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Study of Probiotic Activity of *Lactobacillus Acidophilus and Lactobacillus Rhamnosus on Non-Escherichia Coli Enterobacterales*

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ABSTRACT

Introduction: Probiotic activity of *L. acidophilus* and *L. rhamnosus* was great intend to treat pathogenic organism. The antibiotic resistance problem, it is important to develop *Lactobacillus* based approaches to combat *Citrobacter koseri, Enterobacter cloacae, Serratia marcescens, Morganella morganii,* and *Proteus mirabilis, Proteus vulgaris* and *Klebsiella aerogenes*-induced urinary tract infections, catheter-associated infections, and intestinal tract infections in the current situation.

Aim/Objectives: In this study, our goal was to assess the *in vitro* antimicrobial activity of *L. acidophilus* and *L. rhamnosus* against clinical isolates of MDR, XDR, Non-MDR and ESBL strains of *C. koseri, E. cloacae, S. marcescens, M. morganii, P. mirabilis, P. vulgaris and K. aerogenes*.

Methods: Antibiotic susceptibility of the test strains was determined by Kirby-Bauer disc diffusion method. The antimicrobial activities of treated and untreated *L. acidophilus* and *L. rhamnosus* against the above mentioned MDR, XDR, Non-MDR and ESBL pathogens were determined by agar overlay spot test. Mean zone of inhibition was statistically analyzed using ANOVA single factor.

Results: Among *L. acidophilus* and *L. rhamnosus* the mean value displayed the potent activity against most test isolates with mean zones of inhibition ranging from 4 to 15 mm and the *p*-value <0.05 were considered for significant action with varying result against different organisms.

Conclusion: The *Lactobacillus spp*. as a probiotic may play a promising role in this era of rapidly growing burden of drug resistant organisms.

Key Words: Extensively Drug Resistance, Extended spectrum β-lactamase, Multidrug resistance, Non-multidrug resistance, Probiotics, Zone of Inhibition

INTRODUCTION

Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host were the Probiotic.[1] Probiotic bacteria are proposed to benefit human health mainly by mechanisms of action[2,3] inhibit pathogens through influence on the commensal microbiota.[2,4] Enhance the epithelial barrier function by modulating signalling pathways, [5] modulate host immune responses.[6]

Enterobacterales order of Gram-negative bacteria are among the most common Genitourinary tract, Gastrointestinal tract, and nosocomial pathogens *Enterobacterales* have an immense impact upon human history through urinary tract infections, dysentery, and hospital acquired infections.[7] Many members of the Enterobacterales are well known for acquiring antibiotic resistance, including Extended spectrum β-lactamase, AmpC Production and Extensively drug resistance. Most bacteria can also gain antibiotic tolerance through formation of a biofilm.[8] Proposed biofilm antibiotic resistance mechanisms include the presence of persister cells, microenvironments within the biofilm that prevent the antibiotic efficacy, and reduced antibiotic access to bacteria

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within a biofilm.[9]

Regardless of the mechanism of resistance, the biofilm-associated infections are difficult to treat with antibiotics and tend to be persistent. Antimicrobial capability of efficiently killing bacteria in biofilms would be of considerable use in treating chronic, biofilm-associated infections. Probiotic Lactobacilli can also suppress virulence and dissemination of infectious pathogens. This is typically accomplished through the production of organic acids and antimicrobials, such as bacteriocins, lipopeptides, and surface proteins. Due to their antimicrobial properties, probiotics may be considered for treatment and prevention of infectious diseases caused by oral, enteric, and urinary tract infections.[10]

Citrobacter species, *Enterobacter cloacae*, *Serratia marcescens*, *Morganella morganii* and *Klebsiella aerogenes* may test susceptible to ceftriaxone, cefotaxime, ceftazidime, and ceftaroline, but these agents may be ineffective against these genera with a few days after initiation of therapy due to repression of inducible AmpC β-lactamase. The risk of AmpC depression during therapy is moderate to high with *C. freundii* complex, *E. cloacae* complex, and *K. aerogenes* and appears to be less frequent with *M. morganii* and *S. marcescens*. Therefore, isolates that are initially susceptible may become resistant. Testing subsequent isolates may be warranted if clinically indicated.[11] The antibiotic resistance problem, it is important to develop *Lactobacillus*-based approaches to combat *Citrobacter koseri, Enterobacter cloacae, Serratia marcescens, Morganella morganii, Proteus mirabilis, Proteus vulgaris* and *Klebsiella aerogenes*- induced urinary tract infections, catheter-associated infections, and intestinal tract infections in the current situation.

MATERIALS AND METHODS

The present study was carried out at Department of Medical Microbiology, School of Medical Education, Kottayam, Kerala from August 2022 to April 2023.

Identification of isolates

A total of 82 isolates of Non-*Escherichia Enterobacterales* including *Citrobacter koseri, Enterobacter cloacae, Serratia marcescens, Morganella morganii, Proteus mirabilis, Proteus vulgaris* and *Klebsiella aerogenes* were collected from various diagnostic microbiology laboratories in Kerala. The isolates were confirmed by Gram staining and various biochemical test. Two probiotic lactobacilli (*L. acidophilus* and *L. rhamnosus*) of commercial origin, grown on MRS medium and incubated at 37°C for 48 hours with 5- 10% carbon dioxide.

Detection of probiotic activity of *L. acidophilus* and *L. rhamnosus* on organisms studying.

Probiotic activity was detected using Agar overlay method described by Fleming et al.[12] with minor modifications. Briefly, a layer of MRS agar was prepared and allowed to solidify. Both *L. acidophilus* and *L. rhamnosus* were inoculated to BHI broth and incubated at 37°C overnight. Similarly Test isolates were incubated in BHI broth at 37°C for 24 hours. Then, the surface of MRS agar was spot inoculated with 5µL of an overnight culture of *L. acidophilus* and *L. rhamnosus*. Also, the overnight culture of *Lactobacillus spp.* were adjusted to 6.5–7.0 pH using 1N NaOH and spot were inoculated on MRS agar. Then the plates were incubated for 24 hour at 37˚C in 5 – 10% CO_2. After incubation visible spot appears on the surface of the MRS medium. Then the MRS agar plates with lactobacillus spots were thereafter overlaid with 7ml of molten BHI soft agar (0.75%) cooled to 40-45˚C, which was seeded with 100µL of isolates to be tested. After 24 hours of incubation at 37˚C in 5-10% CO_2, the inhibition zones around lactobacillus spots were diametrically measured and expressed in millimetres and interpreted following Shokryazdan et al.[13] with modifications. The zone of inhibition diameter ≥ 12 mm, ≤11-8 mm, < 8-4 mm and < 4 mm were considered as strong (4+), intermediate (3+), weak (2+) and no inhibition (1+) respectively. The Spot diameter at least required was 4mm.

Antimicrobial susceptibility testing of Non-Esch*erichia coli Enterobacterales.*

Antibiotic susceptibility profiles of the isolates were determined by the Kirby-Bauer disk diffusion as prescribed by CLSI M02 A-13[14] conditions on MHA at 37°C for 24h under aerobic conditions and analysed using interpretive standards of CLSI M100-S33[11] and are categorized into MDR, XDR, Non-MDR and ESBL groups based on CDC/ECDC guidelines.[15]

Antibiotic susceptibility testing – was done by disc diffusion method as recommended by CLSI for the following drugs: Gentamicin(10µg), Ampicillin(10µg), Amikacin(30µg), Aztreonam(30µg), Imipenem(10µg), Amoxycillin-clavulanic acid(20µg), Cefuroxime(30µg), Tetracycline(30µg), Cefixime(30µg), Ciprofloxacin(5µg), Cefoxitin(30µg), Ceftazidime(30µg), Ceftazidime-clavulanic acid(30µg), Cefotaxime(30µg), Cefotaxime-clavulanic acid(30µg).

Data Analysis

The study was approved by the Institutional Ethical Committee at School of Medical Education. The Data was analysed using Excel 2019. Descriptive statistics such as mean and standard error and inferential statistics such as ANOVA single-factor were employed in the present study p-value < 0.05 was considered statistically significant.

RESULTS

The standard strains of *L. acidophilus* and *L. rhamnosus* were cultured on MRS agar shown in figure.1 A & B. *Serratia marcescens* showed red or magenta, non- diffusible pigment on Violet red bile agar by the production of Prodigiosin shown in Figure.1.C. Dienes line of *Proteus mirabilis* on blood agar. The line of demarcation (centre) indicates that the two strains are not related shown in Figure.1.D.

Antimicrobial susceptibility pattern of Non-*Escherichia Enterobacterales*

A total of 82 samples were received. Which included, *Citrobacter koseri* (n=24), *Enterobacter cloacae* (n=18), *Serratia marcescens* (n=11), *Morganella morganii* (n=9), *Proteus mirabilis* (n=9), *Proteus vulgaris* (n=5) and *Klebsiella aerogenes* (n=5). Antibiotic susceptibility pattern showed in table.1. Prevalence of MDR, XDR, NMDR and ESBL isolates showed in Figure 2.

Detection of Probiotic activity of *L. acidophilus* and *L. rhamnosus* on Non *Escherichia coli Enterobacterales*

L. acidophilus and *L. rhamnosus* with zone of inhibition were made by untreated and treated suspensions shown in figure.3. Treated and untreated suspensions of *L. acidophilus* and *L. rhamnosus* gives varying mean zone of inhibition with Non-*Escherichia coli Enterobacterales*. Four stages of comparative activity of *Lactobacillus* could be done.

Probiotic activity of *L. acidophilus* treated and untreated against Non-*Escherichia coli Enterobacterals*

Treated *L. acidophilus* showed intermediate zone had greater activity against *C. koseri* (62%) and *K. aerogenes* (60%) than untreated. Untreated *L. acidophilus* showed prominent activity on intermediate grade against *E. cloacae* (60%), *S. marcescens* (81%) and *P. vulgaris* (80%) than treated. Untreated *L. acidophilus* exhibit considerable remarks on strong zone of inhibition only against *M. morganii* (77%) than treated. Untreated *L. acidophilus* showed excellent action on intermediate zone of inhibition against *P. mirabilis* (55%) than treated. The resistant pattern of Non-*Escherichia coli Enterobacterales* showed varying results with zone of inhibition (Table.2).

Probiotic activity of *L. rhamnosus* treated and untreated against Non-*Escherichia coli Enterobacterals*

Untreated *L. rhamnosus* showed greatest percentage on strong zone of inhibition only against *S. marcescens* (63%) than treated. Treated *L. rhamnosus* against most of the test isolates gives more activity on intermediate zone size than untreated. For example, Treated *L. rhamnosus* inhibit test isolates of *C. koseri* (66%), *E. cloacae* (72%), and *P. vulgaris* (100%). Intermediate zone of inhibition with considerable results by untreated *L. rhamnosus* suspension against *M. morganii* (66%), *P. mirabilis* (66%) and *K. aerogenes* (80%) than treated. *L. rhamnosus* exhibit considerable action on intermediate level zone of inhibition against most of the resistant and NMDR strains (Table.3).

Comparison between probiotic activity of *L. acidophilus* untreated and *L. rhamnosus* untreated against Non-*Escherichia coli Enterobacterales*.

Untreated *L. acidophilus* had substantial action on intermediate zone of inhibition against *E. cloacae* (60%), *S. marcescens* (81%), *P. mirabilis* (55%) and *P. vulgaris* (80%). However untreated *L. acidophilus* had eminent activity on strong zone of inhibition against *M. morganii* (77%). Untreated *L. rhamnosus* against substantial reaction on intermediate zone of inhibition against *M. morganii* (66%), *P. mirabilis* (66%) and *K. aerogenes* (80%). Meanwhile, untreated *L. rhamnosus* had major effects on strong zone of inhibition against *S.marcescens* (63%).

Comparison between probiotic activity of *L. acidophilus* treated and *L. rhamnosus* treated against Non-*Escherichia coli Enterobacterales*.

Treated *L. acidophilus* showed prime reaction with intermediate zone of inhibition against *C. koseri* (62%) and *K. aerogenes* (60%). Treated *L. rhamnosus* had impressive results on intermediate zone of inhibition against *C. koseri* (66%), *E. cloacae* (72%) and *P. vulgaris* (100%).

Overall comparative activity of *L. acidophilus* and *L. rhamnosus*

The comparative activity of treated and Untreated *L. acidophilus*, Treated and untreated *L. rhamnosus*, Untreated *L. acidophilus* and *L. rhamnosus*, and finally Treated *L. acidophilus* and *L. rhamnosus* against Non-*Escherichia coli Enterobacterales* analysed using ANOVA –one way method and was found varying results among different organisms. The significant was found among Treated and untreated *L. acidophilus* against *C. koseri*, Treated and untreated *L. rhamnosus* against *E. cloacae*, Untreated *L. acidophilus* and *L. rhamnosus* against *S. marcescens* , and finally to treated *L. acidophilus* and *L. rhamnosus* against *S. marcescens.* All other comparative activities using other isolates was found not significant.

DISCUSSION

Probiotics are bacteria that help maintain the natural balance of microflora in the intestines. Experiments into the

benefits of probiotic therapies suggest a range of potentially beneficial medicinal uses for probiotics.[16] *Lactobacillus* spp. are metabolize glucose and resulting in an environment that is not conducive to the growth of pathogenic bacteria due to the production of various antimicrobial agents to disrupt pre-formed pathogenic biofilms.[17] Commensal enteric flora are believed to cause diarrhea, although *C. koseri, E. cloacae* and *P. mirabilis* is the species most frequently recovered from Urinary tract, respiratory and wound infections. *Serratia* spp. are capable of survival under very harsh environmental conditions, *P. vulgaris* is more commonly recovered from infected sites in immunosuppressed hosts, particularly those receiving prolonged regimens of antibiotics. The infections are also associated with contaminated medical devices.[17]

Organisms are rapidly inducible production of ß-lactamase during therapy with Cephalosporin (especially with third generation agents) are difficult to treatment as the increasing chances of MDR, XDR and ESBL as has been documented by H.W. Boucher et al.[18] it has been demonstrated that, there is also a growing concern regarding the lack of new antibiotics. The present study showed Except *Acinetobacter spp*. and *Providencia spp*. on ESCAPPM, highlights among 18 *E.cloacae* isolates with 11% MDR,16% XDR and 16% ESBL, *K. aerogenes* with 60% MDR and 20% XDR were recorded, which is contrast to previous finding of Jari intra [19] which exhibited that among all 2277 *Enterobacter spp*. strains, they observed a significant increase in *E.cloacae* Producing ESBL isolates and a significant decrease in *K. aerogenes* producing ESBL among both inpatient and Out patients over the study period ,as well as a constant low value of carbapenem resistant *Enterobacter* spp. Isolates in the period from 2000-2019 is 0.05%. However, the present study investigation revealed a more higher percentage among MDR, XDR and ESBL strains. The variation in antimicrobial percentage due to the referred study showed change in CLSI to EUCAST Guidelines and an increase in fluoroquinolones susceptibility of *Enterobacter* spp. in the application of EUCAST criteria was reported, most probably owing to the elimination of intermediate category. In this study revealed that *C. koseri* all strains are Non MDR has recommended as an alternative modality by Lilianne and Dominguez[20] it has been presents, high level resistance to beta-lactam antibiotics. The variations in clinical specimen types used to explain why the results of different studies have different findings. In this study, *S. marcescens* 9% XDR isolates were reported, which are variant from Tamma.et.al.[21]it has been reported that, test over 300 isolates 15% of *S. marcescens* isolates were positive for High level Amp C production compared to 38% of *Enterobacter spp.* The great difference in XDR isolates were given by in this study. *M. morganii* with 44% XDR and 33% ESBL comparative to Gemma Jimenez-Guerra[22] revealed a low number of isolates *S. marcescens* meant that it was not possible to show its evolution. *P. vulgaris* with 20% XDR and *P. mirabilis* with all strains of Non MDR revealed, *P. vulgaris* exhibited higher range of resistance strains. It has been demonstrated by Licai Mo[23] exerted sensitivity for male and female separately as showed that male and female with same sensitivity except that female patient were more responsive to levofloxacin and male patients were more responsive to sulfamethoxazole. However, in this study is equivalent to each other in *P. mirabilis*. But *P. vulgaris* showed higher resistance. The variations in population, geographic location, duration between investigations, and clinical specimen types and sizes can all be used to explain why the results of different studies have different findings.

The antimicrobial activity of *Lactobacillus* strains was characterized by agar spot test against different test pathogens. In our study, Agar overlay inhibition assay was employed to demonstrate the antimicrobial activity of *L. acidophilus* and *L. rhamnosus* against *C. koseri, E. cloaace, S. marcescens, M. morganii, P. vulgaris, P. mirabilis and K. aerogenes* isolates. Cadirici and Citak[24] compared the two method (agar overlay and agar well diffusion) and found the agar overlay method as the effective one in the evaluation of inhibitory activity of *Lactobacillus* against Test isolates. Also compared that the present study with Toure et al.[25] agar spot test.

In this comparative study of treated and untreated *L. acidophilus* and *L. rhamnosus* statistically analyzing the mean zone of inhibition revealed several significant findings. Of which, *L. acidophilus* exhibit greater inhibitory activity than *L. rhamnosus* against *C. koseri, E. cloacae, S. marcescens, P. vulgaris* and *K. aerogenes*. The *C. koseri and K. aerogenes* gives equivalent range as same as its convention biochemical tests. However, *M. morganii* and *P. mirabilis* gives greater zone on treated test suspension of *L. acidophilus*. Overall, *L. acidophilus* results greater mean size than *L. rhamnosus*. In a similar study by Fernandez et al.[26] using cell free supernatant and live cells, strongest antimicrobial activity was observed when the live cells of *L. rhamnosus* were used. The variation in our study, may be due to different test isolates and here we used standard strains of *L. acidophilus* and *L. rhamnosus*. This is a useful potential alternative modality for treating pathogenic infection. The study by Toure et al.[25] also demonstrated antimicrobial effect of *L. acidophilus* showed greater inhibitory activity against *E. cloacae*.

The *p value* was significant ($p < 0.05$), is considered and similar activity against organisms in some ranges, on *C. koseri* Comparison of *L. acidophilus* Treated and Untreated, On *E. cloacae* Comparison of *L. rhamnosus* Treated and Untreated and On *S. marcescens* both *L. acidophilus* and *L. rhamnosus* Untreated comparison and Treated comparison. The study performed by Debashis and Shyamapada[27] the inhibition values of commercial probiotic lactobacillus strains (*L. acidophilus* and *L. rhamnosus)* against *E. coli* are similar. Such slight difference in antibacterial activity of lactobacilli may

be due to the strain and source variation. In our study we used standard strains of *L. acidophilus* and *L. rhamnosus* but in the later study *L. acidophilus* and *L. rhamnosus* of commercial origin were used. Also the difference in *p*-value due to different isolates with different characterization.

However, it was found that Untreated suspension of *L. acidophilus* exhibit greater zone of inhibition than treated lactobacillus suspensions against *C. koseri, E. cloacae, S. marcescens, P. vulgaris* and *K. aerogenes* Similarly, the findings of S. Tejero – Sarinena et al.[28] demonstrate that lower the pH, higher the inhibition against pathogenic microorganisms. In this study, Untreated *Lactobacillus* strains did have inhibitory activities effects on Test isolates. This indicated that inhibitory effect of *Lactobacillus* strains were due to Lactic acid production. In case of *L. rhamnosus* have greater zone size on *M. morganii* and *P. mirabilis*. Likewise, the findings of Shaaban M et al.[29] in a study using treated (pH-adjusted) supernatants of *L. reuteri* were effective against almost all tested *P. mirabilis* isolates. Thus, the inhibitory action of treated suspension of *Lactobacillus spp.* were may be due to some other factors, like hydrogen peroxide and bacteriocin. *Lactobacillus* treated with 1 NaOH also did affect the inhibitory activities of the *Lactobacillus* strains against the test pathogens. Also the untreated activity by acid tolerance has been considered standard for investigation of Probiotic strains.

CONCLUSION

Untreated *Lactobacillus* strains did have probiotic activity. Effects of the *Lactobacillus* strains were may be due to their organic acid production like Lactic acid, Acetic acid, Formic acid, succinic acid etc. Hence, this study concludes that among *Lactobacillus* organic acid were responsible for action. Our findings emphasized on the use of probiotic *L. acidophilus* and *L. rhamnosus* on clinical isolates of *Citrobacter koseri, Enterobacter cloacae, Serratia marcescens, Morganella morganii, Proteus mirabilis, Proteus vulgaris* and *Klebsiella aerogenes*. The *Lactobacillus spp*. as a probiotic may play a promising role in this era of rapidly growing burden of drug resistant organisms. However, further *in-vivo* and large scale studies are warranted to clarify this before it can be safely applied in health field.

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Table 1: Percentage showed by Antibiotic susceptibility testing

Organisms	S/I/R	AMPICILLIN	AMOXY/CLAV	CEFUROXIME	CEFIXIME	CEFOXITIN	CEFOTAXIME	CEFTAZIDIME	IMIPENEM	AZTREONAM	GENTAMICIN	AMIKACIN	CIPROFLOXACIN	TETRACYCLINE
C. koseri	S		91%	100%		100%			79%	100%	100%	79%	95%	
	I		8%	0		0			16%	0	0	20%	4%	
	R		0	0		0			4%	0	0	0	0	
E. cloacae	S				83%		72%	66%	66%	66%	88%	83%	88%	66%
	I				0		5%	5%	11%	5%	0	11%	5%	5%
	R				16%		22%	27%	22%	27%	11%	11%	5%	27%
S. marcescens	S				100%		36%	63%		100%	90%	90%	90%	9%
	I				0		0	27%		0	0	0	0	18%
	R				0		63%	9%		0	9%	9%	9%	72%
M. morganii	S				44%	0	100%	44%		55%	100%	77%	100%	
	I				11%	0	0	11%		0	0	0	0	
	R				44%	100%	0	44%		44%	0	22%	0	
P. mirabilis	S	100%	100%		100%	77%	100%	100%		100%			77%	
	I	0	0		0	0	0	0		0			22%	
	R	0	0		0	22%	0	0		0			0	
P. vulgaris	S		20%		80%	80%	80%	80%		100%	80%	80%	80%	
	I		60%		0	0	0	0		0	0	0	0	
	R		20%		20%	20%	20%	20%		0	20%	20%	20%	
K. aerogenes	S			20%	60%		100%	80%	40%	100%	80%	80%	60%	100%
	I			0	0		0	0	0	0	0	0	0	0
	R			80%	40%		100%	20%	60%	0	20%	20%	40%	0

#Blank space indicates, that Antibiotic was intrinsic or Expected resistance to that organism, or in this study such drug was not used for test antibiotic susceptibility against that particular organism.

Table 2: Interpretation of *L. acidophilus* treated and untreated against Non-*Escherichia coli Enterobacterales*.

Organisms	MDR/XDR/NMDR/ ESBL	Interpretation of *L. acidophilus* treated and untreated.							
		STRONG		INTERMEDIATE		WEAK		NO INHIBTION	
		L. acidophilus (Treated)	*L. acidophilus* (Untreated)	*L. acidophilus* (Treated)	*L. acidophilus* (Untreated)	*L. acidophilus* (Treated)	*L. acidophilus* (Untreated)	*L. acidophilus* (Treated)	*L. acidophilus* (Untreated)
C.koseri	NMDR (n=24)	20% (n=5)	50% (n=12)	62% (n=15)	45% (n=11)	16% (n=4)	4% (n=1)	0	0
	MDR (n=2)	50% (n=1)	50% (n=1)	50% (n=1)	50 (n=1)	0	0	0	0
	XDR (n=3)	66% (n=2)	66% (n=2)	33% (n=1)	0	33% (n=1)	0	0	0
E.cloacae	NMDR(n=13)	7% (n=1)	7% (n=1)	61% (n=8)	76% (n=10)	30% (n=4)	15% (n=2)	0	0
	ESBL(n=3)	66% (n=2)	66% (n=2)	33% (n=1)	33% (n=11)	0	0	0	0
S.marcescens	XDR (n=1)	0	0	100% (n=1)	0	0	100 (n=1)	0	0
	NMDR (n=10)	10% (n=1)	0	70% (n=7)	90% (n=9)	20% (n=2)	10% (n=1)	0	0
M. morganii	MDR (n=1)	0	0	100% (n=1)	100% (n=1)	0	0	0	0
	XDR (n=4)	50% (n=2)	75% (n=3)	50% (n=2)	25% (n=1)	0	0	0	0
	NMDR (n=4)	100% (n=4)	100% (n=4)	0	0	0	0	0	0
	ESBL (n=3)	33% (n=1)	66% (n=2)	66% (n=2)	33% (n=1)	0	0	0	0
P. mirabilis	NMDR (n=9)	0	0	44% (n=4)	55% (n=5)	55% (n=5)	44% (n=4)	0	0
P. vulgaris	XDR (n=1)	0	0	100% (n=1)	100% (n=1)	0	0	0	0
	NMDR (n=4)	0	0	50% (n=2)	75% (n=3)	50% (n=2)	25% (n=1)	0	0
K. aerogenes	MDR (n=3)	0	0	66% (n=2)	66% (n=2)	33% (n=1)	33% (n=1)	0	0
	XDR (n=1)	0	100% (n=1)	100% (n=1)	0	0	0	0	0
	NMDR (n=1)	100% (n=1)	100% (n=1)	0	0	0	0	0	0

Table 3: Interpretation of *L. rhamnosus* treated and untreated against Non-*Escherichia coli Enterobacterales*.

Organisms	MDR/XDR/ NMDR/ESBL	STRONG		INTERMEDIATE		WEAK		NO INHIBTION	
		L. rhamnosus (Treated)	*L. rhamnosus* (untreated)	*L. rhamnosus* (Treated)	*L. rhamnosus* (Untreated)	*L. rhamnosus* (Treated)	*L. rhamnosus* (Untreated)	*L. rhamnosus* (Treated)	*L. rhamnosus* (Untreated)
C. koseri	NMDR (n=24)	12% (n=3)	37% (n=9)	66% (n=16)	54% (n=13)	20% (n=5)	4% (n=1)	0	0
	MDR (n=2)	0	50 (n=1)	50% (n=1)	50 (n=1)	50% (n=1)	0	0	0
E. cloacae	XDR (n=3)	33% (n=1)	33% (n=1)	66% (n=2)	66% (n=2)	33% (n=1)	0	0	0
	NMDR (n=13)	0	30% (n=4)	76% (n=10)	69% (n=9)	23% (n=3)	0	0	0
	ESBL (n=3)	0	33% (n=1)	66% (n=2)	66% (n=2)	33% (n=1)	0	0	0
S. marcescens	XDR (n=1)	0	100% (n=1)	100% (n=1)	0	0	0	0	0
	NMDR (n=10)	40% (n=4)	60% (n=6)	50% (n=5)	30% (n=3)	10% (n=1)	10% (n=1)	0	0
	MDR (n=1)	0	0	100% (n=1)	100% (n=1)	0	0	0	0
M. morganii	XDR (n=4)	50% (n=2)	50% (n=2)	50% (n=2)	50% (n=2)	0	0	0	0
	NMDR (n=4)	75% (n=3)	25% (n=1)	0	0	0	0	0	0
	ESBL (n=3)	33% (n=1)	33% (n=1)	66% (n=2)	66% (n=2)	0	0	0	0
P. mirabilis	NMDR (n=9)	0	0	66% (n=6)	55% (n=5)	33% (n=3)	44% (n=4)	0	0
P. vulgaris	XDR (n=1)	0	0	100% (n=1)	100% (n=1)	0	0	0	0
	NMDR (n=4)	0	0	75% (n=3)	100% (n=4)	25% (n=1)	0	0	0
K. aerogenes	MDR (n=3)	0	0	33% (n=1)	66% (n=2)	66% (n=2)	33% (n=1)	0	0
	XDR (n=1)	0	0	100% (n=1)	100% (n=1)	0	0	0	0
	NMDR (n=4)	0	0	100% (n=1)	100% (n=1)	0	0	0	0

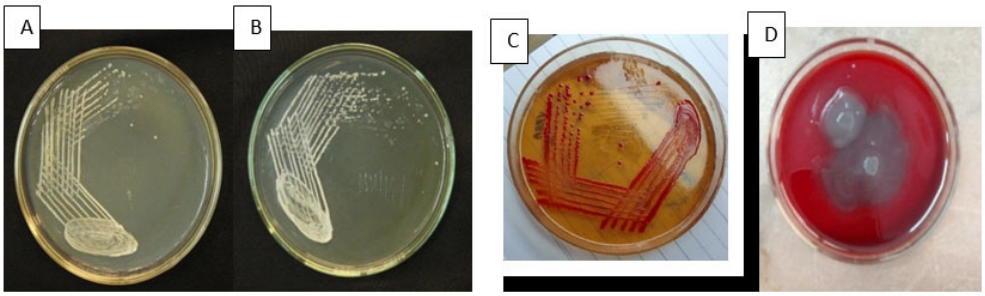

Figure 1: Colonies of *L. acidophilus* (A) and *L. rhamnosus* (B) on MRS agar. Isolated colonies of *Serratia marcescens* on nutrient agar (C). Dienes line of *Proteus mirabilis* on blood agar. The line of demarcation (centre) indicates that the two strains are not related (D).

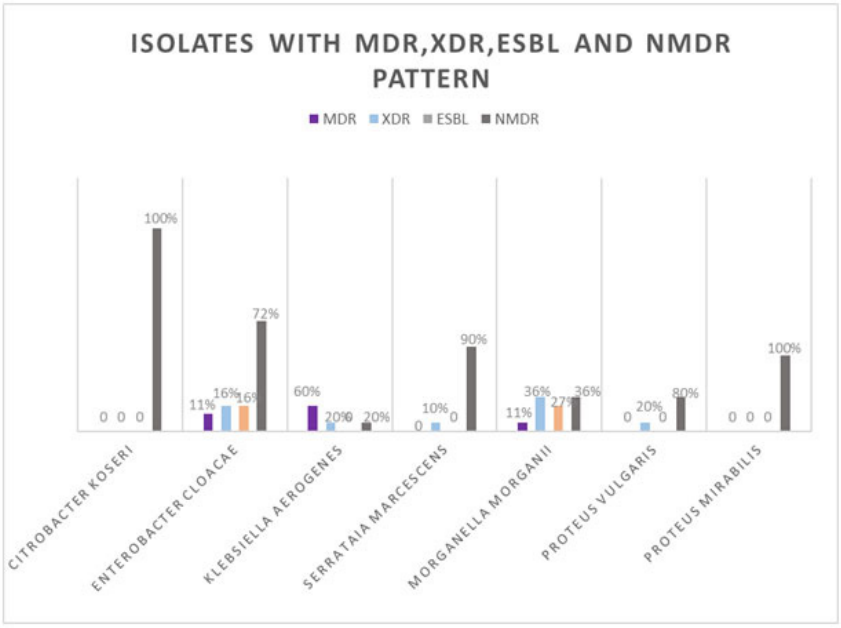

Figure 2: Graphical representation of MDR, XDR and NMDR strains of test isolates.

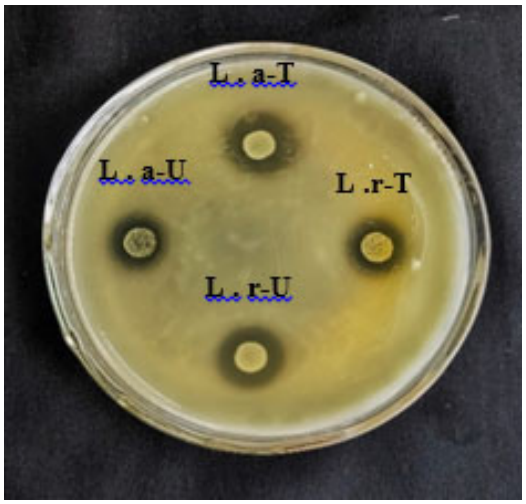

Figure 3: Agar overlay plate of treated and untreated suspensions of *L. acidophilus* (L. a-U).

(L. a-T) and *L. rhamnosus* (L. r-U) (L. r-T) against test isolate.