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A Study on the drug resistance of probiotic strains isolated from commercial probotic products available in the local market of Agra

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ABSTRACT

Probiotics are well known to put positive health effects upon the humans and animals are now being used as medicines to combat the diseases due to their ability to inhibit the pathogenic microorganisms. This study evaluates their susceptibility towards the different drugs which could be an important parameter in the abovementioned regard. For this the probiotic strains were isolated from the commercial probotic products and their drug susceptibility was assessed by using Kirby Bauer disc diffusion method against the readymade antibiotic discs of Amikacin, Azithromycin, Levofloxacin, Ceftazidime, Chloramphenicol, Aztreonam, Amoxycillin/Clavulanate, Ciprofloxacin, Piperacillin/tozobactum, Nitrofurantoin and meropenem. Almost 80% of the probotic strains were found to show resistance for the drugs Ceftazidime Amoxycillin/Clavulanate and Aztreonam but highly sensitive to Ciprofloxacin, Meropenam, Levafloxacin, Chloramphenical, Amikacin and piperacillin/tozobactum while showed intermediate sensitivity for the drugs Nitrofurantoin and Azithromycin. Rest of the 20% strains showed intermediate to high sensitivity against the abovementioned drugs without any resistance case.

(Key words: Probiotics, Antibiotics, resistance and Drug susceptibility).

INTRODUCTION

Probiotics are live microorganisms which confer a health benefits upon the health of the human beings and animals when consumed in appropriate amounts. Probiotics put numerous health benefits including prevention of diarrhea[1] or irritable bowel syndrome[2] pouchitis, ulcerative colitis[3] lactose intolerance [4] urinary tract infections [5]constipation, allergies[6] bacterial & yeast vaginosis [7,8] Probiotics have been found to useful in control of Blood cholesterol, Helicobacter pylori infection[9] and even cancer in some animal models[10]. Probiotics regulate microbial homeostasis by suppression of growth or epithelial binding/invasion of pathogenic bacteria; improve epithelial barrier function, or immune-regulatory activities. Probiotics boon animals by putting beneficial effects on the one hand and by inhibiting the harmful pathogens on the other hand [11]. There is a long lasting list of studies supporting the antimicrobial potential of the probiotics [12, 13, 14] and their ability to enhance the antimicrobial activity of the antibiotics [15, 16, 17]. This antagonistic potential of probiotics has been further evaluated in a view to establish them as an alternative medicine. Besides, the antibiotic & probiotic combination therapy is in vogue. So there is an urgent need to understand their susceptibility towards the various drugs because lesser is the

susceptibility of probotics to the drug greater is the antagonistic effect of the probotic strains with drug when given in combination and *vice versa*. This study is an attempt to understand the antibiotic resistance patterns of the probiotic strains isolated from the commercial probitic products. For this, two probotic products "Darolac" "Prepro" were collected from the local market of Agra and the probotic strains were isolated from them by using the suitable protocol. Now their antibiotic susceptibilities were assessed by using the readymade antibiotic discs.

MATERIALS AND METHODS

Isolation of Probotic strains :
Commercial Probiotic products 'Darolac' and 'Prepro' were used to isolate the different probotic strains used in the study. According to the product content information given on "Darolac" packet, four probiotc strains *Lactobacillus rhamnosus, Saccharonayces boulardii, Lactobacillus acidophilus, Bifidobacterium longum* were present in it. Out of these two strains *Lactobacillus rhamnosus, Saccharonayces boulardii* were isolated for the study. Similarly, Prepro was having the probotic strains: *Streptococcus faecalis , Clostridium butyricum Bacillus mesentricus Lactobacillus acidophilus.* Out of these three strains, *Streptococcus faecalis , Clostridium butyricum* and *Lactobacillus acidophilus* were used in the study.

Darolac:
This product was in form a capsule so the powder from the capsule was suspended in De man,s Rogosa Sharpee (MRS) broth in anaerobic condition at 37^0C for 24 hrs. After incubation a loopful MRS broth was dispensed to MRS agar which was kept in Mc intosch jar with an anaerobic gas packet for 48 hr at 37^oC. Now the *L. rhamnosus* was isolated from the mixed colonies appeared on the plate by repeated sub culturing and confirmed by colony morphology and microscopic examination. *S.boulardii* was isolated from 'Darolac' by preparing its aqueous suspension and inoculating it on sabraoud's agar and keeping at 37^0C for 24hr in aerobic condition. Now the pale yellow colonies were picked by a straight wire and dissolved in normal saline which was further inoculated on sabraoud's agar to isolate the pure colonies of *S.boulardii*.

Prepro:
The powder from the prepro capsule was drained into the two aliquots, one in the cooked meat broth for the isolation of *C.butyricum* and another in the MRS broth for the isolation of: *Streptococcus faecalis* and *Lactobacillus acidophilus.* Both the aliquots were kept at 37^0C for 24hr in anaerobic conditions. After the incubation both the broth were inoculated on the blood agar and kept in Mc Intosch jar at 37^0C for 48hr along with an anaerobe gas packet. *C.butyricum* was isolated from the cooked meat broth inoculated blood agar plate and *S.faecalis* was isolated from MRS broth inoculated blood agar plate. Further the MRS broth was also streaked on the MRS agar kept at 37^0C for 48hr in Mc Intosch jar to isolate the *L.acidophilus.* Pure colonies were obtained by repeated sub culturing in all the cases. All the probiotic strains were confirmed by Gram's staining, cell and colony morphology.

Preparation of probotic suspension:
All the isolated probotic strains were kept at 4^0C in the Brain heart infusion butt slant tubes. To prepare the probotic suspensions for inoculation, the pure colonies of each probotic culture was inoculated in distilled water and the turbidity of the medium was adjusted as Mac farland standard #0.5, now this suspension was used detect the resistance of probotic strains against the various drugs.

Antibiotic susceptibility of probotic strains:
Antibiotic susceptibilities of the all four probiotic strains were detected against the antibiotic discs of Aztreonam(AT^{30}), Ceftazidime(CAZ^{30}), Amoxicillin/Clavulanate($AMC^{20/10}$), Piperacillin/Tozobactum($PIT^{100/10}$), Nitrofurantoin(NIT^{300}), Azithromycin(AZM^{15}), Ciprofloxacin(CIP^5) Meropenem(MRP^{10}), Levofloxacin(LE^5), Amikacin(AK^{30}) and Chloramphenicol(C^{30}) (Hi Media, India) by disc diffusion method [18] according to the national committee for clinical laboratory standards (NCCLS) guidelines. For this the probotic suspensions were swabbed on the M.R.S. agar surfaces. Now the antibiotic discs were placed on Muller Hinton Agar (MHA) surface and kept at 37^0C for 24 hrs. Diameters of zones of inhibition were measured by using a standard caliper from the back of Petri plate.

RESULTS AND DISCUSSION

Both *L.rhamnosus* & *L.acidophillus* and produced round, small creamish colonies on MRS agar and appeared as gram +ve bacilli. A common shape bacilli in smear of Darolac and Prepro was identify as *L.acidophillus. S.boulardii* produced white colonies on sabraoud's agar viewed with characteristic oval shaped cells under microscope. *S.faecalis* viewed as oval cocci in short chain. *C.butyricum* colonies produced narrow zone of complete haemolysis on blood agar and the meat pieces in cooked meat broth turned to pink. Gram positive bacilli with sub terminal spores singly or in chains were identify as *C.butyricum.*

All the probiotic strains were found to be highly sensitive to Levafloxacin and Meropenam (Zone of inhibition 30/35 mm). *Lactobacillus rhamnosus*, *Saccharonayces boulardii*, *Streptococcus faecalis* and *Lactobacillus acidophilus,* were highly resistance to Aztreonam and ceftazidime (0mm) followed by Amoxicillin/clavulanate (6-8mm), Nitrofurantoin(7-20mm) Azithromycin(16-19mm), piperacillin/tozobactum (21- 26 mm) Chloramphenical (22-28 mm), Amikacin(24-29mm) and Ciprofloxacin(12-30mm). *C.butyricum* was not found to show strict resistance against any drug but showed minimum to intermediate sensitivity (15-28mm) for all the drugs except Levafloxacin and Meropenam(table-1,fig.1&2)

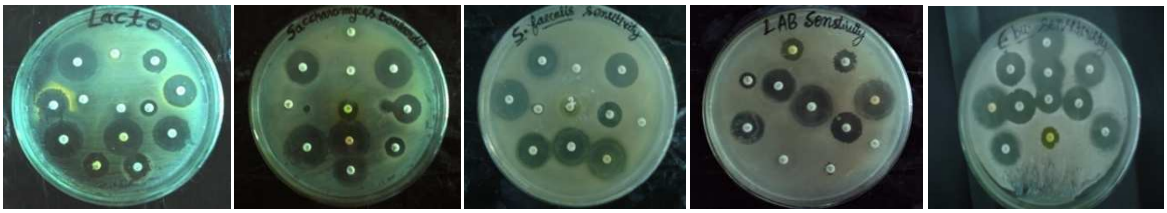

Fig1:- Antibiotic susceptibility of the (a)*L. rhamnosus,* (b)*S .boulardii,*(c) *S .faecalis,* (d) *L. acidophilus* (e) *C.butyricum* against the all 11 drugs used in the study.

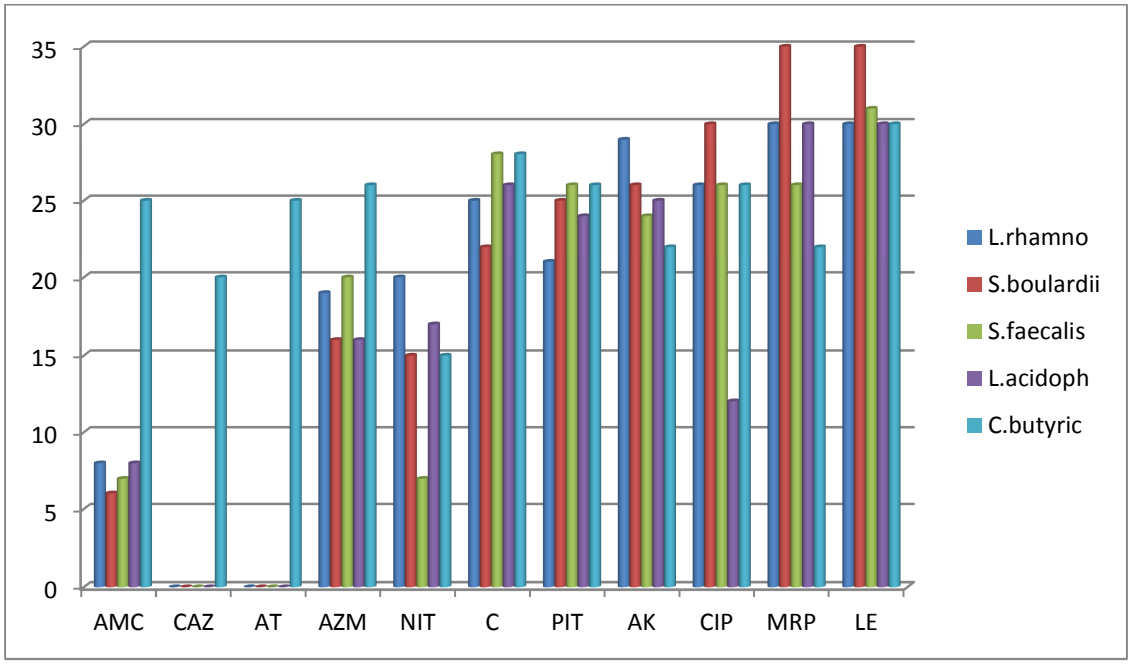

Fig-2: comparision of zone of inhibition of probioric strains against the different drugs used in the study.

Table-1: Zone of inhibition of isolated probotic strains against the 11 antibiotics.

Probiotic strain	Diameter of the zone of inhibition										
	AMC	CAZ	AT	AZM	NIT	C	PIT	AK	CIP	MRP	LE
L.rhamno.	8	0	0	19	20	25	21	29	26	30	30
S.boulardii	6	0	0	16	15	22	25	26	30	35	35
S.faecalis	7	0	0	20	7	28	26	24	26	26	31
L.acidoph.	8	0	0	16	17	26	24	25	12	30	30
C.butyric.	25	20	25	26	15	28	26	22	26	30	30

CONCLUSION

In this study the antibiogram of commonly used probotic strains was prepared against the frequently used antibiotics. Most of the probotic strains were found to resistant against AT, CAZ and AMC indicating their best antagonistic potential with these drugs followed by AZM, NIT,C, PIT, AK, CIP, MRP and LE. *C.butyricum* was found to show intermediate to high sensitivity against the abovementioned drugs but not resistant to any drug, proving its least antimicrobial potential in these drug combinations. It is advisable to use resistance probiotic strains in various drug combinations instead of sensitive one in order to bring their best antimicrobial effect.

REFERENCES

[1] Vanderhoof JA, *J Pediatr Gastroenterol Nutr*., **2000,** 30, S34–S38.
[2] Nikfar S, Rahimi R, Rahimi F, *Dis Colon Rectum* **2008**, 51, 1775–1780.
[3] Bibiloni R, Fedorak RN, Tannock GW, et al, *Am J Gastroenterol* **2005**, 100, 1539–1546.
[4] Alm L, *J Dairy Sci* **1982**, 65, 346–352.
[5] McLean NW, Rosenstein IJ, *J Med Micro-biol*, **2000**, 49, 543–552.
[6] Murch SH, *Lancet* **2001**, 357, 1057–1059.
[7] Sieber R, Dietz UT, *Int Dairy J*, **1998**, 8, 599-607.
[8] Hilton E, Rindos P, Isenberg HD, *J Clin Microbiol*, **1995**, 33, 1433.
[9] Aiba Y, Suzuki N, Kabir AM, Takagi A, Koga Y, *Am J Gastroenterol*. **1998**, 93, 2097–101.
[10] Lee JW, Shin JG, Kim EH, Kang HE, Yim IB, Kim JY, Joo HG, Woo HJ, *J Vet Sci*, **2004,** 5, 41–48.
[11] Gorbach SL, *utr Today*. **1996**, 31(6 suppl), 2-4S.
[12] Hawrelak JA, Whitten DL, Myers SP, *Digestion*. **2005**, 72, 51-56.
[13] de Vrese M, Kristen H, Rautenberg P, Laue C, Schrezenmeir J, *J Dairy Res*. **2011**, 78, 396-403.
[14] Hickson M, *Therap Adv Gastroenterol*. **2011**, 4, 185 –197.
[15] SharmaJ, Chauhan DS, *Int J. current sciences*, **2014,** (in press).
[16] Sharma J, Chauhan DS, *Int. J. Pharm. Life Sci.*, **2015**, 6 (1), 4161-4165.
[17] Sharma J, Chauhan DS, Goyal A, *Advances in applied science research*. **2014,** 5(6):14-18.
[18] Bauer AW, Kirby WM, Sherris JC, *Am. J. Pathol*., **1966**, 45, 493.