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Influence of *Bifidobacterium sp.* over the expresion of antibioresistance profiles of different enteric pathogenic strains

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ABSTRACT

Normal digestive tract microbiota plays an essential role in maintaining homeostasis and normal development of the digestive tract and metabolism, immunity and angiogenesis. Treatment with antibiotics, especially in children, is often associated with significant changes in intestinal microbiota, which can lead to intestinal disbioses. Probiotic bacteria have been used for over a century to promote digestive health. The objective of this study was to investigate the interrelations between probiotic *Bifidobacterium* strains isolated from infants faeces by studying their influence on the expression of antibiotic resistance of enteropathogenic strains recently isolated from digestive tract infections. This study demonstrates that probiotics associated with antibiotics increase enteropapathogens susceptibility to aminoglycosides, beta-lactams, amoxicillin plus clavulanic acid, quinolones and carbapenems.

Keywords: *Bifidobacterium sp., antibioresistance, antibiotics, quinolones, carbapenems, aminoglycosides, beta-lactams.*

1. INTRODUCTION

The pathogenesis of intra-abdominal infections often begins with altered intestinal microbiota [1]. Digestive resident microbiota is normally present only at the extremities of the digestive tract, especially in the terminal area, is very complex and includes aerobic and anaerobic bacteria, fungi and protozoa [2]. The microbial colonization of the infant gut begins immediately after birth and is essential for the development of the intestine and immune system and later well-being. The mechanisms by which probiotic bacteria restore the homeostasis of the intestine after a prolonged treatment with antibiotics or a immunological imbalances consist on optimizing the functionality of the intestinal barrier, the lower production of pro-inflammatory cytokines and prevention of epithelial cell apoptosis induced by cytokines [3]. It has been shown that probiotic bacteria synthesize signaling molecules, that target are not only microbial populations, but also the intestinal epithelial cells, cells with immune function (lymphocytes, dendritic cells) [4]. The rationale for using probiotics is based on the assumption that they modify the composition of colonic microflora and counteract enteric pathogens, by acting locally (at intestinal level) and/or by modulating the immune response. At local level, probiotics compete with pathogens for nutrients and receptors, induce hydrolysis of toxins and receptors, induce production of antimicrobial substances (including peptides of the innate immune system), of organic acids, modulate nitric oxide synthesis, regulate intestinal permeability by modulating the epithelial tight junctions, exhibit a trophic role on the intestinal mucosa, which leads to brush border enzyme activation, stimulation of glucose absorption and exert an antiapoptotic effect on the enterocytes. On the other hand, an increasing body of evidence supports the concept that probiotics modulate the immune response [5]. Diarrhoea is a significant health problem worldwide, especially in the developing world where adequate sanitation facilities are lacking. Globally diarrhoeal diseases account for almost a fifth of all deaths of children below five years of age, with an estimated 2.2 million deaths annually [6]. B The bacterial pathogens most commonly associated with childhood diarrhoea are enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), *Klebsiella sp., Salmonella sp., Yersinia enterocolitica. Pseudomonas aeruginosa* is a rare cause of infectious diarrhea, inducing usually nosocomial infections in immunocompromised hosts [7]. In a time when conventional antibiotics are becoming increasingly less effective for treatment of infections, the relationship between bacteria and antimicrobial resistance is becoming more and more complicated [8]. While antibiotics may play a major part in reducing mortality among severely-ill patients, the ultimate approach against diarrhea in developing countries rests on the need for improving sanitary conditions, maintaining exclusive breastfeeding until the sixth month of life and developing safe and effective vaccines for immune prophylaxis, along with systematic parental education [9].

2. EXPERIMENTAL SECTION

2.1. Microbial strains. In this study were used 12 strains of *Bifidobacterium sp.* and 18 strains of enteropathogenic bacteria (*EPEC, EIEC, Klebsiella sp., Salmonella sp., Yersinia enterocolitica, P. aeruginosa*) isolated from faeces, from 30 patients with ages between 3 days and 5 years. Most of the *Bifidobacterium sp*. strains were isolated from healthy patients.

Isolation was performed on selective media (Selective Agar Bifidobacterium for *Bifidobacterium* strains, Hektoen Enteric Agar and Mac Conkey strains for *Enterobacteriaceae* and *P. aeruginosa* on CLED). For the anaerobic bacteria, after cultivation, the plates were incubated 24-48h in anaerobic conditions. Isolates obtained were identified by MALDI-TOF automated method (Matrix Assisted Laser Desorption of / ionization time of flight) using an analyzer Microflex [10].

2.2. Antimicrobial activity assay. The antimicrobial activity of the probiotic strains on the potential of growing of enteropathogenic strains was assessed using whole culture and sterile culture supernatants of *Bifidobacterium sp*. The *Bifidobacterium sp.* strains were inoculated in thyoglycollate medium under anaerobic conditions and incubated 24h at 37^{o}C; thereafter the cultures were centrifuged at 6000 rpm and supernatants were recovered. From 24 h culture of pathogenic strains were prepared suspensions with 0.5 standards MacFarland density in sterile saline water and streaked on solid Muller Hinton medium as for antibiotic susceptibility testing. Then, amounts of 10 µl of *Bifidobacterium* cultures or supernatants were distributed in spots on the respective media. The plates were allowed to stand at room temperature for the adsorption of the solution droplet in the medium, after which the plates were incubated with caps down for 24 h under the aerobic / anaerobic conditions at 37^{o}C. The inhibition of growth was quantified by the appearance of growth inhibition zones around the *Bifidobacterium sp.* culture fractions spots [11].

2.3. Influence of *Bifidobacterium sp.* culture fractions upon the expresion of antibioresistance markers. Antibiotic sensitivity was tested by disc diffusion method (Kirby-Bauer) according to the recommendations of the Clinical and Laboratory Standards Institute guidelines (CLSI, 2015) [12] using microtablets supplied by Oxoid Ltd. (Basingstoke, UK), an inocul with turbidity of 0.5 McFarland scale and sowing was done on Mueller-Hinton medium. The following antibiotics were tested: imipenem (IPM 10 µg), ertapenem (ETP 10 µg), gentamicin (CN 10 µg), ciprofloxacin (CIP 5 µg), levofloxacin (LEV 5 µg), tetracycline (TE 30 µg), ceftriaxone (CRO 30 µg), ceftazidime (CAZ 30 µg). The antibiogram was performed using pathogenic strains cultured in the presence and absence of *Bifidobacterium breve* supernatant. The reading of the results was carried out after 24 hours by measuring the zones of microbial growth inhibition [13].

2.4. Phenotypic test for the confirmation of beta-lactamase production - DDST (double disk synergy test). The identification of the production of extended spectrum beta-lactamases (ESBLs) was carried out using the double disc diffusion method. Phenotypic confirmation of *E. coli* or *Klebsiella pneumoniae* strains suspected for producing ESBLs was achieved through simultaneous testing by Kirby-Bauer disc diffusion method, the synergy between disks ceftazidime, ceftriaxone and amoxicillin with clavulanic acid disc. Oxoid discs were used containing the combination of amoxicillin / clavulanic acid (20 µg/10 µg), ceftazidime (30 µg) and ceftriaxone (30 µg), placed at a distance of 2 cm (measured between the centers of the discs) on the Mueller-Hinton agar. The plates were incubated for 18-20 hours at 37°C [14].

3. RESULTS SECTION

3.1. Screening of the antimicrobial properties of the *Bifidobacterium* sp. whole culture and soluble fractions. By direct spotting method some *Bifidobacterium* strains / supernatants exhibited a bactericidal effect evidenced by the appearance of a growth inhibition zone. From the 12 strains of *Bifidobacterium* analyzed in this study, only 7 strains showed a growth inhibition effect after direct contact with the enteropathogenic strain cultures (Fig. 1). From the 7 active strains, we selected one *Bifidobacterium breve* strain which exhibited the largest spectrum of antimicrobial activity, being active against 50% of the tested enteropathogenic strains inclusively *Pseudomonas aeruginosa.*

3.2. Antibiotic resistance phenotypes. Most enteric strains isolated from stool presented an expanded sensitivity range. Regarding the influence of the *Bifidobacterium breve* supernatant on antibiotic sensitivity spectrum of enteropathogens strains we observed a decrease of inhibition zones from 2 to 4 mm, without affecting the classification of the enteric strain in one of the three clinical categories, respectively resistant, intermediate and susceptible (fig. 2, 3, 4). Two *Klebsiella pneumoniae* strains were resistant to beta-lactam antibiotics and one of the *Pseudomonas aeruginosa* strains showed the multidrug-resistant profile (table 1). In these strains we noticed an increase in the growth inhibition diameter but without influencing the clinical category.

Figure 1. Antimicrobial activity of different *Bifidobacterium sp.* strains against pathogenic bacterial strains isolated from stool cultures.

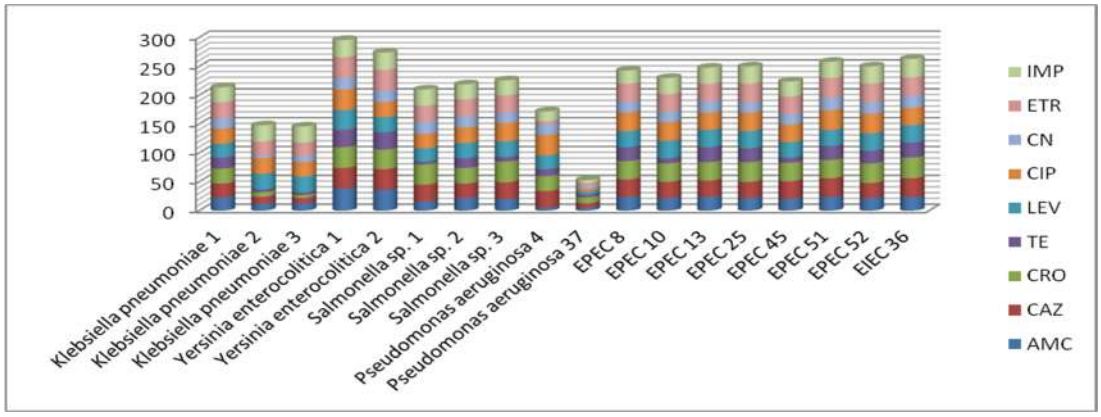

Figure 2.Distribution of antibioresistance markers in bacterial strains isolated from faeces.

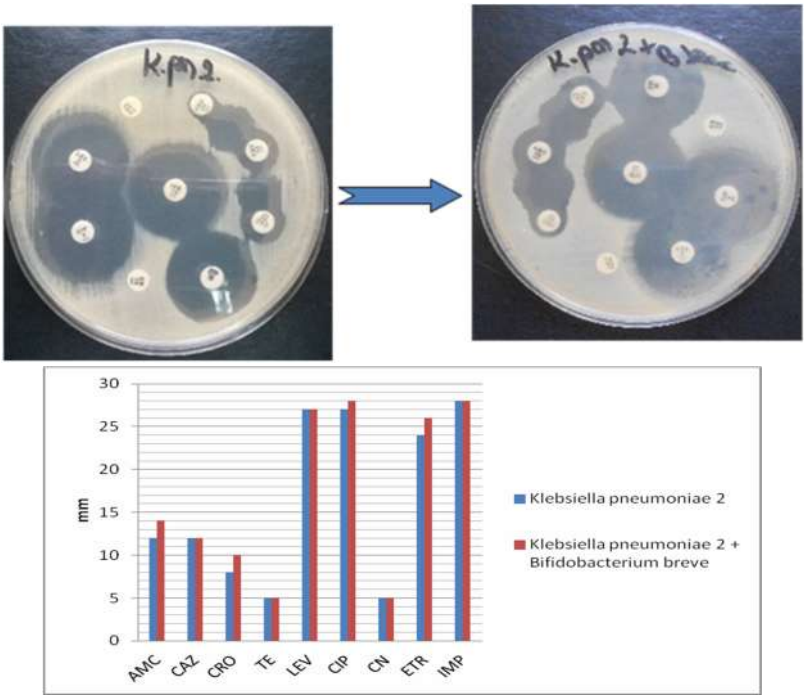

Figure 3. Influence of *B. breve* supernatant on the antibiotic susceptibility *K. pneumoniae 2* strain.

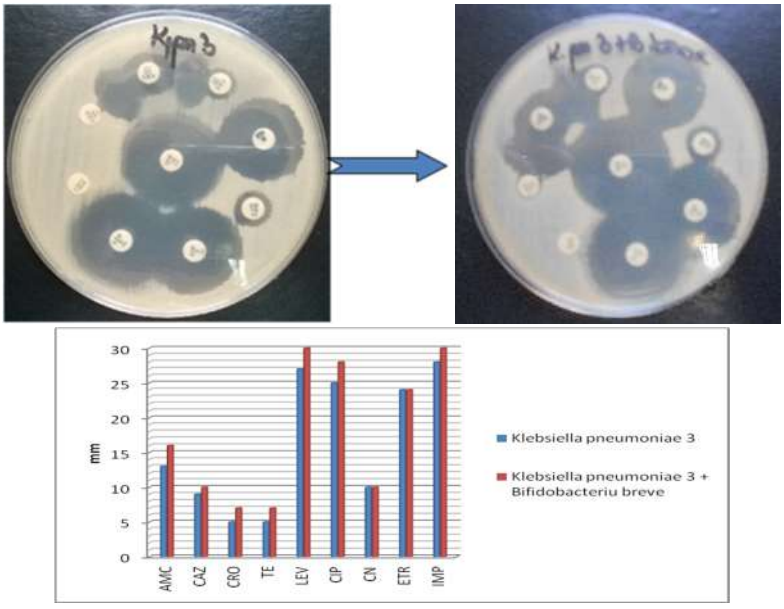

Figure 4. Influence of *B. breve* supernatants on the antibiotic susceptibility of *K. pneumoniae* 2 strain.

Table 1. Antibiograms of *Pseudomonas aeruginosa* strains isolated from stool cultures of the patients

Strains	AMC	CAZ	CRO	TE	LEV	CIP	CN	ETR	IMP
Pseudomonas aeruginosa 4	R	S	S	R	S	S	S	R	R
Pseudomonas aeruginosa 37	R	R	R	R	R	R	R	R	R

4. CONCLUSIONS

The results of this study indicate that the strain of *Bifidobacterium breve* recently isolated from faeces presented a significant probiotic potential, demonstrated by the ability of culture and the sterile supernatant to modulate the growth of enteropathogenic strains. There are plenty of antibiotics currently available for the treatment of acute infectious diarrhea in children. While antibiotics are effective against most bacteria and may help shorten the duration of symptoms, it must always be kept in mind that antimicrobial therapy should be reserved for severe, prolonged or potentially complicated cases, as most patients respond fairly well to supportive therapy, and their indiscriminate use carries the danger of increasing antimicrobial resistance and brings no benefit to patients with mild presentations, as has been shown for uncomplicated salmonellosis [15].

5. REFERENCES

[1] Taur Y., Pamer E.G., The intestinal microbiota and susceptibility to infection in immunocompromised patients, *Curr Opin Infect Dis*., 26, 4, 332-7, **2013.**

[2] Bezirtzoglou E., Stavropoulou E., Immunology and probiotic impact of the newborn and young children intestinal microflora, *Anaerobe*, 17, 6, 369-74, **2011.**

[3] Mennigen R., Bruewer, M., Effect of probiotics on intestinal barrier function, *Annals of the New York Academy of Sciences*, 1165, 183–189, **2009.**

[4] Jayaraman A., Wood, T.H., Bacterial quorum sensing: signals, circuits, and implications for biofilms and disease, *Annual Review of Biomedical Engineering,* 10, 145-167, **2008.**

[5] Alfredo G., Andrea Lo Vecchio, Roberto B.C., Probiotics as Prevention and Treatment for Diarrhea, *Curr Opin Gastroenterol.*; 25(1), 18-23, **2009.**

[6] Iman K.B., Abada Emad A. , Entsar A., Ahmed, Rania S., Labeeb, Enteropathogenic Escherichia coli Associated with Diarrhea in Children in Cairo, Egypt, *The ScientificWorld JOURNAL*, 11, 2613-2619, **2011.**

[7] Porco FV, Visconte EB., *Pseudomonas aeruginosa as a cause of infectious diarrhea successfully treated with oral ciprofloxacin*, Annals Pharmacother, 29, 1122–3, **1995.**

[8] Ditu L.M., Chifiriuc M.C., Bezirtzoglou E., Voltsi C., Bleotu C., Pelinescu D., Mihaescu G., Lazar V., Modulation of virulence and antibiotic susceptibility of enteropathogenic Escherichia coli strains by Enterococcus faecium probiotic strain culture fractions*, Anaerobe*, 17, 448-451, **2011.**

[9] Diniz-Santos D.R., Silva L.R., Silva N., Antibiotics for the Empirical Treatment of Acute Infectious Diarrhea in Children, *The Brazilian Journal of Infectious Diseases,* 10, 3, 217-227, **2010.**

[10] Croxatto A., Prod'hom G., Greub G., .Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology, *FEMS Microbiol Rev,* 36, 2, 380-407, **2012.**

[11] Delcaru C., Alexandru I., Podgoreanu P., Violeta C.C., Bleotu C., Chifiriuc C.M., Bezirtzoglou E., Lazar V., Antagonistic activities of some Bifidobacterium sp. strains isolated from resident infant gastrointestinal microbiota on Gram-negative enteric pathogens, *Anaerobe*, 39, 39-44, **2016.**

[12] Clinical and Laboratory Standards Institute, 35, 3, January **2015.**

[13] Garrido A., Seral C., Gude M.J., Casado C., Gonzaez-Dominguez M., Saenz Y., Castillo F.J., Characterization of plasmid-mediated β-lactamases in fecal colonizing patients in the Hospital and Community setting in Spain, *Microbial Drug Resistance*, 20, 4, 301-4, **2014.**

[14] Jarlier V., Nicolas M.H., Fournier G., Philippon A., Extended broad-spectrum betalactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns, *Rev Infect Dis.,* 10, 4, 867-878, **1988.**

[15] Chiu C.H., Lin T.Y., Ou J.T., A clinical trial comparing oral azithromycin, cefixime and no antibiotics in the treatment of acute uncomplicated Salmonella enteritis in children*., Paediatr Child Health*; 35, 372-4, **1999.**

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