International Journal of Current Medical and Pharmaceutical Research

IJCMPR

ISSN: 2395-6429

Available Online at http://www.journalcmpr.com

DOI: http://dx.doi.org/10.24327/23956429.ijcmpr20170156

RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF LACTOBACILLI AGAINST EXTENDED SPECTRUM B-LACTAMASE PRODUCING ISOLATES FROM POST-OPERATIVE URINARY TRACT INFECTIONS IN CARDIAC PATIENTS

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ARTICLE INFO

Article History:
Received 24th April, 2017
Received in revised form 3rd May, 2017
Accepted 18th June, 2017
Published online 28th July, 2017

Key words:
Lactobacilli, ESBLs, MDRs, Cardiac Patients, Post-operative, infections

ABSTRACT

Purpose: Lactobacilli (LAB) can serve as better supportive supplements in boosting the immune response and act as remedy against life threatening infections. Isolation and characterization of LAB against Extended Spectrum β-Lactamase (ESBL) producing bacteria isolated from postoperative urinary tract infections of cardiac patients.

Methods: ESBL producing isolates were characterized by using phenotypic tests including: combination disc (CD) and double disc synergy test (DDS) and PCR based detection tests. Lactobacilli were isolated from commercial yogurt and milk samples. Characterization was done by using biochemical battery. Well Diffusion assay was used to check antibacterial activity.

Results: Test organisms were obtained from study conducted in Punjab Institute of Cardiology (PIC), Lahore, Pakistan from Oct-2016 to Feb-2017. ESBLs producing strains were isolated from post-operative urinary tract infections (UTIs) in cardiac patients. Fourteen strains were selected as potential probiotics as they possessed strong antibacterial activity against ESBLs. These strains showed variable zone of inhibition, Heat, proteinase k and Sodium dodecyl sulphate (SDS) treatments showed that the antibacterial compound is protein in nature.

Conclusion: Lactobacilli isolated from dairy products may be used against ESBLs. Isolated strains were resistant to low pH and were able to inhibit the growth of ESBLs.

INTRODUCTION

Nosocomial infections are very common in patients who underwent various surgeries. Cardiac patients are also prone to such infections where >30% cases have been reported for development of nosocomial infections in patients who underwent heart surgery [1]. Urinary tract infection (UTI) is one of most occurring nosocomial infections [2]. Such infections affect the economy of the country with enhanced disease burden and high morbidity and mortality rates [3]. Furthermore, many hospitals have recently started reporting the emergence of resistant organisms in their ICUs[4]. Multidrug resistance has serious public health consequences as we are left with very few or no treatment options for life threatening infection [5]. ESBL producing bacteria are of main concern in this decade due to their impact on public health[5]. ESBL producing bacteria being resistant to third generation of cephalosporins are of concern [6]. Severity and impact of MDR issue indicates an urgent need for treatment options other than traditional antibiotics [7]. *Lactobacilli* are beneficial bacteria that reside inside our body and protect us from many opportunistic pathogens capable of causing infections [8]. Due to their ability to tolerate low pH, fermented products are enriched with *Lactobacilli* which may become a good source to protect our body against various infections [9]. *Lactobacilli* spp. are gut indigenous microbiota and they act as probiotics.

In the gut, they are continuously exposed to extreme conditions like low pH, alkaline conditions, so they have intrinsic property to resist severe conditions of gut. There are multiple ways by which probiotics influence our health [8]. This study was conducted to investigate the inhibition activities of *Lactobacilli* against MRSA and ESBL producing isolates from post-operative urinary tract infections in Cardiac patients. Isolated bacteria were also screened for probiotic properties.

MATERIALS AND METHODS

Isolation and Characterization of ESBLS

Punjab Institute of Cardiology (PIC) is a 300 bed tertiary care hospital. It has been providing health care facilities since past two decades. This study was carried out between Oct-2016 and Feb-2017. ESBL production was assessed by using phenotypic tests (combination disc test and double disc synergy test) and molecular detection tests (PCR for CTXM, TEM, SHV and OXA genes). Multidrug Resistant strains which were sequenced in our previous study were used as test organisms here. Test strains, used in the study were *K. pneumonia* (GU594301, KM359143, KR822243 and KR822242), *E. coli* (KR822245, KR822241and KR822244) and *S. aureus* (KC928092 and KC967213) [10, 11].

Isolation and Characterization of *Lactobacilli*: Ten samples of commercially available yogurt and milk were taken (triplicate samples were taken). Samples were serially diluted up to10^{-4} and 50µl of sample was spread on appropriately labeled MRS Agar plates. Incubation of spreaded plates at 37˚C was done for 18-24 hours. After incubation, numbers of colonies on each plate were counted and CFU/ml was calculated for each sample. Fifteencolonies were selected based on morphological features for purification and further proceedings. Gram-stain was performed to check for cellular morphology.

Biochemical Characterization

Catalase and oxidase production test

Tube test was performed to check Catalase production and 3% H_2O_2 was used. Oxidase reagent was applied on filter paper and culture was applied on it and observed for development of purple coloration [12].

Sugar fermentation test

This test was performed by using 9 sugars (glucose, fructose, mannose, sucrose, sorbitol, galactose, arabinose**,** rhaminose, xylose and raffinose. MRS broth was supplemented with each sugar (2%), Durham's tubes were placed in each tube to check for gas production with phenol red as indicator. Inoculated tubes with supplemented sugars were incubated at 37˚C for 18-24 hours. After incubation, results were observed for the sugar fermentation as well as gas production [13].

Potential Probiotics Tests

Temperature tolerance test

Strains inoculated in MRS broth were incubated at different temperatures and observed for growth after 18-24 hours [14].

Acid Tolerance test

This test was performed by using method of Shokryazdan *et al*.[14]. Briefly, Biomass of isolated strains were recovered, washed and inoculated in MRS broth at pH 2.0 and 3.0 and incubated at 37°C for 18 hours. The optimal growth in culture tubes will represent the survival of bacteria in acidic conditions [15].

Salt tolerance test

MRS broth was supplemented with 6.8% NaCl and strains were inoculated in the broth for 18-24 hours at 37˚C. Presence and absence of growth was observed compared with positive and negative control[16]. The test is done to check the salt tolerance ability of Lactobacilli, as the must be able to survive the gut conditions.

Antibacterial Activity of Lactobacilli

Well diffusion assay was performed to check antibacterial activity of *Lactobacilli* strains against ESBL isolated from cardiac patients. Results were observed after 18-24 hours. 20% SDS, Heat and Proteinase k treatmentswere given to check the nature of antibacterial compound [7].

Safety Assessment

Antibiotic Resistance

Antibiotics resistance of *Lactobacilli* was checked by using disk diffusion assay by using panel of 18 antibiotics including ampicillin, amoxicillin, vancomycin, oxacillin, ciprofloxacin, doxycycline, gentamycin, carbenicillin, imipenem, septran, penicillin, teicoplanin, cefoxitin, erythromycin, streptomycin, tetracycline and kanamycin. Results were observed after 18-24hours [17].

Haemagglutination assay

Surface proteins of *Lactobacilli* may induce agglutination of RBCs which is a risk for administration of *Lactobacilli* as probiotic. About 100µl of bacterial suspension was mixed with 100µl of 1% red blood cells on disposable microtiter plates and incubated for 2 hours at 4°C and then for 2 hours at room temperature. Haemagglutination was checked for blood groups (A, B and O). After incubation results was observed macroscopically [18].

Nature of Antibacterial Compound

Nature of compound was checked bySodium dodecyl sulphate (SDS 20%) treatment for 5 minutes,5 µl of Proteinase k for 2 hours and heated at 80ºC for 15 minutes [7]. After respective incubation, treated crude extracts of each strain were placed in labeled wells. Zone of inhibition of treated crude extracts were measured after incubation.

Statistical Analysis

All the experiments were repeated three times and mean values with standard deviation and standard errors were calculated by using SPSS version 14.0.

RESULTS

Isolation and Characterization of Lactobacilli

Fourteen strains were selected on the basis of gram-stain, colony morphology, catalase and oxidase test. All gram-positive rods, catalase negative and oxidase negative strains were processed. Sugar fermentation and gas production tests indicated them to be of *Lactobacilli* group (Table 1).

Screening for potential probiotics

All the strains showed growth at high salt concentration but strains A2 and F1-9 showed best growth. Low pH affects the growth of F1 and F10 strains and they showed little growth under such condition (Table 1). All the strains had good antibacterial property against gram negative ESBL but MRSA was quite resistant towards compounds produced by strain A1, F1, F5-10 (Table 2).

Safety Assessment

Selected *Lactobacilli* strains were 98-100% resistant towards oxacillin, vancomycin, and teicoplanin. While, isolates showed 90-100% sensitivity towards kanamycin, tetracycline, streptomycin, erythromycin, ampicillin, imipenem,

doxycycline, ciprofloxacin (Table 3). No strain showed haemagglutination after incubation with all blood groups separately.

were catheterized within first 24 hours of admission suffered more from death and prolong hospital stays[21].

Table 1 Biochemical and Physiological Characterization of Isolated strains

Lactobacillus strains	*Sugars Used										Gas Production	Growth at different physiological conditions	
	Glu	SUC	FRUC	SOR	GLAC	MAN	ARA	RHM	XYL	RAFI		6.8% NaCl	pH3.0
AB1	+++	+++	+	+++	++	+	+	+	+	-	-	++	+++
AB2	+++	+	+++	+++	+	++	+	+	++	+	-	+++	++
AB3	+++	++	++	++	++	++	+	+	+		-	++	+++
FM1	+++	+++	+	++	+	+++	++	+	+		-	+++	+
FM2	+++	+++	+++	++	++	+++	+	++	+		-	+++	++
FM4	+++	+++	+++	+++	++	+++	+	++	++		-	+++	+++
FM5	+++	+++	++	++	++	+++	+/-	+	++	+	-	+++	+++
FM6	+++	+	+++	+	++	+++	+/-	+	++	+	-	+++	+++
FM8	+++	+++	++	-	+++	+++	+++	++	++	++	-	+++	++
FM9	+++	+++	++	-	-	++	-	-	-	-	-	+++	+++
FY1	+++	++	+++	+++	+++	++	++	++	++	-	-	++	+
FY3	+++	+++	+++	-	++	+++	-	-	-	-	-	++	++
PRO1	+++	+	+	+	+	+	+	-	+	+	-	++	+++
PRO2	+++	++	++	+	-	-	+	++	+	+	-	++	+++

*Glu (Glucose), SUC (Sucrose), FRUC (Fructose), SOR(sorbitol), MAN(Mannose), ARA (Arabinose), RHM (rhaminose), XYL (xylose) and RFI (raffinose).
4% of each sugar was used .+++ (very good growth), ++ (good growth), + (week growth) and – (no growth).

Table 2 Antibacterial Activity of isolates against test organisms

Test Strains	Strain Name	Accession No.	Lactobacilli stains zone sizes in mm											
			AB1	AB2	AB3	FM1	FM2	FM4	FM5	FM6	FM8	FY1	FY3	PRO1	PRO2
A5	K. pneumoniae	GU594301	25±1.27	24±0.97	0± 0.00	0± 0.00	0± 0.00	8±0.6	19±0.22	21±1.5	23±1.38	20±1.26	0± 0.00	0± 0.00	0± 0.00
A23	E. coli	GU594304	22±0.97	18±1.2	0± 0.00	22±0.92	0± 0.00	23±0.7	21±1.6	20±1.27	23±1.6	13±1.20	10±3.11	0± 0.00	10±2.0
2K/25	K. pneumoniae	KM359143	12±0.65	22±0.31	0± 0.00	28±3.15	0± 0.00	15±1.2	10±1.28	22±1.25	0± 0.00	0± 0.00	0± 0.00	5±0.10	5±1.38
14755	K. pneumoniae	KR822243	36±1.2	24±0.63	28±0.7	10±0.7	24±1.2	21±125	22±0.97	25±2.0	32±0.7	0± 0.00	0± 0.00	5±2.10	0± 0.00
18750	E. coli	KR822245	12±0.7	45±0.28	0± 0.00	32±1.2	0± 0.00	0± 0.00	18±0.97	36±0.6	18±1.6	0± 0.00	4±1.6	0±0.00	12±1.28
6385	E. coli	KR822241	12±0.89	36±0.7	0± 0.00	19±0.63	0± 0.00	21±1.5	0± 0.00	25±1.27	23±1.5	23±1.7	14±2.0	12±0.97	7±0.67
17781	E. coli	KR822244	15±0.61	17±1.2	0± 0.00	12±1.25	16±0.6	10±2.1	10±0.21	15±2.1	0± 0.00	0± 0.00	0± 0.00	8±1.62	5±2.17
10554	K. pneumoniae	KR822242	14±2.14	0± 0.00	0± 0.00	9±0.73	0± 0.00	15±2.6	16±1.26	21±1.2	19±1.5	10±2.1	21±2.6	12±1.28	19±1.60
MR-9	MRSA	KC928092	0± 0.00	40±0.72	13±0.78	0± 0.00	50±0.71	30±1.28	32±1.38	0± 0.00	0± 0.00	0± 0.00	0± 0.00	35±0.69	10±0.10
MR-10	MRSA	KC967213	0± 0.00	21±0.12	27±2.98	0± 0.00	32±2.73	22±1.60	32±1.5	0± 0.00	0± 0.00	0± 0.00	0± 0.00	28±2.10	23±0.21

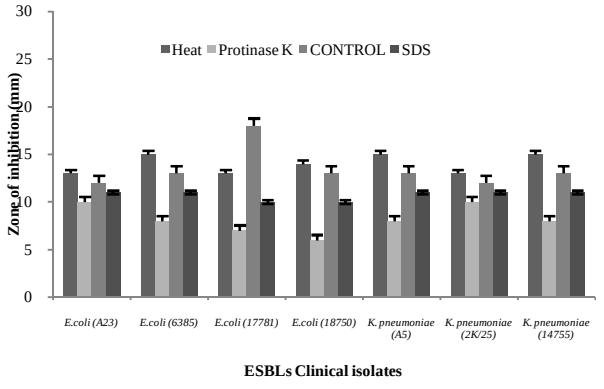

Figure 1 Effect of different treatments on the activity of antimicrobial compound

DISCUSSION

Multidrug resistance is considered as burning public health issue because of its morbidity and mortality rate [19]. Nosocomial and community acquired infections have serious consequences with increased disease burden on economy [20]. Patients with infective endocarditis or implanted pacemakers and cardioverter defiblirrator have extended lengths of stay in CCUs so because of the critical nature of their illness and need for strict fluid monitoring they remain catheterized for most of the duration of hospital stay. According to study, patients who

In this study, we have screened the *Lactobacilli* strains isolated from dairy products for potential probiotic properties helpful in boosting the immunity. Biochemical analysis indicated that glucose, fructose, sucrose and mannose are fermented by all strains while only few strains indicated positive results for xylose, rhaminose and arabinose fermentation. The study by Hedberg *et al*., 2008 indicated that strains that ferment all sugars except for arabinose and xylose belong to *Lactobacillus planterum*[13]. Here, extreme conditions were checked and all strains were resistant to these conditions. Growth at extreme conditions is requirement for probiotic bacteria as they have to survive in gastro intestinal tract [22]. Human stomach is constantly flooded with acidic products which affect its microbial load. Probiotic strains administered must resist to extreme environments like low pH as it helps to colonize them in the gut [23]. Moreover to cope with antibacterial substances in gut, they need to be intrinsically antibiotic resistant. Gueimonde *et al*.,[24] indicated that there are two types of resistance mechanisms, the acquired one and intrinsic ones, within genus *Lactobacilli* which are responsible for final development of antibiotic resistance among these isolates [24].Hence, safety assessment is a big challenge for these strains as they will be competing with gastro-intestinal pathogens where there is a chance of horizontal transfer of resistant genes if they are acquired ones [25, 26].

Table 3 Antibiotic Resistance test to check intrinsic resistance of isolates to antibiotics

Lactobacillus strains	Applied Antibiotics																	
	AM	AMC	VA	OX	CIP	DA	CN	PY	IPM	SXT	P	TEC	FOX	E	SREP	OFX	Tet	K
AB1	R	S	R	R	S	S	R	S	S	R	S	R	R	S	S	R	S	S
AB2	R	S	R	R	S	S	R	S	S	R	S	R	R	S	S	R	S	S
AB3	R	S	R	R	S	S	R	S	S	R	S	R	R	S	S	S	S	S
FM1	S	S	R	R	S	S	R	S	S	R	S	R	S	S	S	R	S	S
FM2	R	S	R	R	S	S	R	R	S	R	R	R	R	S	S	S	S	S
FM4	R	S	R	R	S	S	R	R	S	R	R	R	R	S	S	S	S	S
FM5	R	S	R	R	S	S	R	R	S	R	S	R	R	S	S	R	S	S
FM6	R	S	R	R	S	S	R	R	S	S	S	R	R	S	S	R	S	S
FM8	S	S	R	R	R	S	S	S	S	S	S	R	S	S	S	S	S	S
FM9	R	S	R	R	R	S	S	S	S	S	S	R	S	S	S	S	S	S
FY1	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
FY3	R	S	R	R	S	S	S	R	S	S	S	R	S	S	S	S	S	S
PRO1	S	S	R	R	S	S	S	R	S	S	S	R	S	S	S	S	S	S
PRO2	S	S	R	R	S	S	S	R	S	R	S	R	S	S	S	S	S	S

Here, Ampicillin (AM) amoxicillin (AMC), vancomycin (VA), oxacillin (OX), ciprofloxacin (CIP), doxycycline (DA), gentamycin (CN), carbenicillin (PY), imipenem (IPM), septran (SXT), penicillin (P), teicoplanin (TEC), cefoxitin (fox), erythromycin (E), Streptomycin (SREP), ofloxacin (OFX)tetracycline (tet) and kanamycin (K)

Here we have found no plasmid acquisition in our isolates, hence the resistance pattern is suspected to be intrinsic and not acquired one. It has also been reported that *Lactobacillus* spps are intrinsically resistant towards beta-lactams, penicillins and cephalosporins [24]. Interest in peptide antibiotics has increased greatly during the past decade, as these are believed to be very potent and are biologically and chemically very diverse [7]. Peptide molecules show higher activity and higher specificity towards their target. Moreover, they have few toxicological problems and their accumulation in organs is not observed quite often. There is few drug-drug interaction problems that have been observed for peptide antibiotics [7]. Protease treatment may result in loss of growth inhibition activity due to inactivation of proteinaceous antibacterial compound. Here, we observed more loss of activity with Proteinase k and heat treatment while SDS treatment exhibited minimal loss of activity (Figure 1). Thus, results indicate the active compound to be proteinaceous in nature.

CONCLUSION

Lactobacilli isolated from dairy products may be used as probiotics. Isolated strains were resistant to acid and were able to inhibit the growth of ESBLs. The confirmation of our in vitro results indicates the need of in vivo study to finally use them for humans.

Declarations

Acknowledgement

The authors acknowledge the Department of Microbiology and Molecular Genetics, University of the Punjab and Citi lab and Research Center, Lahore.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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