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## Effectiveness of Lactic Acid Bacteria in the Helpless Condition of Bacterial Resistance

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### ABSTRACT

Lactic acid bacteria are a group of gram positive, non-sporing, cocci or bacilli, which produce lactic acid as the major end product in carbohydrate fermentations. Lactic acid bacteria were isolated from different dairy sources. Disease causing pathogenic bacteria from clinical specimen were collected. Antimicrobial activity of the above pathogens were tested with a set of conventional antibiotics by the disk diffusion method. Antibacterial effects of the lactic acid bacteria against the above bacterial pathogens were done by agar well diffusion method. 10 pathogenic bacteria, resistant to minimum of three antibiotics were selected for the study. Results of antibacterial activity of the lactic acid bacteria in the study revealed, a varying type of inhibition, which was later used for comparison. The study was performed to check whether Lactic acid bacteria could be used in patients with antibiotic resistance.

**Keywords:** Lactic acid bacteria, Bacteriocins, Antibiotics, Antibacterial activity, Antibiotic resistance

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## INTRODUCTION

Lactic acid bacteria and their metabolites play an important role in improving microbiological quality and shelf-life of many food products and provide a good example of biopreservation<sup>1</sup> Most lactic acid bacteria are considered as GRAS (Generally Regarded As Safe) microorganisms and have been extensively studied<sup>2</sup>. Bacteriocins are a group of highly specific antibiotic like substances produced by certain strains of lactic acid bacteria and are active against other strains of the same or different species. Bacteriocins are biologically derived, low molecular weight proteinaceous compound, easily degraded during digestion in human beings. In addition to food biopreservation, bacteriocins are used to inhibit pathogens causing mastitis in cows<sup>3</sup> and have been employed to prevent the formation of biogenic amines<sup>4</sup>. Antibiotics can be of broad spectrum or narrow spectrum of activity and even when their activity is restricted, no preferential effect on closely related strain is observed. Widespread use of antibiotics as prophylactic and therapeutic agents to control bacterial diseases has been associated with the emergence of antibiotic resistance in bacterial pathogens. One of the protective measures against harmful effects of antibiotics is the use of biotherapeutic agents. These are mainly probiotics and lactic acid bacteria is the most exploited probiotic for that purpose. This study is designed to determine the suitability of lactic acid bacteria to kill pathogenic organisms and to compare it with conventional antibiotics.

## MATERIALS AND METHODS

Chemicals and solvents were purchased from SRL, India and bacteriological media were obtained from Himedia, India, respectively. 20 Disease causing pathogenic bacteria from clinical samples were collected from the Microlab diagnostic services at Kozhencherry, India. These samples were processed by standard microbiological methods to confirm their identity. Lactic acid bacteria were isolated from dairy products. De Man Rogosa and Sharpe (MRS) media, purchased from Himedia, India, was used for its isolation. Lactic acid bacteria are gram positive, cocci or bacilli, non spore forming, catalase negative, nitrate non reducing and non motile organisms. All the isolations and identifications were based on the Bergey's Manual of Determinative Bacteriology<sup>5</sup>.

### **Antibiotic Sensitivity Testing**

Disk Diffusion method was used for the antibiotic sensitivity testing of the bacterial pathogens using commercial antibiotics<sup>6</sup>. Mueller-Hinton agar was found to be the best media for the purpose. Bacterial pathogens, growth adjusted to match the 0.5 McFarland turbidimetric standard, were inoculated on the agar surface to produce a bacterial lawn. Commercially available antibiotic discs, were placed on it and the resistant antibiotics and sensitive antibiotics were noted after

overnight incubation at 37 degree centigrade. 10 of the above bacterial pathogens resistant to minimum of three antibiotics were selected for the study .

### Antibacterial activity of Lactic acid bacteria

Agar Well Diffusion method was used for antibacterial activity of lactic acid bacteria against pathogenic bacteria<sup>7</sup>. Mueller-Hinton agar was the selected media. Agar plates were inoculated with bacterial pathogens, on each plate, after adjusting the growth to match the McFarland turbidimetric standard. Wells of 6 mm were cut in the Mueller-Hinton agar, using a cork borer. 50 microlitres of cell free supernatant of lactic acid bacteria were added into each well . Plates were incubated overnight at 37degree centigrade. Diameter of the inhibition zones were measured in millimetres around the wells.

## RESULTS AND DISCUSSION

A total 15 numbers of lactic acid bacteria isolated from dairy sources having antimicrobial activity were selected for this study. The morphological ,physiological and biochemical properties of these were observed. They are known as bacteriocins (bacterial toxins).Identification was based on the methods described in Bergey's Manuel. Bacteriocins produced by lactic acid bacteria are gram positive, catalase negative, nitrate non reducing, non sporing and non motile organisms . Antimicrobial or antibacterial activity testing revealed the sensitive and resistant nature of bacterial pathogens towards antibiotics. Disk diffusion method was done to study that. 10 pathogenic bacteria, causing human diseases, which were resistant to minimum of three antibiotics, selected were given in Table 1.

**Table.1 Pathogenic organisms were tested with antimicrobial drugs to select 10 bacterial strains, resistant to minimum of three antibiotics. Method adopted was disc diffusion method**

No.	Organism	Con. of Drugs in micrograms	Resistant Antibiotics
1	Staph. aureus (A)	20	➤ Ampicillin\Sulbactum AS
		10	➤ Amoxicillin MX
		10	➤ Norfloxacin NX
2	Proteus	25	➤ Co-trimoxazole BA
		5	➤ Ciprofloxacin CP
		10	➤ Norfloxacin NX
		30	➤ Ceftizoxime CI
3	E.coli (A)	75	➤ Cefaperazone CPZ
		10	➤ Cefpodoxim CPD
		10	➤ Gentamycin GM
		30	➤ Tetracycline TE
4	E.coli(B)	20	➤ Ampicillin AS
		30	➤ Chloramphenicol CH
		30	➤ Cefuroxime CXM

5	Staph. aureus (B)	15	➤	Roxythromycin	AT
		1	➤	Cloxacillin	CX
		20	➤	Ampicilin	AS
		30	➤	Cefotaxime	CF
6	E.coli(C)	10	➤	Cefotaxime	CTX
		30	➤	Cefuroxime	CXM
		5	➤	Ciprofloxacin	CP
		10	➤	Norfloxacin	NX
7	Pseudomonas (A)	30	➤	Cefuroxime	CXM
		30	➤	Cefotaxime	CF
		5	➤	Ciprofloxacin	CP
		30	➤	Ceftizoxime	CI
8	Staph. aureus (C)	10	➤	Penicillin	P
		10	➤	Amocycillin	AMX
		20	➤	Ampycillin	AS
9	Pseudomonas (B)	30	➤	Cefadroxil	CFR
		30	➤	Linzolid	LZ
		30	➤	Ceftriaxone	CFX
10	E.coli(D)	10	➤	Cefotaxime	CTX
		5	➤	Cefixime	CFM
		10	➤	Cefpodoxim	CPD
		25	➤	Co-trimoxazole	BA



**Figure 1. Bacterial sensitivity testing done on pathogenic bacteria to collect the resistant strains using disk diffusion method**

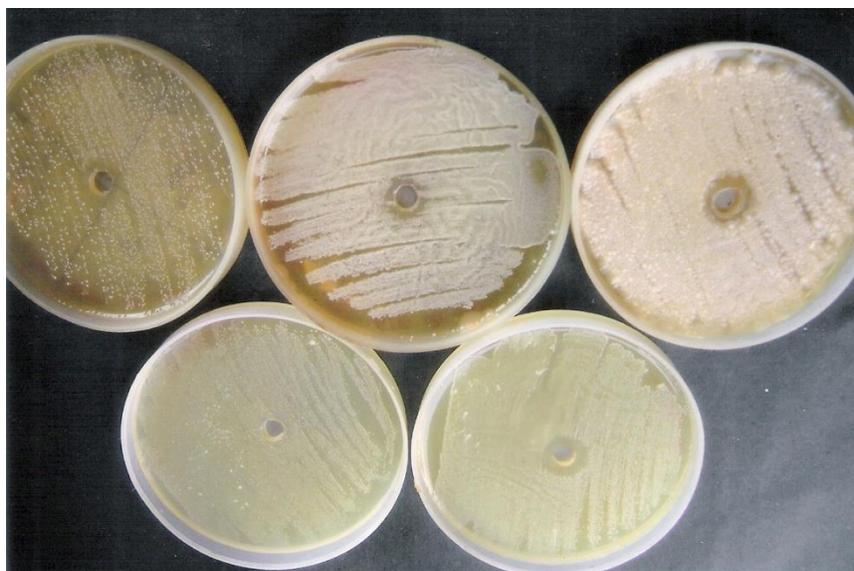
Lactic acid bacteria were used for antibacterial activity studies of these bacterial pathogens, the results of which is given in Table 2. Among LAB bacteriocins, highest inhibition zone was shown by LAB -15th, acting against Staph aureus. Minimum inhibition was shown by LAB-4 and LAB-12 against proteus. LAB-1, LAB-2, LAB-7 and LAB-10 were completely resistant towards all bacterial pathogens

**Table. 2 Lactic acid bacteria tested for its antibacterial activity against bacterial pathogens with antibiotic resistance. Method adopted was Agar well diffusion method.**

	LAB (1)	LAB (2)	LAB (3)	LAB (4)	LAB (5)	LAB (6)	LAB (7)	LAB (8)	LAB (9)	LAB (10)	LAB (11)	LAB (12)	LAB (13)	LAB (14)	LAB (15)
Staph. aureus (A)	R	R	7.8	8.4	8.2	R	R	R	R	R	R	8.6	R	8.6	9
Proteus	R	R	R	7.5	R	R	R	R	R	R	R	7.5	R	R	8.1
E.coli (A)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
E.coli(B)	R	R	R	8.6	R	8.6	R	R	R	R	R	R	R	R	8.1
Staph. aureus (B)	R	R	R	R	R	R	R	8.2	7.8	R	R	R	R	R	R
E.coli(C)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Pseudomonas (A)	R	R	R	R	R	R	R	8.4	8.2	R	R	R	R	8.3	8.6
Staph. aureus (C)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Pseudomonas (B)	R	R	R	8.8	R	R	R	8.6	R	R	R	R	R	R	8.8
E.coli(D)	R	R	R	R	R	R	R	8.2	R	R	8.2	R	R	R	8.6

*Grading: Based on zone diameter*

No inhibition: Negative, Moderate inhibition: (6-9mm)+, Strong inhibition : (10-14mm)++, Very strong inhibition: (15-18mm)+++



**Figure 2. Lactic acid bacteria tested for its activity against bacterial pathogens with anti biotic resistance**

In this study, some of the lactic acid bacteria strains showed moderate inhibition towards bacterial pathogens. Antagonistic effects produced by Lactic acid bacteria towards other organisms may play an important role in maintaining a proper microbial balance in intestinal tract and also in bio-preservation. Many lactic acid bacteria have antimicrobial activity, which can be attributed to the production of bacteriocins. Bacteriocins of LAB are typically cationic, hydrophobic peptides and differ widely in many characteristics like molecular weight, presence of particular group of aminoacids and post-translational modification of certain amino acids. The studies of Blackburn and Projan ,in the production of highly purified nisin preparations ,and enhancement by chelators has led to interest in the use of nisin, the bacteriocin, for human ulcer therapy<sup>8</sup>.In this study, we focussed on the suitability of applying lactic acid bacteria, on disease causing, pathogenic bacteria in the case of antibiotic resistance .Widespread antibiotic resistance is due to excessive or indiscriminate use of antibiotics in this part of the world, or due to chromosomal changes. Antimicrobial resistance makes it difficult to treat some infectious diseases due to excessive use and misuse of antimicrobial drugs, favouring resistant strains of microorganism. This is in consonance with the studies of Howell et al, for the control of mastitis in cattle<sup>9</sup>. This study emphasises the need of antimicrobial susceptibility profile of individual isolates to detect the multidrug resistance pattern to guide the treatment. Valraedes et al proved that bacteriocins and other defensive substances induce immunological responses<sup>10</sup>.

## CONCLUSION

Ignorance of real causative agents, incomplete usage of dose and other irregularities resulted in antibiotic resistance in bacteria, which is an alarming public health issue. This study has shown that lactobacilli isolated from diary sources can be applied, in natural form, modified or as combinations to save the mankind from the clutches of antibiotic resistance. They have potential in combating diseases. Further isolation and purification works are in progress for their in vivo studies. Due to its valuable multifunction properties, bacteriocins are considered as a wonderful product with an expanding and attractive market value. Many developments are in areas of multidrug resistance, quorum sensing, osmoregulation, and bacteriophages. Progress has also been made in the construction of genetically modified LAB which opened a new potential application for these in various industries.

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