ANTIBIOTIC SUSCEPTIBILITY OF POTENTIALLY PROBIOTIC HUMAN FAECAL LACTOBACILLI

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Abstract: Bacteria of the genus Lactobacillus have been proposed as probiotic microorganisms to restore the ecological equilibrium of the gastrointestinal tracts (GIT). The aim of the present study is to determine the antibiotic susceptibility of six human faecal probiotic lactobacilli. The disc diffusion method was performed in Mueller Hinton, LAPTg and MRS agars by the NCCLS (National Committee for Clinical Laboratory Standards) procedure was performed. Due to the absence of a Lactobacillus reference strains, the results were compared to those of *Staphylococcus aureus* MTCC 740. Antibiotic sensitivity was determined with 12 different antibiotics in LAPTg agar, MRS agar and MHA. All human faecal Lactobacilli were sensitive to Chloramphenicol, Ciprofloxacin, Gentamicin, Lincomycin, Pefloxacin, Streptomycin, Intermediate to Kanamycin and resistant to Ampicillin. *Lactobacillus plantarum* (Hef24) and *L. casei* (Hef19) were found resistant to Vancomycin and Rifampicin. *L. fermentum* (Hef2), and *L.plantarum* (Hef4), were found intermediate resistant to Vancomycin. *L. casei* (Hef 19) is only exception that is resistant to Pefloxacin. *L. plantarum* (Hef 24) and *L. casei* (Hef 19) are only two strain resistant to Rifampicin. *L. fermentum* (Hef 3), *L. plantarum* (Hef 24) and *L. casei* (Hef 19) are three strain which are resistant to Vancomycin. The NCCLS method needs to be standardized in an appropriate medium to determine the antimicrobial susceptibility of Lactobacillus. Faecal probiotic lactobacilli do not display uniform susceptibility to antibiotics. Resistance to Ampicillin suggests that lactobacilli could be simultaneously used as a probiotic with Diarrheal treatment. However, the NCCLS procedure needs to be standardized for this genus.

Key words: Antibiotics, Lactobacilli, Probiotics.

INTRODUCTION

Bacteria of the genus *Lactobacillus* have been proposed as probiotic microorganisms to restore the ecological equilibrium of the intestinal, respiratory, and urogenital tracts (Hammes, 1995). This type of bacterial replacement therapy has been widely used as fermented milks to prevent diarrhea in humans and animals (Fuller, 1992). They have also been increasingly considered for their use in women to prevent genital and urinary tract infections (Redondo-Lopez, 1990). It has been found that administration of antimicrobial substances alters the microbial balance of the vagina and suppresses certain bacterial groups. The effect of these substances on autochthonous Lactobacillus is of interest in understanding the development of genital and urinary tract infections related with the lack of these bacteria (Simoes, 2001). The present study was conducted to determine the antimicrobial susceptibility of six candidate human faecal probiotic Lactobacillus strains. These lactobacilli have been previously selected for probiotic properties as surface hydrophobicity (Ocana, 1999), and production of antimicrobial substances. The main aims of knowing the behavior of exogenously applied *Lactobacillus* under the effect of antimicrobial substances are to have an approach of the response of lactobacilli administered to patients subjected to some kind of antibiotic therapy and to consider the concomitant use of lactobacilli and an antibiotic to restore the disrupted ecological environment. Having in mind that a method to study antimicrobial susceptibility of genus *Lactobacillus* has not been standardized yet, different techniques were assayed. The results obtained by using the disc diffusion method with culture media different from Muller Hinton agar proposed by the NCCLS.

MATERIALS AND METHODS

Antibiotics: Twelve different antibiotics: Chloramphenicol (10mcg), Ciprofloxacin (5mcg), Gentamicin (10mcg), Lincomycin (10mcg), Pefloxacin, Streptomycin (10mcg), Kanamycin (30mcg) and Ampicillin (10mcg), Erythromycin (15mcg), Oflaxacin (5mcg) Vancomycin (30mcg) and Rifampicin (5mcg). *L.plantarum* (Hef24) and *L. casei* (Hef19) were found resistant to used were procured from HiMedia, Mumbai.

Growth Media: MRS agars, LAPTg broth/Agar, Muller Hinton broth/Agar

Bacterial Cultures:

Human Faecal *Lactobacillus* sp.

Reference strain of *Staphylococcus aureus* MTCC 740.

The microorganisms used in this study were six Lactobacillus sp. named *L. fermentum*, *L. casei*, *L. plantarum*. All the isolates of *Lactobacillus* were maintained in chalk litmus milk at 4°C. Prior to the assays, they were sub cultured twice in LAPTg broth (Raibaud, 1963), and a third time in the media where the susceptibility to antibiotics assay was going to be performed: MRS (De Man, 1960), LAPTg, or Muller Hinton Agar.

Disc diffusion method: The method to detect antibiotic susceptibility was described by Kirby-Bauer known as Kirby-Bauer disc diffusion method (Bauer and Kirby, 1966), modified by using three different base agar media: Muller Hinton, LAPTg, and MRS agars. Preserved isolates of *Lactobacillus* were subculture twice in LAPTg broth and a third time in MRS, LAPTg, or Muller Hinton broth for 24 hours at 37°C. Suspensions were adjusted to 0.5 McFarland scale (10^8 cfu/mL). Fifteen mL of MRS Agar, Muller Hinton Agar, LAPTg Agar were poured in Petri plates and allowed to solidify. Then, It was overlaid with 5 mL of soft Agar (0.5 % agar) seeded with 200 µL of active culture at 45^0C. Petri plates were allowed to stand at room temperature for 15 min under aseptic conditions. before dispensing the antibiotic Discs. The HiMedia Antibiotic Disc was dispensed on to agar by HiMedia Disc dispenser (HiMedia Ltd, Mumbai). Plates were incubated at 37^0C aerobically for 24h. Diameter (mm) of zone of inhibition Diameter was measured and result was expressed in terms of resistance Intermediate or susceptibility.

RESULTS AND DISCUSSION

Growth of lactobacilli in Muller Hinton broth was not so good and when any type of growth was detected on the agar, it was not clear and the halos were undefined. In LAPTg agar the inhibition halos were sharply defined and the diameters could be easily measured when the microorganisms were inoculated either on the surface or into the agar. All Human faecal Lactobacilli were sensitive to Chloramphenicol, Ciprofloxacin, Gentamicin, Lincomycin, Pefloxacin, Streptomycin. Intermediate to Kanamycin and resistant to Ampicillin. *L.plantarum* (Hef24) and *L. casei* (Hef19) were found resistant to Vancomycin and Rifampicin. *L. fermentum* (Hef2), and *L. plantarum* (Hef4), were found intermediate resistant to vancomycin. *L. casei* (Hef 19) is only exception that is resistant to Pefloxacin. *L. plantarum* (Hef 24) and *L. casei* (Hef 19) are only two strain resistant to Rifampicin. *L. fermentum* (Hef 3), *L. plantarum* (Hef 24) and *L. casei* (Hef 19) are three strain which are resistant to Vancomycin (Table 1). In order to know both LAPTg and MRS agar were appropriate to be used as a base medium in a standardized method for Lactobacillus *sp.* The knowledge of the antimicrobial susceptibility or resistance is of interest to predict the behavior of an exogenously applied probiotic formula in patient's subject to consider the concomitant use of the probiotic and antibiotics for the restoration of the normal GIT flora as well as any type of chemotherapy (Ocana *et al.*, 2006). Although Faecal potentially probiotic lactobacilli do not display uniform susceptibility to antibiotics. But resistance to Ampicillin suggests that lactobacilli could be simultaneously used as a probiotic with Diarrheal treatment. We can conclude that the present standard techniques and the guidelines for the disc diffusion method have been provided by the NCCLS only for selected bacteria or yeasts related with laboratory clinical diagnostic. So the NCCLS procedure needs to be standardized for this genus.

Table 1: Diameters of zone of inhibition (mm) for stains into LAPTg, MRS and MHA with 12 different types of antibiotics.

Strain	Media	Antibiotics											
		C	E	Cf	K	G	S	Pf	L	Of	R	V	A
Staphylococcus aureus (MTCC 740)	MRS	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
	LAPTg	18	26	34	21	20	33	40	40	41	31	20	40
	MHA	23	26	29	21	30	36	40	40	41	33	25	37

L. fermentum **(Hef 2)**	**MRS**	**29**	**32**	**30**	**16**	**23**	**34**	**43**	**28**	**32**	**31**	**16**	**NZ**
	LAPTg	**29**	**30**	**30**	**14**	**20**	**33**	**44**	**29**	**30**	**29**	**17**	**NZ**
	MHA	**27**	**29**	**28**	**13**	**19**	**NG**	**41**	**28**	**30**	**28**	**13**	**NZ**
L .fermentum **(Hef 3)**	**MRS**	**27**	**20**	**28**	**16**	**19**	**28**	**34**	**27**	**31**	**10**	**10**	**NZ**
	LAPTg	**27**	**21**	**31**	**14**	**17**	**22**	**30**	**31**	**35**	**10**	**12**	**NZ**
	MHA	**22**	**20**	**30**	**15**	**21**	**24**	**30**	**31**	**33**	**08**	**10**	**NZ**
L. plantarum **(Hef 4)**	**MRS**	**21**	**28**	**31**	**13**	**39**	**26**	**40**	**29**	**34**	**31**	**19**	**NZ**
	LAPTg	**30**	**29**	**30**	**13**	**20**	**15**	**28**	**29**	**28**	**25**	**17**	**NZ**
	MHA	**27**	**30**	**29**	**13**	**33**	**29**	**38**	**31**	**30**	**25**	**17**	**NZ**
L. plantarum **(Hef 24)**	**MRS**	**21**	**24**	**35**	**13**	**24**	**29**	**38**	**27**	**41**	**10**	**12**	**NZ**
	LAPTg	**24**	**25**	**35**	**12**	**23**	**29**	**36**	**26**	**40**	**09**	**12**	**NZ**
	MHA	**24**	**25**	**32**	**12**	**23**	**30**	**33**	**26**	**37**	**08**	**10**	**NZ**
L. casei **(Hef 16)**	**MRS**	**33**	**22**	**27**	**17**	**20**	**30**	**35**	**35**	**32**	**27**	**29**	**NZ**
	LAPTg	**30**	**22**	**26**	**17**	**19**	**27**	**33**	**35**	**35**	**27**	**28**	**NZ**
	MHA	**29**	**20**	**27**	**16**	**18**	**26**	**33**	**30**	**33**	**25**	**28**	**NZ**
L. casei **(Hef 19)**	**MRS**	**25**	**23**	**12**	**15**	**20**	**15**	**10**	**29**	**37**	**13**	**15**	**NZ**
	LAPTg	**23**	**23**	**12**	**17**	**19**	**20**	**13**	**29**	**30**	**12**	**12**	**NZ**
	MHA	**21**	**24**	**12**	**14**	**20**	**17**	**11**	**29**	**35**	**11**	**16**	**NZ**

Symbols: NG – No growth; NZ- No zone; C – Chloramphenicol; E – Erythromycin; Cf- Ciprofloxacin, K –Kanamycin; G- Gentamicin, S- Streptomycin; Pf- Pefloxacin; L- Lincomycin; Of- Oflaxacin; R –Rifampicin; V- Vancomycin; A- Ampicillin.

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REFERENCES

Bauer, A.W.; Kirby, M.M.; Sherris, J.C. and Tuurck, M. (1966). Antibiotic susceptibility testing by a standarized single disk method. *American Journal of Clinical Pathology*. **45**:493–496.

De Man J.C.; Rogosa M. and Sharpe M.E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology*. **23**: 130–135.

Fuller, R. (1992). Probiotics: their development and use. In: Fuller R, Heidt PJ, Rusch V, Van der Waaij D, eds. *Probiotics: Prospects of Use in Opportunistic Infections.;* Herborn Dill, Germany: Institute.

Hammes, W.; Weiss, N. and Holzapfel, W. (1995). The genera *Lactobacillus* and *Carnobacterium*. In: knmm..Balows A, Tr¨uper H, Dworkin M, Harder W, Schleifer KH, eds. *The Prokaryotes. A Hand*

book on the Biology of Bacteria: Ecophysiology, Isolation, Applications. Vol II. 2nd ed. New York, NY: Springer,1536–1594.

Oca˜na, V.; Bru, E.; de RuizHolgado, AAP and Nader-Mac´ıasME (1999). Surface characteristics of lactobacilli isolated from human vagina. *Journal of General and Applied Microbiology*. **45** (5): 203–212.

Ocana, V.; Silva, C. and Nader-Macıas, M.E. Antibiotic Susceptibility of Potentially Probiotic Vaginal Lactobacilli. *Infectious Diseases in Obstetrics and Gynecology*, Volume 2006, Article ID 18182, Pages 1–6, DOI 10.1155/IDOG/2006/ 18182.

Raibaud, P.; Galpin, J.V.; Duclezeau, R.; Mocquot, G. and Oliver, G. (1963). Le Genre *Lactobacillus* dans le tube digestif du rat. II. Caracters de souches heterofermentaires isolates de rats. "holo" et "gnotoxeniques". AnnMicrobiologie. *Annales de Microbiologie (Annales de L'Institute Pasteur).* **124**: 2223–2235.

Redondo-Lopez, V.; Cook, R.L. and Sobel, J.D. (1990). Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. *Reviews of Infectious Diseases.* **12** (5):856–872.

Simoes, J.A.; Aroutcheva, A.A.; Shott, S. and Faro, S. (2001). Effect of metronidazole on the growth of vaginal lactobacilli in vitro. *Infectious Diseases in Obstetrics and Gynecology*. **9** (1):41–45.

Lactobacillus. Lactobacilli are added as deliberate starters or take part in the fermentation as a result of their being natural contaminants of the starting substrates. From: Encyclopedia of Food Microbiology (Second Edition), 2014. Related termsÂ Lactobacilli are small, slender, nonmotile, gram-positive bacilli in chains. Immature colonies appear smooth, convex, and translucent. 17. Ocana V., Silva C., Nader-Macias M.E. Antibiotic susceptibility of potentially probiotic vaginal lactobacilli. Infect Dis Obstet Gynecol. 2006; 2006: 18182. 18.Â 19. Charteris W.P., Kelly P.M., Morelli L., Collins J.K. Antibiotic susceptibility of potentially probiotic Lactobacillus species. J Food Prot 1998; 61: 12: 1636-1643. 20. Kirtzalidou E., Pramateftaki P., Kotsou M., Kyriacou A. Screening for lactobacilli with probiotic properties in the infant gut microbiota. Anaerobe 2011; 17: 6: 440-443.Â 34. Sandberg-Gertzen H., Kjellander J., Sundberg-GillÃ¥ B., JÃ¤rnerot G. In vitro effects of sulphasalazine, azodisal sodium, and their metabolites on Clostridium difficile and some other faecal bacteria. Scand J Gastroenterol 1985; 20: 5: 607-612. Taking antibiotics along with Lactobacillus can reduce the effectiveness of Lactobacillus. To avoid this interaction, take Lactobacillus products at least 2 hours before or after antibiotics. Medications that decrease the immune system (Immunosuppressants)Interaction Rating: Minor Be cautious with this combination.Talk with your health provider.Â Persistence of colonization of human colonic mucosa by a probiotic strain, Lactobacillus rhamnosus GG, after oral consumption. Appl Environ Microbiol 1999;65:351-4. View abstract.