin (13.0%) and tobramycin (26.1%). Whereas those isolated from infant feces showed comparatively lesser resistance 4.5%, 11.4%, 9.1%, 20.5% and 9.1%, respectively (**Figure 1b**). Resistance to gentamycin and ciprofloxacin was earlier documented [19, 20]. Concerning cell wall synthesis inhibitors, Lactobacilli are reported to be resistant to oxacillin and cephalosporins (cefoxitin and ceftriaxone) [21].

They were also found to show resistance to aminoglycosides (neomycin, kanamycin,

Table 2. Percent antibiotic resistance in target microorganisms isolated from adult and infant fecal samples. Expressed in percentage (%)

Mechanism of Action	Antibiotic	*Lactobacillus* spp.		*Pediococcus* spp.		*Enterococcus* spp.	
		Adult	Infant	Adult	Infant	Adult	Infant
Cell Wall Inhibitors	Ampicillin	10.9	11.4	7.3	7.0	28.2	21.4
	Cefotaxime	13.0	4.5	22.0	9.3	28.2	26.2
	Cefuroxime	26.1	4.5	36.6	0.0	20.5	9.8
	Penicillin	10.9	11.4	12.2	11.6	10.3	11.9
	Vancomycin	0.0	0.0	0.0	0.0	30.8	11.9
Protein Synthesis Inhibitor	Chloramphenicol	10.9	9.1	0.0	0.0	0.0	0.0
	Erythromycin	13.0	9.1	19.5	0.0	30.8	0.0
	Gentamycin	23.9	20.5	26.8	9.3	41.0	4.8
	Tobramycin	26.1	6.8	29.3	2.3	20.5	9.5
DNA Synthesis Inhibitor	Ciprofloxacin	32.6	2.3	26.8	20.9	33.3	9.5

streptomycin, and gentamicin) [22]. There are many species of Lactobacilli which contain intrinsic resistance to vancomycin, erythromycin and tetracycline. The matter of concern is that since Lactobacilli are added to infant food, they can act as reservoirs of antibiotic resistance genes, which could be transferable [23].

Enterococcus spp. also followed a similar pattern where the antibiotic resistance associated with adult fecal samples was higher than those isolated from infant feces. Adult fecal isolates were 30.8% resistant to erythromycin, 20.5% resistant to tobramycin, and 41% resistant to gentamycin. This was significantly higher ($P < 0.05$) than infant fecal isolates, which were sensitive to erythromycin, 9.5% resistant to tobramycin, and 4.8% to gentamycin. Higher resistance was also found against vancomycin (30.8%), ciprofloxacin (33.3%), ampicillin (28.2%), cefuroxime (20.5%) and cefotaxime (28.2%); however, it was not statistically significant in comparison to infant fecal isolates which showed 11.9%, 9.5%, 21.4%, 9.8% and 26.2% resistance against above-mentioned antibiotics, respectively. All the isolates (fecal and adult) were susceptible to chloramphenicol. Infant isolates were 11.9% resistant to penicillin; this was higher than adult isolates, which showed 10.3% resistance (**Figure 1c**). Enterococci showed intrinsic and acquired resistance against many antibiotics [24, 25]. Such intrinsic resistance was reported inlincosamides, nalidixic acid penicillin, polymyxins, quinupristin–dalfopristin, monobactams, and low levels of aminoglycosides. Resistance to high levels of aminoglycosides, high levels of trimethoprim, and high levels of clindamycin, chloramphenicol, tetracyclines, penicillins (due to β-lactamase), fluoroquinolones, macrolides (e.g. erythromycin), glycopeptides and oxazolidinones (linezolid) were acquired [26-27]. Acquired resistance is a major threat in treatment, such a trait was found to be transferred to other *Enterococci* in the gut [28]. Vancomycin resistance is especially important as vancomycin is the last drug option for treating diseases caused by multidrug resistance *Enterococci* [29].

Apart from probiotic use, *Pediococci* are also widely used for the fermentation of meat and vegetables and also in cheese production [30]. According to the EFSA's FEEDAP Panel [31] (European Food Safety Authority Panel on Additives and Products or Substances used in Animal Feed), the bacterial cultures which are used for the production of animal feed should be susceptible to antibiotics used in treating humans bacterial infections. Hence, it is extremely necessary to distinguish antibiotic susceptible and resistant strains. This also emphasizes the importance of safe source or niche of a selection of strains used as probiotics. The results of the study indicate that infant feces could be a better source for isolation of LAB cultures intended to be used as probiotics.

Apart from being used traditionally as starter cultures in dairy products, LAB are also used for the production of animal feed. They also belong to normal flora of the human gut and confer health benefits to the host. During the process of food manufacturing and passage of food through gut, there is a possibility of antibiotic resistance, carried by LAB getting transferred to human pathogenic bacteria [32]. Hence, it is imperative to select strains that have low resistance against antibiotics for human and animal use. From the results of the antibiotic susceptibility in the current study, obtained from a broad range of antibiotics, it was found that the isolated strains of *Lactobacillus*, *Pediococcus* and *Enterococcus* were resistant to various antibiotics. However, antibiotic resistance was lesser in strains obtained from infant fecal samples than adult fecal samples.

Conclusion

Lactobacillus, *Pediococcus* and *Enterococcus* as LAB were isolated from the human fecal samples exhibiting more antibiotic resistance from adult fecal isolate than the infant. The development of antibiotic resistancein LAB can be attributed to the long term exposure of antibiotic as therapeutic agents as well as food habits which pose food safety concerns. Thus, it is essential to see safety measure during antibiotic uptake in day to day life. In addition to this, the low antibiotic-resistant strains from infant could be the choice of strain to avoid the risk of transferof LAB linked antibiotic resistance to human pathogenic bacteria.

Authors Contribution

RP has made a substantial contribution to data analysis and its interpretation. VZ contributed in designing the experiments. BN contributed to data interpretation and all authors RP, VZ and BN contributed equally to drafting and reviewing of the manuscript followed by final approval from BN.

Competing Interests

No competing interests were disclosed.

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Ethical Approval and Consent

The study protocol was approved by an independent ethical committee and performed in compliance with the US Code of Federal Regulations on Good Clinical Practices (21 CFR 10.90, 50, 56, and 812) and the World Medical Association Declaration of Helsinki (1996 amendment). All adult volunteers and parents of infants signed informed consent before sample collection.

References

1. Holzapfel WH, Haberer P, Geisen R, Björkroth J, Schillinger U. Taxonomy and important features of probiotic microorganisms in food and nutrition. The American Journal of Clinical Nutrition. 2001 Feb 1;73(2):365s-73s. https://doi.org/10.1093/ajcn/73.2.365s
2. Thursby E, Juge N. Introduction to the human gut microbiota. Biochemical Journal. 2017 Jun 1;474(11):1823-36. https://doi.org/10.1042/BCJ20160510
3. Srikumar S, Fanning S. The Therapeutic Potential of the "Yin-Yang" Garden in Our Gut. InRole of Microbes in Human Health and Diseases 2018 Dec 4. IntechOpen.
4. Vael C, Desager K. The importance of the development of the intestinal microbiota in infancy. Current Opinion in Pediatrics. 2009 Dec 1;21(6):794-800. https://doi.org/10.1097/MOP.0b013e328332351b
5. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, Belzer C, Palacio SD, Montes SA, Mancabelli L, Lugli GA. The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. Microbiology and Molecular Biology Reviews. 2017 Dec 1;81(4). https://doi.org/10.1128/MMBR.00036-17
6. Makino H, Kushiro A, Ishikawa E, Muylaert D, Kubota H, Sakai T, Oishi K, Martin R, Amor KB, Oozeer R, Knol J. Transmission of intestinal *Bifidobacterium longum* subsp. *longum* strains from mother to infant, determined by multilocus sequencing typing and amplified fragment length polymorphism. Applied and Environmental Microbiology. 2011 Oct 1;77(19) :6788-93. https://doi.org /10.1128/AEM.05346-11
7. van den Elsen LW, Garssen J, Burcelin R, Verhasselt V. Shaping the gut microbiota by breastfeeding: the gateway to allergy prevention? Frontiers in Pediatrics. 2019 Feb 27;7:47. https://doi.org/10.3389/fped.2019.00047
8. Walker WA. Role of nutrients and bacterial colonization in the development of intestinal host defense. Journal of Pediatric Gastroenterology and Nutrition. 2000 Jan 1;30:S2-7. https://doi.org/10.1097/00005176-200000002-00002
9. Feedap P. Opinion of the scientific panel on additives and products or substances used in animal feed on the updating of the criteria used in assessment of bacteria for resistance to antibiotics of human and veterinary importance. European Food Safety Authority Journal. 2005;223:1-2. https://doi.org/10.2903/j.efsa.2005.223
10. Bronzwaer SL. European Antimicrobial Resistance Surveillance System. A European study on the relationship between antimicrobial use and antimicrobial resistance. Emergency and Infectious Diseases. 2002;8:278-82. https://doi.org/10.3201/eid0803.010192
11. Yoon MY, Yoon SS. Disruption of the gut ecosystem by antibiotics. Yonsei Medical Journal. 2018 Jan 1;59(1):4-12. https://doi.org/10.3349/ymj.2018.59.1.4
12. Heller KJ. Probiotic bacteria in fermented foods: product characteristics and starter organisms. The American Journal of Clinical Nutrition. 2001 Feb 1;73(2):374s-9s. https://doi.org/10.1093/ajcn/73.2.374s
13. World Health Organization:WHONET software. Available from: https://whonet.org/
14. World Medical Association Declaration of Helsinki (1996) 48th General Assembly, Somerset West, Republic of South Africa, 1-3.
15. Shioiri, T, Yahagi, K, Nakayama, S. The effects of symbiotic fermented milk beverage containing *Lactobacillus casei* strain Shirota and transgalactosylated oligosaccharides on defecation frequency, intestinal microflora, organic acid concentrations, and putrefactive metabolites of sub-optimal

health state volunteers: a randomized placebo-controlled cross-over study. Bioscience Microflora 2006; 25, 137–146.
16. Clinical Laboratory Standards Institute. M2-M9. Performance standardsfor antimicrobial disk susceptibility tests; approved standard 2006; Ninth edition PA. CLS.
17. Charteris WP, Kelly PM, Morelli L, Collins JK. Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. Journal of Food Protection. 1998 Dec;61(12):1636-43. https://doi.org/10.4315/0362-028X-61.12.1636
18. Klare I, Konstabel C, Werner G, Huys G, Vankerckhoven V, Kahlmeter G, Hildebrandt B, Müller-Bertling S, Witte W, Goossens H. Antimicrobial susceptibilities of *Lactobacillus*, *Pediococcus* and *Lactococcus* human isolates and cultures intended for probiotic or nutritional use. Journal of Antimicrobial Chemotherapy. 2007 May 1;59(5):900-12. https://doi.org/10.1093/jac/dkm035
19. Dec M, Urban-Chmiel R, Stępień-Pyśniak D, Wernicki A. Assessment of antibiotic susceptibility in *Lactobacillus* isolates from chickens. Gut Pathogens. 2017 Dec 1;9(1):54. https://doi.org/10.1186/s13099-017-0203-z
20. Fukao M, Tomita H, Yakabe T, Nomura T, Ike Y, Yajima N. Assessment of antibiotic resistance in probiotic strain *Lactobacillus* brevis KB290. Journal of Food Protection. 2009 Sep;72(9):1923-9. https://doi.org/10.4315/0362-028X-72.9.1923
21. Shazali N, Foo HL, Loh TC, Choe DW, Rahim RA. Prevalence of antibiotic resistance in lactic acid bacteria isolated from the feces of broiler chicken in Malaysia. Gut Pathogens. 2014 Dec 1;6(1):1. https://doi.org/10.1186/1757-4749-6-1
22. Coppola R, Succi M, Tremonte P, Reale A, Salzano G, Sorrentino E. Antibiotic susceptibility of Lactobacillus rhamnosus strains isolated from Parmigiano Reggiano cheese. Le Lait. 2005 May 1;85(3):193-204. https://doi.org/10.1051/lait:2005007
23. Zhou JS, Pillidge CJ, Gopal PK, Gill HS. Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. International Journal of Food Microbiology. 2005 Feb 1;98(2):211-7. https://doi.org/10.1016/j.ijfoodmicro.2004.05.011
24. Mathur S, Singh R. Antibiotic resistance in food lactic acid bacteria—a review. International Journal of Food Microbiology. 2005 Dec 15;105(3):281-95. https://doi.org/10.1016/j.ijfoodmicro.2005.03.008
25. Miller WR, Munita JM, Arias CA. Mechanisms of antibiotic resistance in *Enterococci*. Expert Review of Anti-infective Therapy. 2014 Oct 1;12(10):1221-36. https://doi.org/10.1586/14787210.2014.956092
26. Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in *Enterococcus*. Virulence, 3 (5), 421–433. https://doi.org/10.4161/viru.21282
27. Kimiran-Erdem A, Arslan EO, Yurudu NO, Zeybek Z, Dogruoz N, Cotuk A. Isolation and identification of enterococci from seawater samples: assessment of their resistance to antibiotics and heavy metals. Environmental Monitoring and Assessment. 2007 Feb 1;125(1-3):219-28. https://doi.org/10.1007/s10661-006-9506-0
28. Rathnayake IU, Hargreaves M, Huygens F. Antibiotic resistance and virulence traits in clinical and environmental *Enterococcus faecalis* and *Enterococcus faecium* isolates. Systematic and Applied Microbiology. 2012 Jul 1;35(5):326-33. https://doi.org/10.1016/j.syapm.2012.05.004
29. Ahmed MO, Baptiste KE. Vancomycin-resistant *Enterococci*: a review of antimicrobial resistance mechanisms and perspectives of human and animal health. Microbial Drug Resistance. 2018 Jun 1;24(5):590-606. https://doi.org/10.1089/mdr.2017.0147
30. Reuter G. Present and future of probiotics in Germany and in Central Europe. Bioscience and Microflora. 1997;16(2):43-51. https://doi.org/10.12938/bifidus1996.16.43
31. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal. 2012 Jun;10(6):2740. https://doi.org/10.2903/j.efsa.2012.2740
32. Schjørring S, Krogfelt KA. Assessment of bacterial antibiotic resistance transfer in the gut. International journal of microbiology. 2011 Oct;2011. https://doi.org/10.1155/2011/312956