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Enhancement of antimicrobial activity of antibiotics by probiotics against *Escherichia coli*-An *invitro* study

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ABSTRACT

Escherichia coli is the commonest organism responsible for urinary tract infection and diarrhoea specially in developing countries like India. Probiotic strains (Lactobacillus rhamnosus, Saccharomyces boulardii, Streptococcus faecalis and Lactobacillus acidophilus) are found to have antagonistic activity against E.coli (MTCC 443 and isolated human clinical strain). This study deals with enhancement of zone of antibiotics (Amoxicillin/clavulanate, Amikacin, Ceftazidime and Piperacillin/Tozobactum) by above mentioned Probiotic strains against E.coli Potentiation of antimicrobial activity of antibiotics by probiotic strains was investigated by using Kirby bauer disc diffusion method, keeping the antibiotic discs as positive control. Maximum zone enhancement was produced by Streptococcus faecalis in combination with Ceftazidime with the enhancement of zone by 24 mm and 18 mm against the MTCC and clinical strains of E.coli respectively. S.faecalis produced 14 mm enhancement in combination with Amoxicillin/Clavulanate against E.coli MTCC 443 followed by 10 mm by S.boulardii and L.rhamnosus in combination with Amoxicillin/Clavulanate against the E.coli (MTCC 443 and clinical strains). No enhancement was seen against probiotic strains and Piperacillin tozobactum combinations, while marginal enhancement was observed by Amoxicillin/Clavulanate in combination with the given probiotic strain. 75% tests showed enhancement of zone while in 21.87% tests zone diameter remained the same. Only 3.125% tests has shown decrease in zone diameter. The positive outcome of this study definitely indicates the therapeutic utility of the Probiotics.

Key words: Antagonistic effect, Probiotics, Antibiotics, *Lactobacillus rhamnosus, Saccharomyces boulardii, Streptococcus faecalis* and *Lactobacillus acidophilus*

INTRODUCTION

Use of probiotics for the betterment of human and animals is an established fact now. Recently a plenty of studies have been emerged in support of their antimicrobial effect from the good quality clinical trial with randomized placebo controlled design and results from the properly performed invitro studies[1,2,3,4,5]. Probiotics with a variety of application are reported to enhance the intestinal health and immune system, as well as anti carcinogenic, anti diarrheal and hypocholesterolaemic effects, improve lactose utilization[6,7]. Lactobacilli are known to produce many types of bacteriocins like acidophilin acidolin, lactocidin, lactobrevin[8,9]. These organic acids not only lower the pH thereby affecting the growth of pathogens but also are toxic to microbes. Besides producing antimicrobial toxins,

probiotics have ability to adhere to cells, reducing pathogenic bacteria adherence hence causing pathogen exclusion. Probiotics interact with epithelial cells and dentritic cells and immunomodulatory effect. Many *Lactobacilli, streptococcus* and *saccharomyces* species have been reported to found safe for the prevention and treatment of various infectious diseases[10,11].

Indiscriminate use of costly antibiotics leading to the emergence of multi drug resistance in pathogenic bacteria is a major clinical concerned throughout the world[12]. The severe side effects of antibiotic therapy has raised the demand for an alternative safer therapeutic agent[13]. Probiotic could be a good candidate in this regard[14]. The antimicrobial activities either *invivo* or *invitro* against the wide range of pathogens including *E.coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* have been reported by the various species of *lactobacilli, sachharomyces* and *streptococcus*[15,16]. Researches related to the use of probiotic as a complete alternative of antibiotic are in early phases. So, it is envisaged that a judious combination of antibiotics & probiotics must be used to treat acute phase of the infection as the enhancement in the antimicrobial activity of the antibiotic by probiotic strain will not only cut the duration but also the cost of the antibiotic treatment booning the poor people in developing countries on one hand and reducing the increasing drug resistance in pathogenic micro-organism on the other hand.

Present investigation is an attempt to evaluate the enhancement of antimicrobial activity of antibiotic by probiotics and to investigate the interaction between probiotic strain and antibiotics with a view to finding a suitable probiotic strain for use in both preventive and therapeutic purposes. This study evaluates the potentiation of antimicrobial activity of the Antibiotics, Amoxicillin/Clavulanate (AMC), Amikacin (AK), Ceftazidime (CAZ) and Piperacillin/Tozobactum (PIT) by the probiotic strains *Lactobacillus rhamnosus, sachharomyces boulardii, streptococcus faecalis* and *Lactobacillus acidophilus invitro* against standard and the clinical isolates of *E.coli.*

MATERIALS AND METHODS

Bacterial isolation and cultivation :

Probiotic strains *L.rhamnosus* and *S.boulardii* were isolated from commercially available capsule 'Darolac'. For this, half of capsule was suspended in 2 ml of MRS broth in anaerobic condition and kept at 37^0C for 24 hrs while another half was suspended in normal saline and inoculated on sabraoud's agar and kept at 37^0C for 24 hrs in aerobic condition. After incubation a loopful MRS broth was dispensed to MRS agar and kept in Mc intosch jar with an anaerobic gas packet for 48 hr at 37^oC. *S.boulardii* was isolated from sabraoud's plate while *L.rhamnosus* was isolated from MRS plate. Simillarly *S.faecalis* and *L.acidophillus* were isolated from the commercially available product 'Prepro' with the only difference that the *S.faecalis* was subculture on blood agar from the mixed colonies appeared on MRS agar. Pure colonies were obtained by repeated plating. All the probiotic strains were confirmed by Gram's staining, cell and colony morphology.

Culture of *E.coli*. MTCC443 was collected from Imtech, Chandigarh, India. The clinical isolate of *E.coli* was obtained from the Department of Microbiology, S.N. Medical College, Agra (India) and was confirmed by using standard morphological, cultural and biochemical reactions. *E.coli* stock was prepared by inoculating it to Brain heart infusion agar slant in screw capped tubes and stored at 4^oC.

Antibiotic resistance :

Antibiotic resistance of probiotic strains was assessed using antibiotic discs (Hi Media, India) by using disc diffusion method[17] according to the national committee for clinical laboratory standards (NCCLS) guidelines. Probiotic suspension 0.5 *Mc.farland* standard was inoculated by swabbing the MHA surface 3 times by rotating at 60^o to ensure even distribution. After 10 min. antibiotic discs of Amikacin (30ug), Ceftazidime (30ug), Meropenem (10ug), Azithromycin (15ug), Aztreonam (30ug), Nitrofurantoin (300ug), Amoxicillin/Clavulanate (20/10ug), Piperacillin/Tozobactum (100/10ug), Ciprofloxacin(5ug), Levofloxacin (5ug) and Chloramphenicol (30ug) were placed on Mueller hinton agar (MHA) surface and kept at 37^0C for 24 hrs.

Antagonistic activity :

The antagonistic activity of antibiotic and antibiotic & probiotic combination was determined by modified disc diffusion method according to the NCCLS guidelines. Probiotic test inocula was prepared by inoculating pure well isolated colonies into the brain heart infusion broth (BHIB) and kept at 37^0C for 24 hrs. The two MHA plates of 120 mm diameter were swabbed by *E.coli,* MTCC 443 and *E.coli* from clinical sample separately for each of the 4 probiotic strain and kept for 3 hr. at 37^0C. Now the readymade antibiotic disc of AMC, AK, CAZ and PIT were

dipped into the probiotic test inocula and kept for 1 hr at 37^0C to allow the maximum absorption. The MHA plates were seeded with the above antibiotic disc impregnated with probiotic along with plain antibiotic disc taking as positive control. Now the MHA plates were kept at 4^oC for 1 hr to allow the proper diffusion. The two MHA plates were now kept at 37^0C for 24 hrs. Zone of inhibition were measured by using a caliper micrometer against the back of the petri plates[17].

MIC of probiotic strains :

For testing the Minimum Inhibitory Concentrations (MIC) of probiotic strains, 3 steril blank discs of 6 mm diameter were transferred with 20 ul of their respective serial suspensions i.e. the suspension of turbidity equal of ≠ 1.0, (3×10^8 cfu/ml), 1/10 (3×10^7 cfu/ml) and 1/100 (3×10^6 cfu/ml). These discs were kept at 37°C for 1 hr. so that to absorb in their full capacity. These impregnated discs now contained approximately 6×10^6 cfu/disc (for Mac Farland standard ≠ 1.0), 6×10^5 cfu/disc (for 1/10 serial suspensions) and 6×10^4 cfu/disc (for 1/100 serial suspension).

Now a petriplate of MHA was swabbed with *E.coli* MTCC443 and clinical isolate of *E.coli* and kept at 37°C for 3 hrs. After it the probiotic discs were placed gently on the surface of MHA plate, along with the sterile water disc (negative control). The plates were kept at 4°C for 1 hr diffusion and then incubated at 37°C for 24 hrs zones of inhibition were measured **.**

RESULTS AND DISCUSSION

Bacterial isolation and cultivation :

L.rhamnosus and *L.acidophillus* were isolated from 'Darolac' and 'Prepro' cultivated on MRS. Both the *Lactobacilli* showed round, small colonies without any pigment and white to cream in colour. Both appeared as gram +ve bacilli. *S.boulardii.* produced dense, smooth and white colonies on Sabrauds's agar and produced characteristic oval shaped cells under microscope. *S.faecalis* appeared as gram +ve cocci in chains. *E.coli* produced circular, moist, smooth and non mucoid colonies on nutrient agar and viewed as gram -ve bacilli. The biochemical kit testing for *E.coli* was found to positive for almost all the carbohydrate utilization tests as it showed more than 90% positivity towards glucose, arabinose, lactose, sorbitol, mannitol and 11-89% positivity towards rhamnose and sucrose but was negative for adonital. *E.coli* gave positive Indole and methyl red but negative *Voges proskauer* and citrate utilization test (IM ViC, + + - -).

Antibiotic susceptibility of probiotic strains :

All the probiotic strains were highly resistance to Aztreonam and Ceftazidime as the zone of inhibition was 0 mm in both the cases. Next in the row was Amoxicillin/Clavulanate combination, where the zone of inhibition was 6-8 mm. Azithromycin and Nitrofurantoin showed comparatively larger zone of inhibition with the maximum diameter of zone around 19 to 20 mm. Chloramphenical and Piperacillin/Tozobactum produced the even larger zone (upto 28 mm) but their sensitivity was still lesser than Leavofloxacin and Meropenam and results are shown in Table-1.

Table-1 : Antimicrobial activity of antibiotics and antibiotics + Probiotic combination against *E.coli*

Test Microorganism	***E.coli* MTCC 443**												***E.coli* clinical isolate**											
	Diameter of the zone of Inhibition (in mm)												**Diameter of the zone of Inhibition (in mm)**											
	L. rham			***S. boul.***			***S. face.***			***L. acido.***			***L. rham.***			***S. boul***			***S.fae.***			***L. acido.***		
Drugs	A	A+L rham	E	A	A+ S. boul.	E	A	A+S. face.	E	A	A+ L. acido.	E	A	A+ L. rham.	E	A	A+ S. boul	E	A	A+ S.fae.	E	A	A+ L. acido.	E
AMC	6	15	9	6	16	10	6	20	14	6	10	4	18	28	10	12	22	10	15	18	3	15	25	10
AK	27	28	1	28	30	2	30	32	2	30	31	1	32	34	2	30	31	1	32	32	0	32	34	2
CAZ	0	16	16	0	16	16	0	24	24	0	18	18	6	20	14	0	16	16	0	18	18	0	20	20
PIT	27	27	0	26	26	0	26	25	-1	26	26	0	30	30	0	30	30	0	31	31	0	28	31	3

Antagonistic activity :

The antagonistic activity of all the 4 probiotic strains, *L.rhamnosus*, *S.boulardii*, *S.faecalis* and *L.acidophillus* were assessed against the both *E.coli*, MTCC443 and clinical isolate of *E.coli*. The drugs, $AMC^{20/10}$, AK^{30}, CAZ^{30}, $PIT^{100/10}$ and AK was taken as +ve control. The synergistic activity 0f all these drugs and probiotic combinations were compared with the antagonistic activity of antibiotic drug used to see the enhancement of zone by probiotic

counterpart. Almost in all the cases maximum enhancement was shown by CAZ & probiotic combinations followed by probiotic combination with the drugs AMC, AK and PIT.

MIC of probiotic strains :

The Minimum Inhibitory Concentrations (MIC) of all the 4 probiotic strains assessed against both *E.coli*, MTCC443 and clinical isolates keeping sterile water disc as -ve control and the drug AK (referred from the synergistic activity section) as +ve control. Maximum Inhibitory activity was shown by the Mac farland standard #1.0(3x108 cfu/ml) followed by 3x107 cfu/ml and 3x106 cfu/ml by almost all the probiotic strain in both the cases (Table-2).

Table-2: Antimicrobial activity and minimum inhibitory concentration of probiotic strains against *Escherichia coli* (MTCC) & clinical isolated

S. No.	Name of the serial suspensions	Zone of inhibition (in mm)							
		L. rhamnosus		*S. boulardii*		*S. faecalis*		*L. acidophilus*	
		MT	CI	MT	CI	MT	CI	MT	CI
1.	Antibiotic drug (AK)	27	32	28	30	30	32	30	32
2.	M.F.S. ≠ 1	20	15	20	17	18	14	18	13
3.	1/10 suspension	6	6	7	6	7	6	6	6
4.	1/100 suspension	0	7	0	7	0	8	0	6
5.	Sterile d.w. disc	0	0	0	0	0	0	0	0

(MT- MTCC; CI- CLINICAL ISOLATED)

This study has used 4 probiotic strains isolated from the commercial probiotic products. The synergistic effect of all probiotic strains in combination with drugs AMC, AK, CAZ and PIT was recorded against the *E.coli* MTCC 443 and clinical isolates to evaluate the potentiating role of probiotic in the antimicrobial activity of the drug in combination. The antibiogram of each probiotic strain indicates that resistance of the probiotic strain to some antibiotics could be used for preventive and therapeutic purposes in controlling some infections[18].

All the probiotic strains showed maximum enhancement of zone with ceftazidime as the enhancement of the zone went upto 24, 18 and 16 mm. Ceftazidime combinations were followed by AMC (max. 14 mm), AK (max. 2 mm) and PIT where no enhancement was observed except in combination with *L.acidophilus* against *E.coli* clinical isolates. *S.faecalis* showed best enhancement of the zone almost with all the drug combinations except with PIT where the reduction of zone by 1 mm was noticed against the *E.coli* MTCC443. *S.boulardii* showed better enhancement of the zone than *L.rhamnosus* and *L.acidophillus* but lesser than *S.faecalis* against *E.coli*. MT CC443. On the controrary *L.acidophillus* showed best enhancement with all the drugs against the clinical isolates *E.coli* followed by *L.rhamnosus, S.boulardii* and *S.feacalis*. In 75% cases i.e. 3 out of 4 probiotic strains, *L.rhamnosus, S.boulardii* and *S.faecalis* produced maximum enhancement o0f the zone against the *E.coli* MTCC443 strain while in only 25% of cases i.e. by *L.acidophillus* the better enhancement was recorded against the clinical isolates of *E.coli*.

This study has attempted to establish the role of probiotics in prophylaxis and in the treatment of diseases by cutting down the duration and cost of antibiotic therapy as the patients can be switched over to probiotic therapy only when the acute phase of the treatment is treated with antibiotic probiotic combination.

CONCLUSION

In conclusion all the probiotics have shown the antimicrobial activity against the standard and clinical isolate of *E.coli* alone as well as in combination with other drugs. Probiotic strains resistance to the drug in combination showed maximum enhancement as compared to the sensitive one. Out of total 32 invitro tests 75% showed enhancement of zone diameter but no enhancement was seen 21.87 % cases while reduction in zone size was recorded in 3.125 % tests. No reduced zone was seen against the clinical isolate giving rise to75% cases with enhanced zone diameter recording 25% cases with unaffected zone diameter.

REFERENCES

[1] Simone Rosario C Cid, Ma Carina Cruz, Vincent Faustino, Alexander O Tuazon, *PIDSP Journal*, **2005,** 9 (2), 2342.
[2] Jacobsen CN, *Appl. Environ. Microbio*., **1999,** 65**,** 4949.

[3] Millette M, Luquet FM, Lacroix M, *Appl. Microbio.*, **2006,** 314.
[4] Naheed M, Mehdi PA, *J. Appl. Sci***., 2006,** 6 (12), 2629.
[5] Bhutada SA, Dahikar SB, Tambekar DH, *International Journal of Pharmaceutical Sciences Rev.*, **2011,** 9 (2), 2143.
[6] Gibson GR, Probert HM, Van Loo J, Rastall RA, *Nut. Res. Rev.*, **2004**, 17**,** 259.
[7] Sumaryati Syukur, Endang Purwati, *Biotechnology Probiotic*, **2013,** 9**,** 978.
[8] Alvarez-Olmos MI, Oberhelman RA, *Clin Infectious Diseases***, 2001,** 32 (11), 1567.
[9] Sarika AR, Lipton AP, Aishwarya MS, *Adv. J. Food Sci. Technol*., **2010,** 2(5), 291.
[10] Chapoy P, *Ann. Pediatr (Paris),* **1985,** 32, 561.
[11] Saggioro A, *J. Clin. Gastroenterol*, **2004,** 38 (6), S104.
[12] De La Cochetiere MF, Durand T, Lalande V, *Microb. Ecol*., **2008**, 56, 395.
[13] Mirnejad R, Jafari H, Ardebilli A, Babavalian H, *African J. Microb. Res***.**, **2012,** 4, 2283.
[14] Jamalifar H, Rahimi H, Samadi N, Shahverdi A, Sharifian Z, Hosseini F, *Iran J. Microbiol*., **2011**, 3(1), 21.
[15] Gopal Pramod K, Prasad Jaya, Smart John, Gill Harsharanjit S, *International Journal of Food Microbiology*, **2001,** 67 (3), 207.
[16] Karska-Wysocki B, Bazo M, Smoragiewicz W, *Microbiol. Res*., **2010**, 165 (8), 674.
[17] Bauer AW, Kirby WM, Sherris JC, *Am. J. Pathol*., **1966**, 45, 493.
[18] El-Naggar MYM, *Asian Network for Scientific Information Biotechnol*., **2004**, 3 (2), 173.