International Journal of Research in Science advanced Technology and management studies (IJRSTMS)

ISSN: 2459-425X • Website: www.ijrstms.com

ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA AGAINST MULTIPLE DRUG RESISTANT UROPATHOGENS

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ABSTRACT

Urinary tract infections (UTIs) are the common bacterial infections that affect various areas of the urinary system in both females and males. Globally, the morbidity rates associated with UTI are very high due to occurrence of antimicrobial resistance in pathogens causing UTI.Many studies have been conducted to overcome the resistant in UTI pathogens. One of the alternatives for treating UTI pathogens areLactic acid bacteria (LAB) commonly known as probiotics. The present research aims to explore the bio therapeutic application of LAB over Uropathogens in order to reduce the overreliance on antibiotics. In the present study, LAB was isolated from different dairy products viz. buttermilk, yoghurt and curd. Four different LAB isolates were identified as *Lactobacillus plantarum*, *Lactobacillus rhamnosus, Lactobacillus casei* and *Lactococcus lactis.* The results revealedthat maximum antimicrobial activity was given by *Lactobacillus plantarum* and *Lactobacillus rhamnosus* against most of the Uropathogens. Hence, Probiotics could be utilized as one of the best natural therapeutics for prevention as well as cure of urinary tract infections.

Key words: **UTI, LAB, Antimicrobial resistance, Probiotics, Uropathogens**

I. INTRODUCTION

Urinary tract infections are the frequent infections affecting most of the population. It mostly includesCystitis, Pyelonephritis and Urethritis. The infection is mostly caused by one or more microorganisms belonging to aerobic or facultative bacteria, yeast and molds or sometimes viruses also. Most of the studies had reported that 80 to 85% UTI is caused due to *E.coli.* Similarly other Uropathogens reported are *Candida spp., Staphylococcus spp., Klebsiella spp., Enterococci spp., and Proteus mirabilis.* [1-3]

Females are more vulnerable to UTI as compare to males due to urogenital tract anatomy. It is reported that every year more than 40% women develop UTI. [4] The recurrence of UTI has also been reported. However, the mortality rates are less but morbidity is high. [5]The symptoms of UTI includefrequent and urgent need of urination,burning or painful micturition, bad-smelling urine, pus or blood in urine, lower abdominal pain,pressure or cramps.If the infection spreads to the kidneys, symptoms could include fever, chills, nausea, vomiting, and fatigue in addition to mid-back pain (to the right or left of the spine). The risk group includes sexually active young women, patients undergoing genitourinary

instrumentation or catheterization and several other populations.Typically ampicillin or a combination of trimethoprim and Sulfamethanoxazole [TMP/SMX] are the usual treatments for this infection. However, development of antibiotic resistance in pathogens associated with

UTI makes the antibiotic therapy failure.Hence, search for new therapeutic agent to treat UTI is needed.

Previous studies had reported that lactic acid bacteria (LAB) known for their probiotic activity has potential to inhibit Uropathogens.[6-7] They act as a shield against infection forming a natural barrier in urinary system and can prevent the occurrence of UTI. Numerous antibacterial properties are displayed by lactic acid bacteria due to production of organic acids, and other compounds such as bacteriocins and antifungal peptides. [8-10] So far there is little information on the LAB isolated from dairy products against organisms implicated in UTI. Hence, the present study aims to explore the potential of LAB to treat Uropathogens to decrease the over reliance on antimicrobials.

II. MATERIAL AND METHOD

2.1. Isolation of Uropathogens -

The urine samplesof UTI positive patients were collected from different pathology laboratories located at Washim city area and immediately transported to Microbiology laboratory, R. A. College Washim Under aseptic condition. The urine samples were enriched in sterile nutrient Broth in aseptic condition. Then the broth was allowed to incubate at 37°c for 24 hours. After 24 hours, the turbidity was observed for the enrichment of Uropathogens.

The enriched sample was spread on different Selective media plates viz. EMB, MSA, MacConkey, Cetrimide and Potato dextrose agar plates for the isolation of Uropathogens.After incubation, uropathogens were identified by adopting conventional identification methods viz. Staining techniques (Gram staining and Lactophenol cotton blue staining), motility, colony characters Biochemical tests, IMViC, Sugar fermentation tests, enzyme assay etc. and were confirmed by comparing with Bergey's manual of systematic bacteriology.

2.2. Antibiotic Susceptibility Test of Uropathogens -

The antibiotic susceptibility of uropathogens was determined by Kirby Bauer disc diffusion technique suggested by CLSI (Clinical Laboratory Standards Institute). The uropathogens were separately inoculated in sterile nutrient broth tubes andincubated at 37°c for 3 to 4 hours and the turbidity was compare with 0.5 McFarland standards. The uropathogens were spread on the surface of sterile Muller- Hinton Agar plates. The antibiotic discs namely Tetracycline (30µg) , Azithromycin (15µg) , Cephalexin (30µg) , penicillin (10U) were placed aseptically on the surface of seeded agar plates and incubated at 37°c for 18 to 24 hours. After incubation the plates were observed for the zone of inhibition and the diameter of zones wasmeasured using HiAntibiotic Zone ScaleC. [11]

2.3. *Isolation and identification of Lactic Acid Bacteria from Dairy products*-

The sealed dairy samples viz. Yoghurt, Curd and Buttermilk was purchased from Local Dairy located in Washim city area and transported to R. A. College, Microbiology Laboratory.

1mlYoghurt and Curd sample were separately diluted in 9 ml. sterile distilled water by serial dilution.0.1 ml. each dairy samples were spread on MRS agar plates. These Petri plates were then incubated at 37° C for 48 hrs.in bacteriological incubator.After incubation the diversified colonies grown over the MRS media were streaked on MRS agar plates. The isolated and purified cultures were subjected for conventional identification using different tests viz. Gram staining, motility, colony characters, biochemical and sugar fermentation pattern, etc. The bacterial cultures were confirmed by comparing the characters with Bergey's manual of bacteriology.

2.4. Antimicrobial activity of LAB against Uropathogens-

The antimicrobial activity of LAB against uropathogens was determined by Kirby Bauer disc diffusion technique suggested by CLSI (Clinical Laboratory Standards Institute) as describe earlier. [12]

III. RESULT AND DISSCUSION

The purpose of this study was to evaluate the antimicrobial activity of Lactic Acid Bacteria on the MDR pathogens associated with Urinary tract infection. The uropathogens were isolated from urine samples and identified *as E.coli, Proteus species, Klebsiella species, Staphylococcus species, Pseudomonas species* and*Candida species*respectively.The antibiotic susceptibility of these Uropathogens was analysed.

Table.1 and fig.1 represents the findings on the antibiotic susceptibility patternof isolated Uropathogens. From the table it is observed that all the Uropathogens showed resistance against the tested antibiotics. In tetracycline, the minimum zone diameter (7 mm) was exhibited by*Pseudomonas species* and maximum zone of inhibition (11 mm) was shown by*Staphylococcusspecies* and *Candida spp.* respectively. The mean zone ofinhibition against tetracycline was found to be 9.83 mm.

In case of Penicillin, the zone of inhibition (9 mm) was at par for *Pseudomonas species*, *Proteus species* and*Klebsiella species* respectively and maximum zone of inhibition was 11 mm shown by *Staphylococcus spp.* and *Candida spp.*Hence, the mean zone diameter is 9.83 mm which denotes the resistance of uropathogensConsidering Azithromycin, the minimum diameter of zone of inhibition was 9mm shown by *Proteus spp. and Klebsiella*. The maximum zone of inhibition was 15 mm shown by *Staphylococcus spp.*Hence, the mean zone diameter is 11.16 mm which denotes the resistance of uropathogens.

Similarly, in Cephalexin minimum zone was 9 mm shown by *Pseudomonas species* and *Staphylococcus species* while the maximum zone of inhibition was 11 mm shown by *E.coli*. Hence, the mean zone diameter is 9.83 mm. The maximum zone of inhibition was exhibited by *Staphylococcus species* (11.5 mm) followed by *E Coli* (11.25 mm) and *Candida spp.* (10.5 mm). *Proteus and Klebsiella* showed at par results of inhibition zone against all the antibiotics. *Pseudomonas spp.* exhibited least zone of inhibition (8.75 mm) depicting its high resistance towards antibiotics (Fig.2), from the above results, it is concluded that all the Uropathogens are Multidrug Resistant (MDR). The results on present studies are in accordance with the experimental findings of most of the research workers enlighten same line of research. [13-16]

Table1:AntibioticsusceptibilitypatternofUropathogens

Antibiotics / **Microorganism**	**Tetracycline (30µg)**	**Penicillin (10U)**	**Azithromycin (15µg)**	**Cephalexin (30µg)**	**MeanZone ofinhibition**
	Zoneofinhibitioninmm				
E.coli	10(R)	10(R)	14 (I)	11 (R)	11.25
Proteus spp.	10(R)	9(R)	9(R)	10(R)	9.5
Klebsiella spp.	10(R)	9(R)	9(R)	10(R)	9.5
Pseudomonas spp.	7(R)	9(R)	10(R)	9(R)	8.75
Staphylococcusspp.	11(R)	11(R)	15 (I)	9(R)	11.5
Candida spp.	11(R)	11(R)	10(R)	10(R)	10.5
Mean zone ofinhibition	9.83	9.83	11.16	9.83	

{R-ResistantandI-Intermediate}

Figure1: AntibioticsusceptibilitypatternofUropathogens

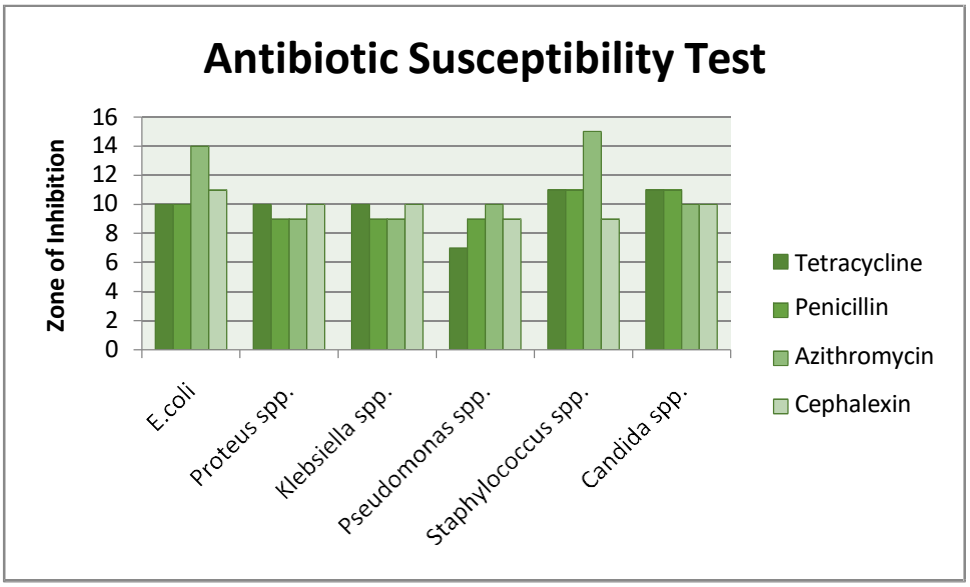


Figure2:Meanzoneofinhibitionexhibitedbyuropathogens

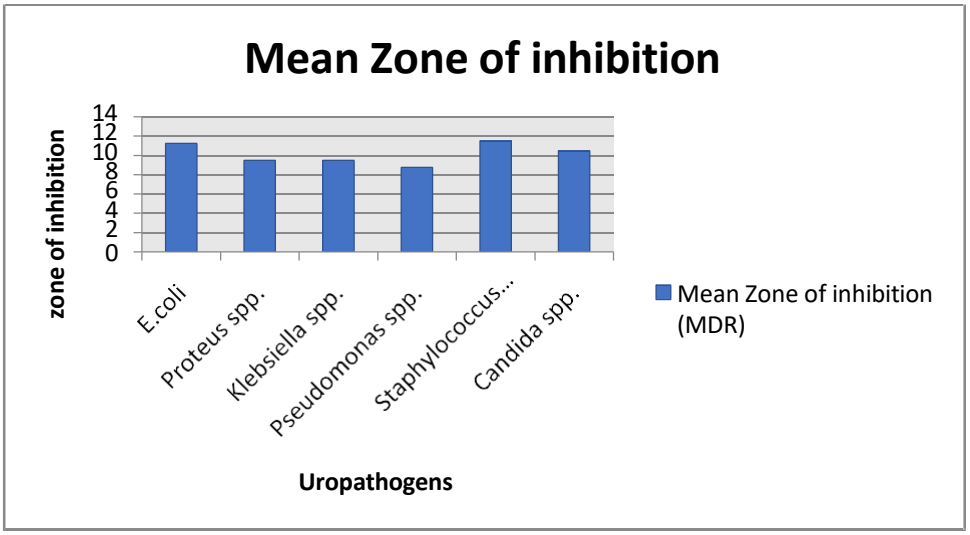


Table 2 represents the results on identification of LAB isolated from different dairy samples. From the table it is observed that four different LAB belonging to different species were isolated from the dairy samples. The isolates were labelled according to their source of isolation as Y1 (Yoghurt), C1 (Curd), B1 & B2 (Buttermilk). From the observations obtained by Biochemical tests, catalase test, Carbohydrate Utilization test, cultural characteristics and Gram Staining processes, the Y1 isolate identified as *Lactobacillus casei*, C1 as *Lactococcus lactis*, B1 & B2 as *Lactobacillus rhamnosus and Lactobacillus plantarum* respectively. [17]

Table2:-IdentificationofLABbyconventionalmethod

Culturalcharacters	**Isolatesfromdairysamples**			
	Y1	**C1**	**B1**	**B2**
Size	1.1x4.0mm	1mm	1.0-1.5mm	0.9×3um
Shape	Convex, Shortchains	SmallCircular	Round	SmallCircular
Colour	Opaquewithoutpigment	Whitecreamy	White	White
Type	Smooth	Smooth	Smooth	Liquid
Gramnature	Grampositive	Grampositive	Grampositive	Gram positive
Shape	Rod	Cocci	Rod	Rod
BiochemicalCharacters				
Indole	-	-	-	-
Methylred	-	-	-	-
Voges-Proskauer	-	-	-	-
Citrate	+	+	-	-
Catalase	-	-	-	-
Glucose	+	+	-	+
Lactose	+	+	+	+
Sucrose	+	-	-	+

Possiblespecies	***Lactobacilluscasei***	***Lactococcuslactis***	***Lactobacillusrhamnosus***	***Lactobacillusplantarum***

Table.3 represents the findings on antimicrobial activity of LAB against UTIPathogens. From the table it was observed that *Lactobacillus plantarum* followed by*Lactobacillus rhamnosus* isolated from buttermilk showed more antimicrobial activityagainst uropathogens. The average zone of inhibition exhibited by both was found tobe 12.83 mm and 12.66 mm respectively. *Lactobacillus casei* isolated from yoghurt showedaverage zone diameter of 12.16 mm and *Lactococcus lactis* from curd showed averagezone of 10.33 mm respectively. Hence, buttermilk was found to possess more benefit ascompare to curd and yoghurt. However, the variations in findings might be possiblebecause the growth of LAB and its beneficial effects are dependent on productioncondition, environmental temperature, external factors, quality of raw material forproduct preparation etc.

The mean zone of inhibition given by *Staphylococcus spp* .against all LAB was found to be (13.75 mm) followed by *E.coli* (12.25 mm), *Klebsiellaspp. (*12.5 mm), *Proteus spp.* (12 mm) and*Candida spp.* (11.25 mm). *Pseudomonas spp.* showed least zone of inhibition (10.25 mm) (Fig 4). Hence, LAB was found to be effective against uropathogens.

The results on present studies are in accordance with the experimental findingsof most of the research workers enlighten same line of research. They reported that Lactobacilli can prevent the adherence, growth and colonization ofuropathogenic bacteria.[18-22]

Table3:-AntimicrobialActivityofLacticAcidBacteriaagainstUropathogens.

LAB	***Lactobacillus casei***	***Lactococcuslactis***	***Lactobacillusrhamnosus***	***Lactobacillus plantarum***	**Meanzone ofInhibition(mm)**
Uropathogens	Zoneofinhibitioninmm.				
E.coli	13	10	14	12	12.25
ProteusSpp.	14	10	11	13	12
Klebsiella	12	10	13	15	12.5
Pseudomonas spp.	9	12	11	9	10.25
Candida spp.	10	11	11	13	11.25
Staphylococcus spp.	15	9	16	15	13.75
AverageZone(mm)	12.16	10.33	12.66	12.83	

FIG3: AntimicrobialActivityofLacticAcidBacteriaagainstUropathogens

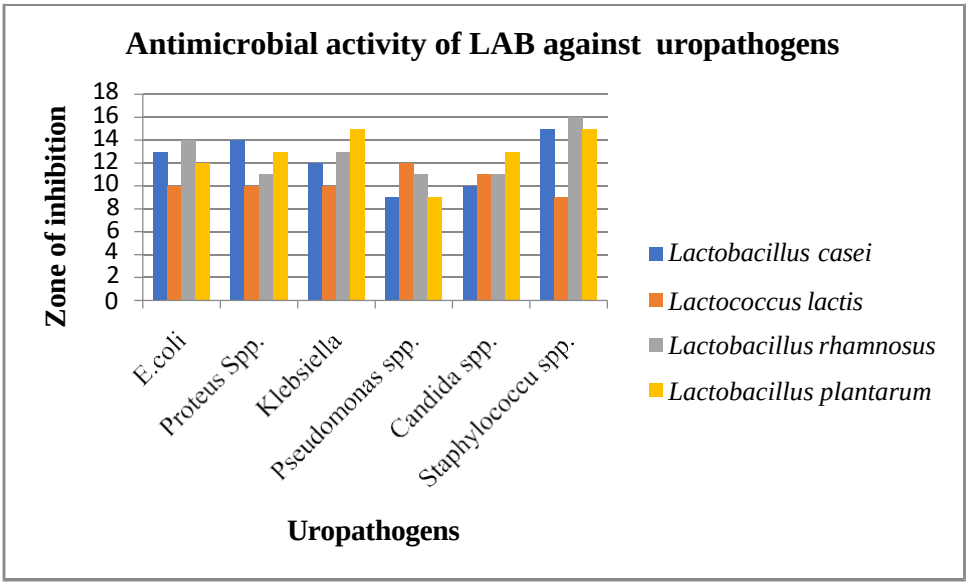
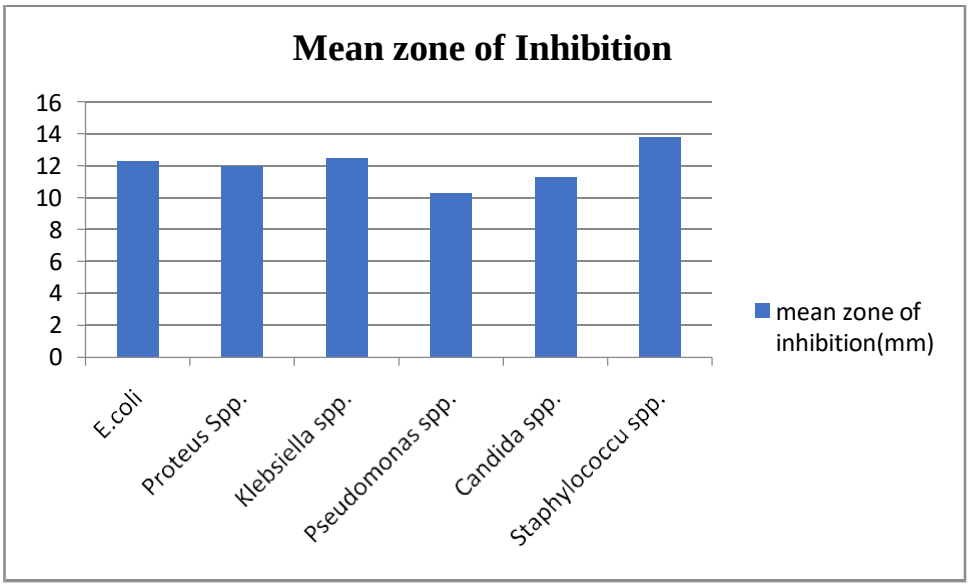


FIG4:Mean AntimicrobialactivityofLABagainstUropathogens.



IV. CONCLUSION

The present study concluded that the species of Lactic acid bacteria isolated from different dairy products viz. Yoghurt, Curd and Buttermilk were found to be a perfect substitute to antibiotics used for the treatment of multiple drug resistant UTI pathogens such as *Candida spp., Klebsiella spp., E.coli,Pseudomonas spp.and Staphylococcus Spp.* Hence, Lactic acid bacteria can prove to be useful if consumed as Probiotics against Urinary tract infections.

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