DR-1970
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RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume Issue : ()

# Evaluation of Efficiency and Extent of Inhibitory Characteristics of *Lactobacillus* spp. (*Lactobacillus plantarum*) against Selected Pathogenic Bacteria

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10.18805/ajdfr.DR-1970

## ABSTRACT

**Background:** Despite the improved modern technologies to inactivate pathogens, there has been a continuous surge of food-borne diseases involving many bacterial pathogens like *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Salmonella*, *Campylobacter*, *Listeria etc.* Physical methods of reduction of microbial load in raw foods have been known to negatively impact the organoleptic properties of the products hence reducing their acceptability. Therefore, knowing that food may be laden with many pathogens, the biocontrol methods may be an effective alternative to chemical and physical methods of preservation for eliminating pathogens.

**Methods:** The pathogens to be tested in the experiment were recovered from various food sources and clinical samples *viz*., milk, raw chicken and a faecal sample, chicken. For evaluation of inhibitory characteristics of *Lactobacillus* spp. (*Lactobacillus plantarum*) against selected pathogenic bacteria the grown culture of pathogens (*S. aureus*, *E. coli*, *B. cereus*) was added with *Lactobacillus* spp. at different concentrations and incubated at 24, 36 and 48 hrs and mean CFU units were evaluated. All of the treated groups were compared with the concurrent untreated groups of respective bacteria.

**Result:** After 24 hrs of incubation at 37°C, the mean colony forming units (CFU/ml) of *S. aureus, E. coli, B. cereus* in the cultured groups treated with $10^8$, $10^7$, $10^6$, $10^5$ and $10^4$/ml of L. *plantarum* were $2.56 \times 10^8$, $2.78 \times 10^8$, $2.90 \times 10^8$, $3.03 \times 10^8$ and $39.19 \times 10^8$; $0.81 \times 10^8$, $1.09 \times 10^8$, $1.36 \times 10^8$, $1.85 \times 10^8$ and $24.62 \times 10^8$; $0.31 \times 10^8$, $0.43 \times 10^8$, $0.60 \times 10^8$, $0.69 \times 10^8$ and $7.12 \times 10^8$, respectively. Henceforth, the mean colony forming counts of all the three pathogens increased when the concentration of *L. plantarum* decreased.

**Key words:** *Bacillus cereus*, Biocontrol, *Escherichia coli*, Food-borne diseases, Incubation, Organoleptic, *S. aureus*.

## INTRODUCTION

Despite the improved modern technologies to inactivate pathogens, there has been a continuous surge of food-borne diseases involving many bacterial pathogens like *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Salmonella*, *Campylobacter*, *Listeria etc.* These pathogens come into contact with foods during harvest or slaughtering, processing, storage and packaging. For reducing pathogenic bacteria physical treatments such as UV light, high pressure, dry heat and steam have been encouraged as the use of antibiotics has been restricted over the years due to the risk of antibiotic-resistant bacteria entering the human food chain and leading to negative impact on human antimicrobial treatment. However, such methods have been known to negatively impact the organoleptic properties of the products thus reducing their acceptability (Ravi *et al*. 2017). Many of the chemical preservatives are known potential carcinogens and have many other ill effects on human health. Thus there has been an increasing need to develop novel strategies to reduce bacterial pathogens in foods and still satisfy consumer demand for minimally processed foods with low concentrations of chemical preservatives (Garcia *et al*. 2008). Therefore, knowing that food may be laden with many pathogens, the biocontrol methods may be an effective alternative to chemical and physical methods of preservation for eliminating pathogens.

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**How to cite this article:** Ahmad, P.R., Rather, M.A., Nabi, B., Sofi, O.M.U.D., Hafiz, M., Fayaz, I.B. and Zehgeer, M.M. (2022). Evaluation of Efficiency and Extent of Inhibitory Characteristics of *Lactobacillus* spp. (*Lactobacillus plantarum*) against Selected Pathogenic Bacteria. Asian Journal of Dairy and Food Research. DOI: 10.18805/ajdfr.DR-1970.

**Submitted:** 16-06-2022 **Accepted:** 20-09-2022 **Online:** 05-10-2022

Because of the wide spread association of bacteriocins, nisin and pediocin with foods and is generally recognized as safe (GRAS) status, the use of *Lactobacilli* spp. and/or their metabolites as natural drugs or antimicrobial agents have attracted considerable interest in recent years (Reis *et al*. 2012). *Lactobacillus plantarum* is one of the most important species of LAB, with proven health benefits and antagonistic properties against the various pathogen (Arena *et al*. 2016). In this regard, the present study has been undertaken to determine the efficiency and extent of inhibitory characteristics of *Lactobacillus* spp. (*Lactobacillus plantarum*) against selected pathogenic bacteria.

## MATERIALS AND METHODS

In the current study, the reference strain of *Bacillus cereus* (NCTC 11145) was available with the Division of Veterinary Public Health, Faculty of Veterinary Science and Animal Husbandry (F.V.Sc and A.H), Alusteng, SKUAST-K. The standard strains of *Staphylococcus aureus* (ATCC 25293) and *Lactobacillus plantarum* (ATCC 8014) used in the study were procured from Hi-media, Mumbai. The characterized shiga toxin *Escherichia coli* was available with the Division. The strains were maintained on nutrient agar slants by subculturing every fortnight and were tested for purity.

A total of 60 samples comprising of 30 samples of milk (15 mastitic milk, 15 raw milk), 15 samples of raw chicken and 15 faecal samples from sheep were collected randomly from the different areas of district Srinagar. The milk and faecal samples were collected in sterile vials. The raw chicken samples were collected aseptically in sterile zip lock polythene bags and transported in ice to the Division of Veterinary Public Health, F.V.Sc and A.H, Shuhama and processed for isolation and identification of *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*. Five samples from each source were collected separately for the isolation of the pathogens, the details of the samples are provided in Table 1.

### Isolation and identification of *Staphylococcus aureus*

Briefly, for isolation of *S. aureus*, the samples were enriched in buffered peptone water (BPW) and incubated at 37°C for 18-24 hrs. A loop full of inoculum from the enriched medium was directly streaked on Baird-Parker Agar (BPA) plates containing egg-yolk tellurite emulsion. The inoculated plates were incubated at 37°C for 24 hrs. The typical jet-black colonies surrounded by white halo on BPA were considered as presumptive positive for *Staphylococcus* spp. These presumptive positive isolates were preserved on nutrient agar slants, stored at 4°C till further processing and were identified and confirmed based on cultural, morphological and various biochemical characteristics as per the standard protocol (Cruickshank *et al*. 1970).

**Table 1:** Type and Nature of samples collected for isolation of *S. aureus, B. cereus* and *E. coli.*

| Sample type | Nature of sample | No. of samples |
|---|---|---|
| Milk from cattle | Raw milk 15 | 30 |
| | Mastitic milk 15 | |
| Raw chicken | Chicken | 15 |
| Sheep faeces | Faeces | 15 |
| Total | | 60 |

The presumptive *Staphylococcus* isolates were subjected to morphological and biochemical characteristics for further confirmation. The biochemical tests included catalase reaction, oxidase testing, indole reaction, methyl red test, voges-proskauer test and coagulase test (Cruickshank *et al*. 1970).

### Isolation and identification of *Escherichia coli*

Isolation and identification of *E. coli* was done by using standard bacteriological procedures (Cowan and Steel, 1974). The milk, meat and faecal samples were enriched overnight at 37°C in buffered peptone water and were subsequently inoculated on MacConkey's agar (Hi- Media, Mumbai) and incubated aerobically for 24 hr. The presumptive colonies (lactose fermenting pink colonies) were picked up and re-cultured on Eosine Methylene Blue (EMB) agar and again incubated aerobically for 24 hr. Colonies producing characteristic greenish metallic sheen colour were considered as *E. coli.*

The well-separated colonies were picked up on nutrient agar slants as pure culture and subjected to standard morphological and biochemical tests such as oxidase test, catalase test, tryptophan degradation (indole test), glucose degradation (Methyl red/Voges-Proskauer test), citrate and urease utilization test and ability to ferment triple sugar iron for further confirmation.

### Isolation and identification of *Bacillus cereus*

Polymyxin-pyruvate-egg yolk-mannitol-bromothymol blue agar (PEMBA) media was used for isolation of *B. cereus*. The samples were processed as per the method described by Shinagawa (1990) and Tallent *et al*. (2012) with suitable modifications. Approximately 10 ml/gm of the sample, was inoculated in 50 ml of brain heart infusion broth (BHIB) containing polymyxin B (10,000 U per 100 ml). The raw chicken was homogenized with BHIB. The enrichment of samples in BHIB was carried out at 35°C for 16-18 h. After enrichment, a loopful was streaked on PEMBA plates and incubated at 35°C for 24 h. The plates showing crenate to fimbriate peacock blue colored colonies (3-5 mm) surrounded by a blue zone of egg yolk hydrolysis against a green background and other related species were purified and taken on nutrient agar slants

The confirmation of *B. cereus* was carried out as described by Rhodehamel and Harmon (2001). Briefly, the presumptive colonies of *B. cereus* and closely related species were collected and subjected to morphological and biochemical tests for identification of species. Gram reaction, cell morphology, spore production, motility, reduction of nitrate, Christensen's citrate utilization, Voges-proskauer reaction, aerobic and anaerobic utilization of glucose, mannitol fermentation and hemolysis were the tests employed for identification and conformation.

### *Lactobacillus plantarum* as a biocontrol agent against food-borne pathogens

The procedure consisted of the following steps:

#### Probiotic preparation

This step included the preparation of 0.5 Mac Farland solution (0.05 ml of 1.175% barium chloride dihydrate and 9.95 ml of 1% sulfuric acid) of *Lactobacillus* spp. in sterile normal saline solution. Briefly, 0.5 Mac Farland solution (Appendix-I) was taken and *Lactobacillus plantarum* colonies from the plates were dissolved in 9 ml of sterile NSS one by one till turbidity matched with standard 0.5 Mac Farland solution. The number of *Lactobacillus plantarum* organisms at 0.5 Mac Farland were calculated by plating on selected agar (ManRogosa Sharpe agar) by serial dilution and the total viable count was evaluated. The concentration of *L. plantarum* equivalent to 0.5 Mac Farland was approx. $10^8$ per ml.

The aliquots of this tube were prepared by serial dilution to get different concentrations of *L. plantarum* solution ($10^8$/ml, $10^7$/ml, $10^6$/ml, $10^5$/ml and $10^4$/ml). The efficacy of these five concentrations of *L. plantarum* was tested against standard concentrations of selected pathogenic microorganisms (*S. aureus*, *E. coli* and *B. cereus*).

#### Efficacy of *Lactobacillus plantarum* against pathogenic bacteria

The efficacy of *L. plantarum* was observed against three foodborne pathogens (*S. aureus*, *E. coli* and *B. cereus*) recovered naturally from various foods. To determine the efficiency of *L. plantarum* against *S. aureus*, briefly, the inoculums of *S. aureus* were prepared as given in Table 2. Briefly, a standard 0.5 Mac Farland solution of *S. aureus* was prepared in sterile normal saline solution thereby containing approximately $1.5\times10^8$ microorganisms per ml. Then six tubes containing 15 ml of nutrient broth were taken and all the six tubes were inoculated with 1 ml of the standard solution of *S. aureus* ($10^8$/ml) and 1 ml of sterile NSS. The first tube was kept as a control with 1ml of *S. aureus* inoculum and 1 ml of NSS. In the rest of the five tubes, 1ml each of different standard concentrations ($10^8$/ml, $10^7$/ml, $10^6$/ml, $10^5$/ml and $10^4$/ml) of *L. plantarum* (mentioned above in probiotic preparation) were added. Then, all the six tubes were incubated at 37°C and viable counts of *Staphylococcus aureus* on selective media (Baired Parker Agar) was determined after 24, 36 and 48 hours to see the degree of reduction/increase in growth/number of organisms brought about by presence of different concentrations of *Lactobacillus* spp. as compared to the control.

A similar procedure was adopted to determine the inhibition of the growth of *B. cereus* and *E. coli* by *L. plantarum*.

#### Antibacterial characteristics of *L. plantarum* against selected pathogenic micro-organisms

An evaluation of the antibacterial activity of L. *plantarum* against the selected pathogenic bacteria was evaluated as per the standard protocol (Tagg and McGiven, 1971). Briefly, the isolated *L. plantarum* strain was grown in MRS broth (pH 6.5) inoculated with 1% of an overnight culture and incubated at 37°C for 18-24 hours. After incubation, cells were removed from the broth by centrifugation (6000 rpm for 20 min, 4°C). The cell free supernatant was sterilized by filtering through a 0.22 milli pore filter. The antimicrobial spectrum of the cell free supernatant of different *L. plantarum* strain was determined using the disc diffusion method.

## RESULTS AND DISCUSION

The present study was undertaken to determine the efficiency of *Lactobacillus plantarum* as a biocontrol agent against some foodborne pathogens *viz*. *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*. The extent of antibacterial activity of *L. plantarum* against these pathogens was also studied.

#### Occurrence of *S. aureus*, *E. coli* and *B. cereus* in foods and clinical sample

Out of 20 samples comprising of raw milk (5), mastitic milk (5), raw chicken meat (5) and sheep faecal samples (5) that were screened for the presence of *S. aureus*, 9 turned out to be positive for *Staphylococcus* spp. and of theses 9 isolates, 6 were identified as *S. aureus* (Table 3) based on morphological and biochemical characteristics. One isolate each was recovered from raw chicken and sheep faecal samples and two isolates each were recovered from raw milk and mastitis milk samples, respectively. The isolates of *S. aureus* on Baird-Parker Agar (BPA) showed typical jet black colored colonies surrounded by white halo zone (Lecithinase activity). On Gram staining, the gram-positive cocci arranged in bunches were seen under a microscope. The *Staphylococcus* isolates characterized biochemically gave a positive reaction for catalase, methyl red test and Voges-Proskauer while as negative reaction was observed for oxidase activity and indole reaction. On the basis of these tests, out of 20 samples, 9 isolates of *Staphylococcus* spp.

**Table 2:** Effect of different concentrations of *L. plantarum* on counts of standard concentration of *S. aureus* ($10^8$/ml).

| Group | Combination of micro-organisms | 24 hrs | 36 hrs | 48 hrs |
|---|---|---|---|---|
| Control | *S. aureus* $10^8$/ml (0.5 McFarland) | Count | Count | Count |
| A | 1 ml each of *S. aureus* ($10^8$/ml) and *L. plantarum* ($10^8$/ml) in 15 ml nutrient broth | Count | Count | Count |
| B | 1 ml each of *S. aureus* ($10^8$/ml) and *L. plantarum* ($10^7$/ml) in 15 ml nutrient broth | Count | Count | Count |
| C | 1 ml each of *S. aureus* ($10^8$/ml) and *L. plantarum* ($10^6$/ml) in 15 ml nutrient broth | Count | Count | Count |
| D | 1 ml each of *S. aureus* ($10^8$/ml) and *L. plantarum* ($10^5$/ml) in 15 ml nutrient broth | Count | Count | Count |
| E | 1 ml each of *S. aureus* ($10^8$/ml) and *L. plantarum* ($10^4$/ml) in 15 ml nutrient broth | Count | Count | Count |

were identified. These 9 isolates were subjected to coagulase test and 6 coagulase positive isolates were recognized, thereby confirmed as *S. aureus*.

Similarly, for isolation of *E. coli*, raw milk (5), mastitic milk (5), raw chicken meat (5) and sheep faecal samples (5) were screened and 7 isolates were recovered (Table 3). The isolates of *E. coli* were recovered from raw milk (2), meat (2) and faecal samples (3) and from the mastitis milk none of the isolates of *E. coli* could be recovered. Of the 7 isolates recovered 4 isolates were selected for experimental testing of which one each were from raw milk and chicken and two isolates included those recovered from sheep faecal samples. All the isolates that produced a characteristic greenish metallic sheen on Eosine Methylene Blue (EMB) were presumptively considered as *E. coli* and were small rod shaped when seen under a microscope. The *E. coli* isolates characterized biochemically revealed positive reactions for catalase, indole and methyl red. While as isolates were negative for oxidase, voges- proskauer, citrate and urease utilization and triple sugar iron fermentation activity.

For isolation of *B. cereus*, 5 samples each of raw milk, mastitic milk, raw chicken meat and sheep faecal samples were screened and in total 5 isolates were recovered. The isolates were recovered from raw milk (2), raw chicken (2) and faecal samples (1). Of the 5 isolates, 4 were tested in the experiment of which, 2 were recovered from raw milk and 1 isolate each was taken from raw chicken and faecal samples. All presumptive isolates of *B. cereus* showed typical fimbriate or crenate peacock blue colored colonies with egg yolk reaction (Lecithinase activity) on PEMBA medium. On morphological characterization all the isolates were found to be Gram positive, rod shaped, spore formers and were devoid of toxin crystals. All the isolates were motile and strongly hemolytic (β-hemolytic) on 5 per cent sheep blood agar. The presumptive isolates of *B. cereus* were further characterized using biochemical tests. The isolates reduced nitrate, were also positive for VP and citrate utilization tests. All the isolates fermented glucose both aerobically and anaerobically and none of the isolates fermented mannitol.

The inhibitory effect of *L. plantarum* was observed on *in vitro* growth of selected pathogens (*S. aureus*, *B. cereus* and *E. coli*). The enumeration of these pathogens was carried out after different incubation periods (24 hrs, 36 hrs and 48 hrs). The standard concentration of pathogens ($10^8$/ml) was grown along with varying concentration of *L. plantarum* ($10^4$ to $10^8$/ml). The mean colony forming counts of *S. aureus* in the controls after 24 hrs of incubation were found to be $622.66 \times 10^8$ CFU/ml and in treatment groups the counts were $2.56 \times 10^8$ CFU/ml, $2.78 \times 10^8$ CFU/ml, $2.90 \times 10^8$ CFU/ml, $3.03 \times 10^8$ CFU/ml and $39.19 \times 10^8$ CFU/ml treated with $10^8$, $10^7$, $10^6$, $10^5$ and $10^4$/ml of *L. plantarum*, respectively. The mean counts of *S. aureus* in control groups were statistically higher compared to treatment groups. After 36 hrs of incubation the mean counts of *S. aureus* in different treatment groups with concentrations of *L. plantarum* as $10^8$, $10^7$, $10^6$, $10^5$ and $10^4$/ml the counts were $2.04 \times 10^8$ CFU/ml, $2.44 \times 10^8$ CFU/ml, $2.39 \times 10^8$ CFU/ml, $2.97 \times 10^8$ CFU/ml and $74.60 \times 10^8$ CFU/ml, respectively. In the control group, the counts after 36 hrs of incubation were $7466.66 \times 10^8$ CFU/ml. Therefore, the difference in the mean counts of control group and all the treatment groups was statistically significant. The mean counts of *S. aureus* in the control group after 48 hrs of incubation were $902.66 \times 10^8$ CFU/ml. The mean counts of *S. aureus* after 48 hrs in different treatment groups with concentration of *L. plantarum* as $10^8$, $10^7$, $10^6$, $10^5$ and $10^4$/ml were $0.11 \times 10^8$ CFU/ml, $0.14 \times 10^8$ CFU/ml, $0.17 \times 10^8$ CFU/ml, $0.23 \times 10^8$ CFU/ml and $3.87 \times 10^8$ CFU/ml, respectively. Again the difference in the counts of *S. aureus* between all the treatment groups and the control group were statistically significant.

For *E. coli* the mean colony forming units in the control group after 24 hours of incubation were $446.66 \times 10^8$ CFU/ml

**Table 3:** Occurrence of *S. aureus*, *E. coli* and *B. cereus* in various samples.

| Food-borne pathogen | Type of sample | No. of samples collected | No. positive | Per cent occurrence |
|---|---|---|---|---|
| *S. aureus* | Raw Milk | 5 | 2 | 40 |
| | Raw Chicken | 5 | 1 | 20 |
| | Mastitis milk | 5 | 2 | 40 |
| | Sheep faeces | 5 | 1 | 10 |
| Subtotal | | 20 | 6 | 30 |
| *E. coli* | Raw milk | 5 | 2 | 40 |
| | Raw chicken | 5 | 2 | 40 |
| | Mastitis milk | 5 | 0 | 0 |
| | Sheep faeces | 5 | 3 | 60 |
| Subtotal | | 20 | 7 | 35 |
| *B. cereus* | Raw milk | 5 | 2 | 40 |
| | Raw chicken | 5 | 2 | 40 |
| | Mastitis milk | 5 | 0 | 0 |
| | Sheep faeces | 5 | 1 | 20 |
| Subtotal | | 20 | 5 | 25 |
| Grand total | | 60 | 18 | 30 |

and were 0.81 × $10^8$ CFU/ml, 1.09 × $10^8$ CFU/ml, 1.36 × $10^8$ CFU/ml, 1.85 × $10^8$ CFU/ml and 24.62 × $10^8$ CFU/ml, when grown with $10^8$, $10^7$, $10^6$, $10^5$ and $10^4$/ml, of *L. plantarum*, respectively. The counts in the control group were significantly higher compared to all the treatment groups. After 36 hrs of incubation mean colony forming units of the control group was 5720.00 × $10^8$ CFU/ml. The treatment groups with concentration of *L. plantarum* as $10^8$, $10^7$, $10^6$, $10^5$ and $10^4$/ml, was 1.13 × $0^8$ CFU/ml, 1.64 × $10^8$ CFU/ml, 2.20 × $10^8$ CFU/ml, 2.54 × $10^8$ CFU/ml and 24.84 × $10^8$ CFU/ml, respectively. A significant difference was there in the mean counts of *E. coli* between the control and treatment groups at 36 hrs of incubation. The mean colony forming counts of *E. coli* in control group after 48 hrs of incubation was 624.66 × $10^8$ CFU/ml. The counts of *E. coli* in the treatment groups after 48 hrs of incubation were 0.10 × $10^8$ CFU/ml, 0.12 × $10^8$ CFU/ml, 0.14 × $10^8$ CFU/ml, 0.18 × $10^8$ CFU/ml and 2.26 × $10^8$ CFU/ml when grown with $10^8$, $10^7$, $10^6$, $10^5$ and $10^4$/ml of *L. plantarum*, respectively. The difference in the counts of treatment and control was statistically significant.

After 24 hrs of incubation the mean colony forming counts of *B. cereus* in the control group was 83.60 × $10^8$ CFU/ml and in the treatment groups the counts of *B. cereus* were 0.31 × $10^8$ CFU/ml, 0.43 × $10^8$ CFU/ml, 0.60 × $10^8$ CFU/ml, 0.69 × $10^8$/ CFU/ml and 7.12 × $10^8$ CFU/ml, when grown with *L. plantarum* with concentration of $10^8$, $10^7$, $10^6$, $10^5$ and $10^4$/ml, respectively. Statistically, it was seen that the mean colony forming units of control group of *B. cereus* varied significantly with *B. cereus* treated with different concentrations of *L. plantarum.* Similarly, the counts of *B. cereus* were highest in the control group (1330 × $10^8$ CFU/ml) compared to all the treatment groups. The counts of treatment groups with the concentration of *L. plantarum* as $10^8$, $10^7$, $10^6$, $10^5$ and $10^4$/ml were 1.22 × $10^8$ CFU/ml, 1.31 × $10^8$ CFU/ml, 1.60 × $10^8$ CFU/ml, 1.73 × $10^8$ CFU/ml and 6.86 × $10^8$ CFU/ml, respectively. All the counts of treatment groups were statistically lower compared to control groups. The counts of *B. cereus* in the control group after 48 hours of incubation were 1434.66 × $10^8$ CFU/ml. In the treatment groups with different concentration of *L. plantarum viz*. $10^8$, $10^7$, $10^6$, $10^5$ and $10^4$/ml the counts of *B. cereus* were 1.30 × $10^8$ CFU/ml, 1.31 × $10^8$ CFU/ml, 1.46 × $10^8$ CFU/ml, 1.57 × $10^8$ CFU/ml and 7.56 × $10^8$ CFU/ml, respectively. The difference in the counts of *B. cereus* in the control group and all the treatment groups was statistically significant.

Food-borne diseases are of global concern and the World Health Organization estimated that diarrheal diseases are responsible for around 1.9 million child deaths every year (WHO, 2008). Despite improved hygiene and sanitation, there is an increased incidence of food-borne diseases around the globe and more so in developing countries. Many technologies have been employed to combat the food-borne pathogens involving physical and chemical methods, but they are with many disadvantages. These conventional preservative methods may be at the cost of food quality, for example, heat treatments are associated with deterioration of organoleptic properties, extensive use of antimicrobials have led to the development of resistant bacteria and chemical preservatives have a negative effect not only on sensory parameters but also on health as many of them are carcinogenic. Therefore, the consumers prefer organic food which is free from all sorts of chemicals and the addition of chemical preservatives prevents consumers to buy these products. Hence, there is a need for new strategies that fulfill consumer demand and ensure food safety. One such promising approach is the use of a natural antagonist towards pathogenic bacteria to control food-borne diseases as well as bacterial contamination in foods by a process called as biocontrol. The biocontrol methods may tackle the drawbacks of current processing and preservation technologies and is likely to be acceptable to consumers. One such method involves the use of beneficial bacteria to control harmful bacteria, the classical example being Lactic Acid Bacilli (LAB).

The main properties of beneficial/probiotic microorganisms involve equilibrating the endogenous microflora, in protecting the gut from pathogen invasion by competitive exclusion and production of antimicrobial molecules and in stimulating mucosal immunity. Of the various species of LAB, *Lactobacillus plantarum* is one of the most versatile species with valuable use in milk industry and recognized probiotic features (Da Silva Sabo *et al*. 2014; Guidone *et al*. 2014). Concurrently, because of the increasing attention of consumers for healthy and natural food the food industry is prompted towards scientific research to investigate the application of natural compounds for the processing of food products, in order to eliminate or reduce chemical additives used as antimicrobial agents. Thus, in recent decades, several lines of research have tried to find the natural solution to the chemical problem. Among these, the selection of microbial molecules or bacterial strains able to produce such compounds to be used as antimicrobials and preservatives, proved that Lactic Acid Bacteria (LAB) could be suitable candidates for biocontrol (Da Silva Sabo, 2014; Suskovi *et al*, 2010).

The present study was carried out to study *In-vitro* antagonistic effect of *Lactobacillus plantarum* on the growth of some important food-borne pathogens *viz. Staphylococcus aureus, Escherichia coli* and *Bacillus cereus* recovered from foods of animal origin and clinical sources. The isolation and identification of these pathogens were carried out by standard microbiological protocols. The pathogenic isolates recovered from various foods of animal origins were also characterized for virulence properties involving molecular methods. The experiment was also carried out on these pathogens, in order to assess the extent of antibacterial effect of *L. plantarum* on the pathogenic isolates involving disc diffusion assay.

*Staphylococcus aureus* is widely distributed across the globe and is linked to an array of infections in humans and animals. The organism is profoundly known for its pathogenicity and ability to diminish the impact of antimicrobials. The organism causes infections, ranging from mild superficial skin to severe and fatal diseases. In the present study, of the 20 samples comprising of raw milk (5), mastitis milk (5), raw chicken (5) and sheep faecal samples

(5) screened for isolation of *S. aureus,* 6 turned out positive making an overall occurrence of 30%. The results concur with the observations of Philip *et al*. (2006) reporting an occurrence of 31.6% from foods of animal origin. Among all the categories of the samples processed, the highest occurrence (40%) of *S. aureus* was found in mastitis milk samples. Similar results from mastitic milk were reported by Sharma *et al*. (2015) and Awad *et al*. (2017), with 33.7% and 42% occurrence of *S. aureus*, respectively. The highest recovery of *S. aureus* from mastitis milk samples indicates that the pathogen is one of the prime causes of mastitis in bovines in this part of the world. The reports of *S. aureus* being one of the leading causes of mastitis in cattle has been reported by other authors as well (Reshi *et al*. 2015, Wells *et al*. 1998). Of the raw milk samples, 2 (40%) were positive for *S. aureus*. The results are in agreement with the findings of Suelam *et al*. (2012), reporting 30% occurrence of *S. aureus* in raw milk samples.

## CONCLUSION

From the present study it could be concluded that bio-control and bio-preservation strategies can be adopted against food-borne pathogens to cope with the problems related to the chemical preservatives and antibiotics use in animal farming and food processing. Searching and developing novel probiotics could prove better for the treatment of food-borne bacterial pathogens. This antimicrobial/antagonistic ability of probiotics can be efficiently used as antimicrobials as well as antagonist against pathogenic bacteria. This may pave way for the use of beneficial bacteria as biocontrol agents and as preservatives in food industry with immense health benefits.

### Conflict of interest

There is no conflict of interest among the authors.

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