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International Journal of Current Research
Vol. 7, Issue, 02, pp.12321-12324, February, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

A COMPARATIVE STUDY ON THE ANTIMICROBIAL ACTIVITY OF ANTIBIOTICS AND ANTIBIOTIC AND PROBIOTIC COMBINATIONS AGAINST *KLEBSIELLA PNEUMONIAE*

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ARTICLE INFO

Article History:
Received 18th November, 2014
Received in revised form
09th December, 2014
Accepted 05th January, 2015
Published online 26th February, 2015

Key words:
Probiotics,
Antibiotics,
Antagonistic Effect,
Klebsiella Pneumoniae

ABSTRACT

Probotics are microorganisms which has a potential to put positive health effect upon the health of human and animals. Their antagonistic potential is being utilized to overcome the increasing drug resistance of common pathogens. Present study is an attempt to further evaluate their antagonistic potential against resistance pathogen *Klebsiella Pneumoniae*. For this the synergistic antagonistic activity of probiotics and antibiotic combinations was carried out by using Kirby Bauer disc diffusion method. Out of the total tests in 68.75% cases probiotics were found to enhance the antagonistic activity of antibiotics while in 31.25% antimicrobial activity of antibiotics remained the same. None of the probotic strain caused reduction in the antimicrobial activity of the antibiotics in combination. Probiotics must be tried as an agent to overcome the leading drug resistance of the pathogens.

INTRODUCTION

Probiotics are defined as "A live microbial food supplement which beneficially affects the host animal by improving its intestinal microbial balance". Members of the genera *Lactobacillus* and *Bifidobacterium* are mainly used, but not exclusively, microorganism other than Lactic acid bacteria, which are being used in probiotic preparation include *Bacillus* sp; yeasts (eg- *Saccharomyces boulardii* and *Saccharomyce cerevisiae*) and filamentous fungi (eg-*Aspergillus oryzae*, *pentosaccus, EnteroCoccus faecium, Pediococcus, P. clamnosus P.halophilus, clostridium, C.butyricum, Streptococcus faecalis*) etc. Probiotics have been reported to be effective in Preventing various kind of diorrhoea (**Pillai and Nelson, 2008**), Crohn's disease, Ulcerative colitis (**Kruis *et al*., 2001**), pouchitis (**Gionchetti *et al*., 2000**), Irritable Bowel Syndrome (**Mac Farlane and Cummings 2002**), Necrotizing Enterocolitis, Lactose intolerance (**Marteau *et al*., 1990**), Hepatic diseases, Arthritis, Allergies, Cancer, Cardiovascular diseases, Hypertention, Mucosal immunity, urinary tract infection, Genital disorders, Bacterial and Yeast Vaginitis, HIV, Immunity, Control of Blood Cholesterol and Helicobacter pylori infection (**Kabir *et al*.,1997; Johnson *et al*., 2004**). Probiotics do so by regulating the intestinal microbial homeostasis, immuno-modulation, pathogen exclusion, and by putting antimicrobial effect on the pathogens (**Christensen *et al*., 2002**). A plenty of *in vivo* (**Favier *et al*., 2003; Indrio *et al*., 2008, Yap *et al*., 2008**) and *in vitro* (**Jacobsen, 1999; Bhutada *et al*., 2011**) studies have been reported from all over the world advocating the antimicrobial effect of the probiotics.

Also there have been reports regarding the role of probotic strains in potentiation of antimicrobial activity of the antibiotics by probiotics (**Sharma and Chauhan 2014; Sharma *et al*., 2014; Sharma and Chauhan 2014**). *Klebsiella* with the ability to produce extended-spectrum beta-lactamases (ESBL) are resistant to many classes of antibiotics. So, the use of probiotics as a therapeutic agent sounds reasonable. This study examines the potential of probotic strains, *Lactobacillus rhamnosus, Saccharomyces boulardii, Streptococcus faecalis* and *Lactobacillus acidophilus* to enhance the antagonistic activity of antibiotics, Nitrofurantoin, Amoxicillin/clavulanate, Chloramphenical and Leavofloxacin against *Klebsiella Pneumoniae* MTCC 618 and it, s clinical isolate.

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MATERIALS AND METHODS

Probotic strains

Commercial Probiotic products 'Darolac' and 'Prepro' were used to isolate the probotic strains *L.rhamnosus, S.boulardii* and *S.faecalis, L.acidophillus* respectively. Powder from both 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 and kept in Mc intosch jar with an anaerobic gas packet for 48 hr at 37oC to isolate the colonies of *L.rhamnosus* and *L.acidophillus. S.boulardii* was isolated from 'Darolac' by inoculating it's aqueous suspension on sabraoud's agar and keeping at 37^0C for 24hr in aerobic condition.

S.faecalis was isolated from 'Prepro' by inoculating it's MRS suspension on blood agar from the mixed colonies appeared on MRS agar keeping at 37^0C for 24hr. Pure colonies were obtained by repeated sub culturing. All the probiotic strains were confirmed by Gram's staining, cell and colony morphology. *Klebsiella Pneumoniae* MTCC 618 Culture was collected from Imtech, Chandigarh, India. The clinical isolate of *Klebsiella* was taken from the Department of Microbiology, S.N. Medical College, Agra (India) and confirmed by biochemical test. All the bacterial strains were stocked in Brain heart infusion agar slant at 4^oC.

Probiotic susceptibility to the drugs

Antibiotic susceptibilities of the all four probiotic strains were detected by using readymade antibiotic discs (Hi Media, India) through disc diffusion method [21] according to the national committee for clinical laboratory standards (NCCLS) guidelines. For this, the probotic suspension of Mc far land standard (M.F.S.) # 0.5 was prepared and swabbed on the surface of M.R.S. agar surface. The antibiotic discs of Ceftazidime, Amoxicillin/Clavulanate, Meropenem, Aztreonam, Nitrofurantoin Levofloxacin, Azithromycin Piperacillin/Tozobactum, Amikacin Ciprofloxacin and Chloramphenicol were placed on Muller Hinton Agar (MHA) surface and kept at 37^0C for 24 hrs. Zones of inhibition were measured.

Antimicrobial activity of probiotic strains

Antimicrobial activity of probiotic strains were assessed by soaking the plain discs of 6mm diameter with the 20ul of dilutions 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) so the each disc now contained approximately 6×106 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 these discs were transferred to the MHA petriplates swabbed with *K. Pneumoniae*, MTCC 1688and clinical isolate of *K. Pneumoniae*. and with the sterile water disc as negative 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 by using a standard caliper.

Synergistic antimicrobial activity

The Synergistic antimicrobial activity antibiotic & probiotic combination was assessed by modified disc diffusion method according to the NCCLS guidelines. For this the readymade antibiotic disc of NIT, AMC, C and LE were dipped in the 24 hrs old probotic suspensions and kept for 1 hr at 37^0C to allow the maximum absorption. Now 60ml molten MHA was poured into the petriplates of 120 mm diameter and plates were swabbed by *Klebsiella Pneumoniae* MTCC

618 and its clinical counterpart separately and kept for 3 hr at 37^0C. Now the probotic strain laden antibiotic discs were gently placed along with plain antibiotic disc taking as positive control and were kept at 4^oC for 1 hr to allow the proper diffusion. Both the plates were now kept at 37^0C for 24 hrs. Zones of inhibition were measured.

RESULTS AND DISCUSSION

Both *L.rhamnosus* and *L.acidophillus* appeared as gram +ve bacilli and showed round, small creamish colonies on MRS agar. *S.boulardii*. with characteristic oval shaped cells produced white colonies on sabraoud's agar. *S.faecalis* viewed as oval cocci in short chain. *Klebsiella* seen as gram- ve bacilli and produced large mucoid pink to red red colonies on MacConkey, s agar. The biochemical kit testing for *Klebsiella* was found to be positive Indole, methyl red, Voges proskauer citrate utilization (IM ViC, ++++) and carbohydrate utilization test. All the probiotic strains were highly resistance to Aztreonam and Ceftazidime Amoxicillin/Clavulanate, showed intermediate sensitivity to Azithromycin, Nitrofurantoin, Piperacillin/Tozobactum but found highly sensitive to Chloramphenical, Amikacin, Ciprofloxacin, Leavofloxacin and Meropenam. Antimicrobial activities of probiotic strains *L.rhamnosus, S.boulardii, S.faecalis* and L.acidophillus were detected against both *Klebsiella Pneumoniae* MTCC 618 and its clinical counterpart taking the drug $AMC^{20/10}$ as +ve control and sterile distill water disc as -ve control. Maximum Inhibitory activity was shown by the Mac farland standard #1.0(3x108 cfu/ml) as the zone sizes were 11-12mm against standard and 7-9mm against clinical isolate by all the four probotic strains, followed by 3x107 cfu/ml and 3x106 cfu/ml for both the standard and clinical strains.

Synergistic antimicrobial activity of all the 4 probiotic strains, *L.rhamnosus, S.boulardii, S.faecalis* and *L.acidophillus* in combination with the drugs $NIT^{300}AMC^{20/10}$, LE^5 and C^{30}were assessed against the both *Klebsiella Pneumoniae* MTCC 618 and clinical isolate keeping plain antibiotic drug used as +ve control.

Zones of inhibition were measured and compared to see the enhancement of zone by probiotic strains. Maximum enhancement was shown by AMC& probiotic combinations(up to 12mm) followed by probiotic combination with the drugs, NIT (up to 5mm), C and LE (up to 2mm).No test was seen with reduction in zone size. (Table 1, Fig.1, 2, 3 and 4).

Table 1. Antimicrobial activity of antibiotics and antibiotics + Probiotic combination against *K.Pneumoniae*

Test Micro organism	*K.Pneumoniae* MTCC 618												*K.Pneumoniae* 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+ Lrham.*	E	A	S.boul.	E	A	*A+ S.fae.*	E	A	*A+ Lacido.*	E
NIT	24	28	4	27	30	3	27	30	3	25	27	2	27	32	5	27	27	0	23	28	5	27	29	2
AMC	22	30	8	20	27	7	20	28	8	20	25	5	15	22	7	15	18	3	12	18	6	13	25	12
C	35	37	2	39	39	0	37	39	2	39	39	0	37	39	2	34	34	0	36	37	1	36	37	1
LE	38	38	0	40	41	1	40	42	2	40	40	0	39	39	0	35	35	0	39	39	0	35	35	0

(A) (B) (C)

Fig.1. Antimicrobial activity of (A) antibiotics and *L. rhamnosus* + antibiotics combination against *K.pneumoniae* (clinical) (B) antibiotics and *S.feacalis* + antibiotics combination against *K.pneumoniae* (MTCC)618 (C) antibiotics and *L. acidophillus* + antibiotics combination against *K.pneumoniae* (MTCC)618

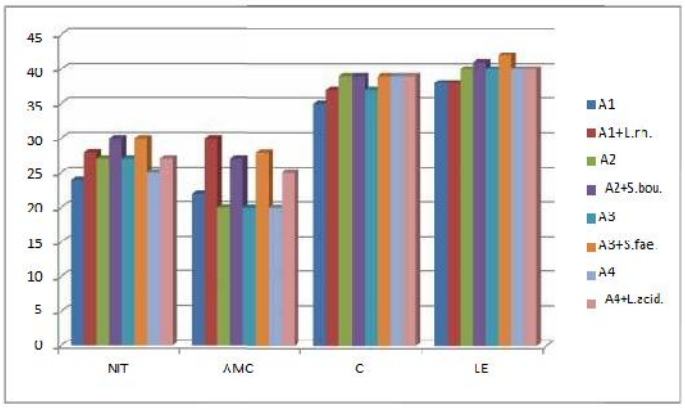


Fig. 2. Comparison of zone of inhibition of antibiotics and antibiotics + Probiotic Combination against *K.pneumoniae* (MTCC) 618

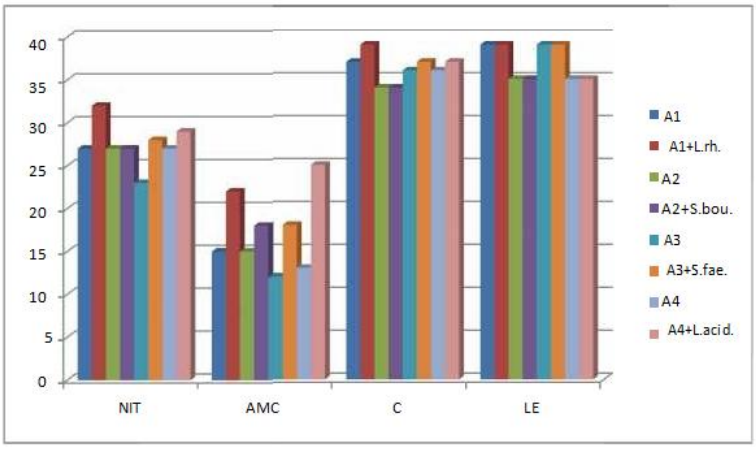
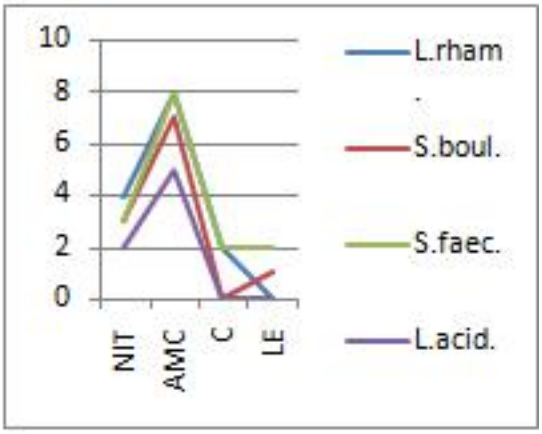
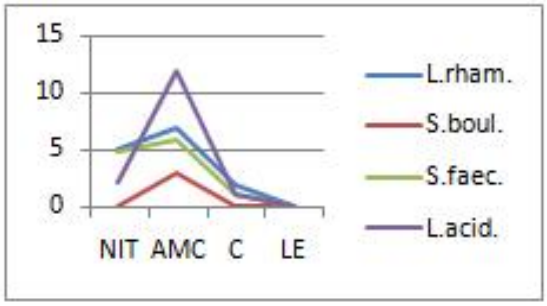


Fig. 3. Comparison of zone of inhibition of antibiotics and antibiotics + Probiotic combination against *K.pneumoniae* (clinical)



(A)



(B)

Fig.4. comparative enhancement of zones of inhibitions by probiotic strains against (A) *K.pneumoniae* (MTCC)618 (B) *K.pneumoniae* (clinical) for the given drugs.

Conclusion

This study was carried out to see the probotic potential of enhancing the antimicrobial activity of the antibiotics by comparing the zone of inhibition of antibiotics alone and the antibiotic and probotic combination. 75% combinations showed enhancement in zone size against the standard strain while 62.5% cases showed enhancement against the clinical strain. Zone diameter did not change in rest of the cases. Reduction in zone size was not seen in any combination. So, it can be concluded that there should be no problem in trying these combinations to fight with the emerging resistance of *Klebsiella Pneumoniae.* Further more *in vitro* and *in vivo* studies must be done to make this study useful to the patients suffering with the lack of treatment due to antibiotic resistance.

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