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In vitro* study on the role of probiotic strains in potentiation of antimicrobial activity against *Staphylococcus aureus

Jagriti Sharma^{1*} and D.S. Chauhan²

1, Department of Microbiology, School of Life Sciences, Dr. B.R. Ambedkar University
Khandari Campus, Agra, (UP) - India

2, Department of Microbiology & Molecular Biology, National JALMA Institute for Leprosy and other
Mycobacterial Diseases, Agra, (UP) - India

Abstract

Probiotic strains *Lactobacillus rhamnosus*, *Saccharomyces boulardii*, *Streptococcus faecalis* and *Lactobacillus acidophilus* have been found to enhance the antagonistic activity of antibiotics (Amoxycillin/Clavulanate, Azithromycin, Ciprofloxacin and Levofloxacin) against the standard and clinical isolates of *staphylococcus*. Probiotic strains were isolated from the commercial probiotic products "Darolac" and "Prepro" by using suitable protocol. For this study the Kirby Bauer disc diffusion method was used. Zone of inhibition of antibiotics was enhanced in 84.375% tests by probiotic strains against the standard and clinical isolates of *staphylococcus*. No enhancement was seen in 12.5% of tests while reduction of zone was observed only in 3.125% tests. This study endorses the role of Probiotic strains in reducing the multiple drug resistance of *staphylococcus*.

Key-Words: Antagonistic effect, Probiotics, Antibiotics

Introduction

Probiotics are live microorganisms which confer beneficial effect on the host when consumed in appropriate amounts.⁽¹⁾ The main probiotic genera include *Lactobacilli*, *Streptococcus*, *Bifidobacterium*, *Saccharomyces*, *Bacillus*, *Faecalibacterium*, *Clostridium*, *Propionibacterium*, *Bacteroids*, *Enterococcus*, etc.. Probiotic strains have been found to be effective in the prevention of diarrhea, pouchitis, antibiotic associated diarrhea, ulcerative Colitis, hypercholesterolemia, allergies and colon cancer was reduced in animal models remission of abdominal pain.⁽²⁻⁵⁾ Risk of colon cancer was reduced in animal models by the use of probiotics.⁽⁶⁾ The chief mechanisms of Probiotic action include regulation of intestinal microbial homeostasis, Immunomodulation, pathogen exclusion and antimicrobial activity.⁽⁷⁻¹⁰⁾ There are many studies reporting the antagonistic role of probiotics *in vivo* and *in vitro* conditions.⁽¹¹⁻¹⁴⁾ Also the probiotic strains have been found to enhance the antimicrobial activity against the *P. aeruginosa* and *E.coli*.^(15,16)

Staphylococcus aureus is one of the major resistance pathogens. Community acquired methicillin resistance *S. aureus* (CA-MRSA) has emerged as an epidemic causing fatal diseases like necrotizing pneumonia, severe sepsis and necrotizing fasciitis.⁽¹⁷⁾ Apart from MRSA *S.aureus* has been found to show the resistance to penicillin, erythromycin and tetracycline. So there is urgent demand for some alternative medicine which not only show antagonistic activity but also put positive health effects on the host. Serious attempts are being made to put probiotics as an alternative to antibiotics to some extent, if not completely. So, the studies portraying probiotics as an agent to enhance the antimicrobial activity of antibiotics could gain much interest. Present study is an effort in the same direction. This study was carried out to see the role of various probiotic strains in potentiating the antimicrobial activity of the Antibiotics, Amoxycillin/Clavulanate (AMC), Azithromycin (AZM), Ciprofloxacin (CIP) and Levofloxacin (LE) against the standard and clinical strain of *S.aureus*.

Material and Methods

Probiotic strains and test pathogen

Probiotic strains *Lactobacillus rhamnosus* and *Saccharomyces boulardii* were isolated from commercially available capsule 'Darolac' by inoculating half of ampoule in MRS broth in anaerobic condition and another half to the Sabraoud's

* Corresponding Author

E.Mail: microjagriti@gmail.com

agar in aerobic condition and incubate at 37°C for 24 hrs. Now a loopful of MRS broth was dispensed to MRS agar and kept in Mcintosh jar with an anaerobic gas packet for 48 hr at 37°C. *S.boulardii* & *L.rhamnosus* were isolated from sabraoud's and MRS plate respectively. *Streptococcus faecalis* and *Lactobacillus acidophilus* were isolated from the commercial product 'Prepro' by subculturing on blood and MRS agar respectively from the mixed colonies appeared on MRS agar. Pure colonies were obtained by repeated sub culturing of a single colony from each plate. All the probiotic strains were confirmed by Gram's staining, cell and colony morphology. Test pathogen, *Staphylococcus aureus* MTCC737 was obtained from Imtech, Chandigarh, India while the clinical isolate of *Staphylococcus* was collected from the Department of Microbiology, S.N. Medical College, Agra (India) and confirmed by cultural and biochemical test. Bacterial Stocks were kept in Brain heart infusion agar slant at 4°C.

Drug susceptibility of the probiotic strains

Antibiotic resistance of probiotic strains was assessed by swabbing probiotic suspension of M.F.S.#0.5 on MHA surface against the antibiotic Amikacin, Ceftazidime, Meropenem, Azithromycin, Aztreonam, Nitrofurantoin, Amoxicillin/Clavulanate, Piperacillin/Tozobactam, Ciprofloxacin, Levofloxacin and Chloramphenicol by using disc diffusion method[18] according to the national committee for clinical laboratory standards (NCCLS) guidelines.

Potentiation of antagonistic activity of drugs

Probiotic test inocula was prepared by inoculating MRS broth with probiotic cultures and kept it for 24 hrs at 37°C in anaerobic conditions. Now the turbidity of this inocula was maintained at M.F.S.# 1.0. The turbidity of *S.aureus* suspension was adjusted at M.F.S.# 0.5. Petri plates of 120 mm diameter were poured with 60ml of molten MHA and swabbed by *S.aureus* suspension and kept for 3 hrs at 37°C. The readymade antibiotic disc of AMC, AZM, CIP and LE were dipped in probiotic test inocula and kept for 1 hr at 37°C to absorb in their full capacity. These discs were now placed on MHA plates along with plain antibiotic disc taking as positive control. MHA plates were kept at 4°C for 1 hr to allow the proper diffusion. Then kept at 37°C for 24 hrs. Zone of inhibition were measured.

Antagonistic activity of probiotic strains

The antagonistic activity was determined by preparing the serial dilutions of M.F.S. # 1.0, (3×10^8 cfu/ml), 1/10 (3×10^7 cfu/ml) and 1/100 (3×10^6 cfu/ml) and 20ul of each was transferred to sterile disc of 6mm so contained approximately 6×10^6 cfu/disc (M.F.S.# 1.0), 6×10^5 cfu/disc (1/10 dilution) and 6×10^4 cfu/disc

(1/100 dilution). Now the probiotic discs were dispensed on MHA surface swabbed with *S.aureus* suspension of the M.F.S. # 0.5, taking the sterile water discs as negative control and antibiotic AMC as positive control. The plates were kept at 4°C for 1hr for diffusion and then at 37°C for 24 hrs zones of inhibition were measured.

Results and Discussion

Probiotic strains and test pathogen

L.rhamnosus & *L.acidophilus* appeared as gram +ve bacilli showing round, white to creamish small colonies without any pigment. *S.boulardii* viewed as oval shaped cells under microscope (100X). *S.faecalis* viewed as oval cocci in pair or short chain. The biochemical kit testing for *S.aureus* appeared as gram +ve cocci in clusters, produced circular, smooth, convex and opaque colonies on nutrient agar and gave IMViC(---+). It utilized glucose, lactose and sucrose but did not utilize adonitol, arabinose, sorbitol, mannitol and rhamnose.

Drug susceptibility of the probiotic strains

Almost all the probiotic strains were found to be highly sensitive to Chloramphenicol, Amikacin, Ciprofloxacin, Levofloxacin and Meropenam (zone of inhibition 28-35mm) but resistance to Aztreonam, Ceftazidime and Amoxicillin/Clavulanate, while showed intermediate sensitivity for Azithromycin, Nitrofurantoin and Piperacillin/Tozobactam.

Antagonistic activity of probiotic strains

The (MIC) of *L.rhamnosus*, *S.boulardii*, *S.faecalis* and *L.acidophilus* was assessed against clinical and *staphylococcus aureus* MTCC 737 taking sterile water disc as -ve control and the drug AMC ^{20/10} as +ve control. Maximum antibiotic activity was shown by the M.F.S. #1.0(11-12mm) followed by serial suspension 1/10(6-10mm) and 1/100(0-8mm) by both strains against almost all the probiotic strains.

Potentiation of antagonistic activity of drugs

The potentiation of antimicrobial activity was determined by measuring the difference zones of inhibition produced by all the 4 probiotic strains, *L.rhamnosus*, *S.boulardii*, *S.faecalis* and *L.acidophilus* in combination with the drugs AMC, AZM, CIP and LE against the antibiotic drug used as +ve control for standard and clinical isolate of *S.aureus*. Maximum enhancement was produced by AMC with all the probiotic combinations (upto 8mm) followed by probiotic combination with the drugs CIP(6mm), LE(3mm) and AZM(3mm to -1mm). (Table-1, fig-1&2)

Conclusion

Out of total 32 *in vitro* tests 84.375% showed enhancement of zone diameter but no enhancement

was seen 12.5 % cases while reduction in zone size was recorded in 3.125 % tests. 100% cases showed enhancement of zone size against standard isolate while the clinical isolate showed increase in zone diameter in 68.75% tests. No change was seen in 25% tests and reduction of zone was recorded only in 6.25% tests against the clinical isolate of *staphylococcus*. These results definitely may help to establish probiotic strains as an agent to enhance the antimicrobial activity of antibiotics against *s.aureus* thus helping antibiotics to fight with the increasing drug resistance of *s.aureus*.

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Table 1: Antimicrobial activity of antibiotics and antibiotics + Probiotic combination against *S. aureus*

Test Micro organism	<i>S.aureus</i> MTCC 443												<i>S.aureus</i> clinical isolate											
	Diameter of the zone of Inhibition (in mm)												Diameter of the zone of Inhibition (in mm)											
Drugs	<i>L. rham</i>			<i>S. boul.</i>			<i>S. faec.</i>			<i>L. acido.</i>			<i>L. rham.</i>			<i>S. boul</i>			<i>S.fae.</i>			<i>L. acido.</i>		
	A	A+L rham	E	A	A+ S. boul.	E	A	A+S. faec.	E	A	A+L. acido.	E	A	A+. rham.	E	A	A+ S. boul	E	A	A+ S.fae.	E	A	A+L. acido.	E
AMC	12	18	6	12	18	6	13	18	5	15	20	5	15	18	3	15	21	6	13	21	8	15	22	7
AZM	24	27	3	28	29	1	25	26	1	25	27	2	24	24	0	27	30	3	25	24	-1	25	28	3
CIP	28	30	2	23	24	1	28	29	1	33	35	2	26	27	1	23	26	3	29	29	0	29	35	6
LE	31	32	1	32	33	1	32	35	3	35	36	1	32	32	0	32	33	1	35	35	0	36	36	0



(a)



(c)

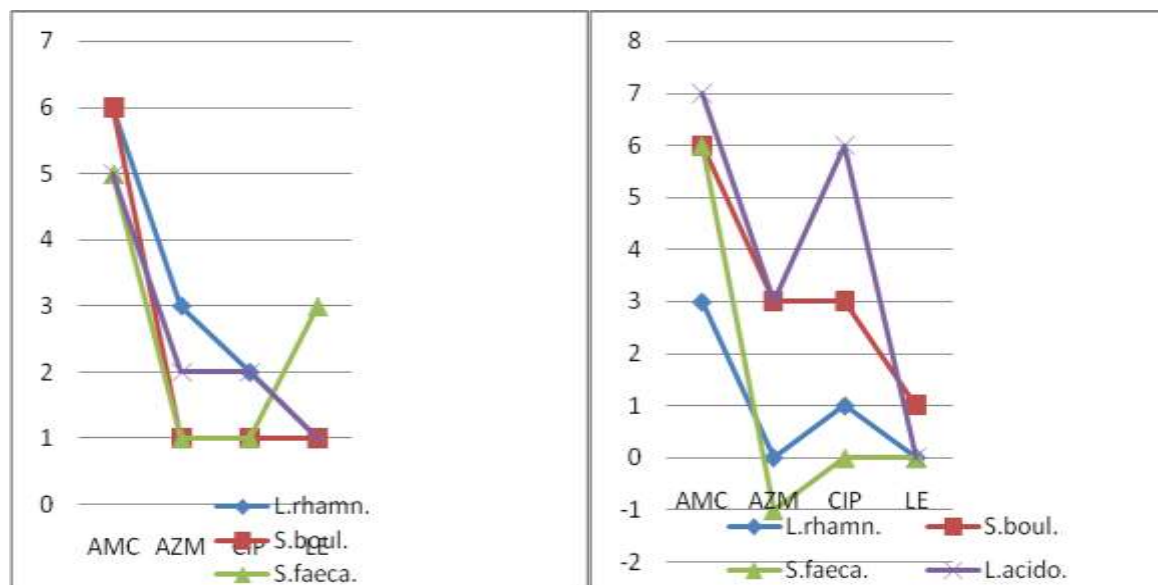


(b)



(d)

Fig. 1: Antimicrobial activity of antibiotics and (a) *L. rhamnosus* + antibiotics combination (b) *S. boulardii* + antibiotics combination (c) *S. faecalis* + antibiotics combination against *S. aureus* MTCC737 (d) Antimicrobial activity of antibiotics and *L. acidophilus* + antibiotics combination against *S. aureus* (clinical) for the given drugs



(a) (b)
Fig. 2: Comparative enhancement of zones of inhibitions by probiotic strains against (a) *s.aureus* MTCC 737)
(b) *s.aureus* (clinical) for the given drugs

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