Probiotic Activity of *L. acidophilus* against Major Food-borne Pathogens Isolated from Broiler Carcasses.

Sherein* I. Abd El-Moez[1], Ahmed F.Y.[2], Samy A.A. [1], Aisha R.Ali[3]

1. Department of Microbiology and Immunology - National Research Center Cairo Egypt.
2. Department of Animal Reproduction and A.I. - National Research Center Cairo Egypt.
3. Serology unit -Animal Health Institute Cairo Egypt

*shereinabdelmoez@yahoo.com, yfahmed54@yahoo.com, Ayman_Samy@hotmail.com

Abstract*: C.jejuni, E.coli* and *S. typhimurium* are the principal food borne pathogens in poultry industry. The first experiment tested the effectiveness of different strains of Lactobacillus as *in vitro* as probiotic against *C. jejuni, E.coli* O157 *and S. typhimurium* Result showed that *L.acidophilus* isolated from colostrums of mare and goat showed the widest inhibition zone against *C. jejuni, E.coli* O157 *and S. typhimurium* strains compared to the use of *L.acidophilus* isolated from goat and cattle milk. The second experiment evaluate the efficiency of *L. acidophilus* isolated from mare colostrums showing highest *in vitro* inhibition activity against tested strains as *in vivo* probiotic against *C. jejuni* isolated from broiler carcasses. The result showed great inhibition of *C. jejuni, E.coli* O157 *and S. typhimurium* strains by the use of *L.acidophilus* in comparing to the use of antibiotics. In the second experiment; four groups of adult albino rats were used; group (1) control negative, group (2) rats orally administrated by *L. acidophilus* only from the start of experiment till the 14th day, group (3) rats challenged only with *C. jejuni* and group (4) orally administrated by *L. acidophilus* from the start of experiment till the 14th day at the 7th day they were challenged with *C. jejuni*. Result showed that the third group showed the highest rate of reisolation of *C.jejuni* (0.80±0.16 from fecal swabs and 0.84±0.17 from the internal organs) as well as major pathological lesions in the tested organs in the form of granulomatus reaction in the lung tissue infiltration of inflammatory cells in the lung tissue thickening of wall of blood vessels with alveolar emphysema. Congestion hemorrhages of renal blood capillaries and coagulative necrosis of the renal tissue as well as degeneration and necrosis of hepatocytes with proliferation of fibrous tissue. The forth group pretreated with L. acidophilus Showed lower rate of isolation of *C.jejuni* (0.08±0.02 from fecal swabs and 0.04±0.01 from internal organs. The pathological findings of the internal organs showed minor lesions in the form of interstitial pneumonia and inflammatory cellular infiltration in the lung Swelling and degeneration of renal epithelium and hepatocytic degeneration with infiltration of inflammatory cells. The second group which was only treated with *L.acidophilus* showed no reisolation of *C.jejuni* as well as no pathological lesions were detected except a minor lesion in the liver in the form of diffused vacuolar degeneration in hepatocytes. Results develop a safe method for competing food borne pathogens in edible animals and suggest the need for probiotics to hinder the spread of highly pathogenic zoonotic bacteria transmitted by animal food by products. [Nature and Science 2010; 8(3):69-78]. (ISSN: 1545-0740).

Key words: *C. jejuni;* L. *acidophilus;* probiotics; *in vivo,* antibiotic sensitivity, rat.

1. Introduction

Foodborne diseases (FBD) represent an important public health problem that significantly affects people's health and withdraw serious socio-economic implications (Younus et al, 2007) .The principal food borne pathogens are (*C. jejuni, Cl. perfringens E. coli O157:H7 L. monocytogenes* Salmonella spp *S. aureus and T. gondii*) (Oliver et al, 2009) Our study will take into account C. jejuni as one of the major pathogenic bacteria in poultry industry. It is recognized that FBD produce economic losses in developing as well as developed countries (Lengsfeld et al, 2007). The problem of FBD and its associated costs is multi factorial and its prevention and control requires a multidisciplinary (Sivapalasingam et al, 2004). Campylobacter is the most common cause of human intestinal infectious disease in many countries (Lengsfeld et al, 2007). Early studies established that this disease was primarily due to thermotolerant *C. jejuni* and *C. coli* are one of the major causes of bacterial food borne enteric infection. The reported incidence of campylobacteriosis continued to rise. Campylobacter is ubiquitous in the environment and can be recovered from the faeces of most domestic and wild animals (Lengsfeld et al, 2007).

Preventing the emergence and spread of

antimicrobial resistant food borne pathogens requires avoiding misuse and overuse of antibiotics thus there is a need to develop a safe method for competing food borne pathogens as well as to raise the immune response of the animals to compete infections. Nowadays the use of probiotics should be a main target to hinder the spread of food borne pathogens and to raise the immune response of the animals which were proved to have different beneficial aspects as it Adhere to surfaces of the host mucous membrane. Release endogenous microbicides (lactic acid bacitracin and hydrogen peroxide) competitive with pathogens (deplete nutrients) and modulate the host immune response (Musa et al, 2009).

Our study aim to develop a safe method for raising the animal immune response to be capable to compete pathogenic infection and in turn to get safe animal by products for human consumption and as well to restrict over misuse of antibiotics.

2. Materials and Methods

Isolation and enumeration of intestinal microflora

Thirty broiler carcasses were aseptically collected immediately after defeathering from a commercial processing markets carcasses were. Carcasses were placed in sample bags in an ice box containing crushed ice and transported to the lab within 30 min. Carcasses tested samples were diluted with saline solution (1:10) and mixed using Stomacher for 1–2 min. After dilution 100 µl of each sample was plated onto the following media: MacConkey agar no 3 (0xoid) for *E. coli* Selenite -F- broth (Oxoid) incubated at 37°C for 16-18 hrs then streaked onto Salmonella-Shigella agar (Oxoid) plates for isolation of Salmonella; plates were incubated at 37°C for 24–48 hr Charcoal agar media (Oxoid) for Campylobacter isolation and kept at 37°C for 24–48 hrs at microaerophilic condition another plate was incubated at 42°C for 48 hrs for the isolation of *C.jejuni* (Quinn et al, 2002) Individual colonies from inoculated plates were picked and separately inoculated onto slope agar for further investigations.

Identification of the isolates was carried out by Gram's Method (Cruickshank et al, 1975) and biochemical tests (Quinn et al, 2002).

Cultivation of the collected samples:

Goat milk samples as well as colostrums were collected under possible aseptic conditions in a sterile cork screw tubes then were streaked onto De Man-Rogosa-Sharpe agar (Oxoid) M.R.S. agar plates using layer plate method for anaerobic incubation (Oxoid Manual, 1982). Plates were incubated at 37°C for 48-72 hrs (Collins and Lyne, 1976).

Isolation and purification of *Lactobacillus* species:

The suspected colonies from M.R.S. plates were picked and separately inoculated into tubes each containing 5 ml M.R.S. broth .The tubes were incubated at 37ºC for 24 hrs under 5% CO_2 tension. After incubation each broth sample was examined microscopically culturally according to (Konman et al 1983, Sneath et al, 1986) and biochemically identified (Quinn et al, 2002) using Oxidase test Catalase production test Indole test Nitrate reduction test Triple sugar iron agar medium used for H_2S production test Sugar fermentation test using the following sugars fructose melibiose mannose cellibiose mannitol sucrose and lactose Gelatin liquefaction test Arginine hydrolysis test production of gas (CO_2) from glucose production of ammonia from arginine fermentation of ribose from gluconate ability of growth at 15ºC and 45ºC.

***In vitro* use of *Lactobacillus* as probiotic; Well diffusion assay (Sgouras et al, 2004):**

Mueller Hinton agar plates divided into three groups; group (A) inoculated with *C.jejuni* group (B) inoculated with *E.coli* O157 and group (C) inoculated with *S. typhimurium* and wells were drilled out using pasture pipettes 50 µl aliquots of cell free cultures supernatant in fresh M.R.S. broth of *L.acidophilus* isolated culture was suspended in the agar wells. Plates were incubated for 48 to 72 hrs under microaerophillic conditions at 37°C inhibition zones around wells showed positive.

***In vivo* use of *Lactobacillus* as probiotics (Strompfova et al, 2005):**

L. acidophilus strain showing positive zone of inhibition by *in vitro* sensitivity test was selected for evaluating its probiotics activity in rats to evaluate its *in vivo* probiotics activity against *C. jejuni* by reisolation of *C. jejuni* from fecal swabs at different intervals after inoculation and reisolation from the different organs after scarification of the animals.

Preparation of *L. acidophilus* culture (Strompfova et al, 2005):

L. acidophillus (isolated from goat colostrums) was inoculated into MRS broth (Oxoid) and incubated at 37°C for 24 hrs then the bacterial cells were harvested by centrifugation at 2000 g for 10 min at 4°C and the bacterial pellet was resuspended in a saline solution (0.85% pH 7.0) to obtain the concentration 10^8cfu/ml. The culture was stored at 4°C before application.

Experimental animals

Twenty non pregnant adult albino rats weighing about 300- 400 grams were used for experimental studies. They were divided into four groups; 5 animals each. Group (1) control negative given saline solution (0.85% pH 7.0) group(2) was orally administered *L. acidophilus* strain (0.5 ml per day; 1.5 x 10^8 cfu/ml of saline solution) with a syringe for 14 days group (3) was challenged with *C. jejuni* at the day 7 with dose (1ml; $5x10^9$ of viable organism / ml) as one oral dose. Group (4) was orally administered *L. acidophilus* strain (0.5 ml per day; 1.5 x 10^8 cfu/ml of saline solution) with a

syringe for 14 days and at the day 7 they were orally administered with *C. jejuni* (1 ml; $5x10^9$ of viable organism / ml) as one oral dose. All groups of animals were fed the commercial diet and had access to feed and water. All animals were examined for the reisolation of *C.jejuni* from faecal samples collected from each rat on days 9 12 15 and 18 and at the end of experiment (day 21). All animals were scarified at the end and their internal organs were tested for the reisolation of *C.jejuni* and the internal organs were pathologically examined for detection the severity of lesions in challenged group with *C.jejuni* only in comparing with findings in group pretreated with *L.acidophilus* before challenging with *C.jejuni*.

Statistical analysis: Statistical analysis was carried out using "Student t" test and Analysis of Variance as outlined by (Snedecor and Cochran, 1980).

3. Results

On screening for the most common pathogenic bacteria isolated from poultry meat to human thirty poultry meat samples were collected form different markets in Egypt for screening of Campylobacter Salmonella as well as *E.coli*. Biochemical analyses and serotyping were carried out for identification of *C.jejuni S. typhimurium* and *E.coli* O157. As shown in **Table (1)** the rate of isolation of tested bacteria from poultry carcasses varied greatly; the most predominant isolates were Campylobacter spp. which was isolated with an incidence of 0.70±0.13 from which *C. jejuni* predominate which was isolated with an incidence of 0.63±0.12 followed by *E.coli* which was isolated with an incidence of 0.57±0.10 among which *E.coli* O157 showed an incidence of 0.67±0.01 then Salmonella spp. (0.33±0.06) among which *S. typhimurium* was isolated with a rate of 0.13±0.02.

Table (2) and figures (1 2 and 3) showed that the most effective antibiotics that the different tested stains showed high sensitivity against them were in order as follows; ofloxacin 5 µg/ml with an incidence (100.00 %) followed by tobramycin 10 µg/ml (97.37%) then garamycin 10 µg/ml (65.79%) then amoxycillin/ clavulinic acid 30 µg/ml (39.47%) then Tetracycline 30 µg/ml (23.68%) and finally Erythromycin 15 µg/ml (15.79%). **Figure (4 5 and 6)** showed that all tested strains showed great sensitivity toward *L.acidophilus* isolated from mare and goat colostrums followed by different milk samples from goat and cows by testing using agar well diffusion test.

As shown in **Table (3)** the incidence of diarrhea reaches 0.00% in group (1) which was used as control negative and group (2) treated with *L. acidophilus* only. In group (3) 80.00% of the tested rats showed severe diarrhea with reisolation of *C.jejuni* from diarrhatic cases in this group. Mortality rate was 0.00% in all groups while it reaches 20.00% in group (3). The morbidity rates due to *C. jejuni* in group (4) previously treated with *L.acidophilus* before challenging with *C.jejuni* showed diarrhea with an incidence of 20.00% associated with the reisolation of *C.jejuni* from the fecal sample of diarrhatic rat.

Results in **Table (4)** revealed that the rate of isolation of *L. acidoplilus* and *C.jejuni* from feacal sample reaches 0.00±0.00 in group (1) 0.80±0.16 and0.00±0.00 in group (2) 0.72±0.14 and 0.08±0.02 in group (3) and finally 0.00±0.00 and 0.80±0.16 in group (4) respectively. While the rate of isolation from internal organs of rats after death or scarification was 0.00±0.00 in group (1) 0.20±0.04and 0.00±0.00 in group(2) 0.20±0.04and 0.04±0.01in group (3) and finally 0.00±0.00 and 0.84±0.17 in group (4) respectively

Lesions occurred in the internal organs of dead or sacrificed rats infected with *C.jejuni* alone or after administration of *L.acidophilus* showed great difference in the rate of isolation of *C.jejuni* as well as in the pathological finding. Group 4 which was challenged with *C. jejuni* only and not given probiotics showed granulomatus reaction in the lung tissue infiltration of inflammatory cells in the lung tissue thickening of wall of blood vessels with alveolar emphysema **(fig.7)** Kidney tissue showed congestion and hemorrhages of renal blood capillaries **(fig.10)** and coagulative necrosis of the renal tissue **(fig.11)** degeneration and necrosis of hepatocytes and proliferation of fibrous tissue in the liver tissue was shown **(fig.14)**. Macroscopically the livers showed hepatomegaly and sever congestion kidney showed large abscesses while the lungs showed sever pneumonia. On the contrary group 4 which was pretreated with *L.acidophilus* before being challenged with *C.jejuni* lesions were less severe in comparing with group 3; lesions were in the form of interstitial pneumonia and inflammatory cellular infiltration in the lung **(fig.8)** swelling and degeneration of renal epithelium in the kidney **(fig.12)** and hepatocytic degeneration with infiltration of inflammatory cells in the liver **(fig.15)**. While group 2 given only *L.acidophilus* showed that lung **(fig.9)** and kidney **(fig.13)** tissues appears within normal limits while liver **(fig.16)** showed diffused **v**acuolar degeneration in hepatocytes.

4. Discussion

Thirty poultry carcasses were collected form poultry markets in Egypt for screening of for Campylobacter and Salmonella as well as *E.coli*. Serotyping was carried out for identification of *S. typhimurium* and *E.coli* O157. As shown in **Table (1)** the rate of isolation of tested bacteria from poultry carcaces varied greatly; the most predominant isolates were Campylobacter spp. which was isolated with an incidence of 0.70±0.13 from which *C. jejuni*

predominate which was isolated with an incidence of 0.63±0.12. The following predominating bacteria was *E.coli* which was isolated with an incidence of 0.57±0.10 among which *E.coli* O157 showed an incidence of 0.67±0.01 while the rate of isolation of Salmonella spp. was 0.33±0.06 among which *S. typhimurium* was isolated with a rate of showed rate of 0.13±0.02. These results agree with (Schlundt et al, 2004) who described five of the most important emerging food-borne zoonotic pathogens: *Salmonella* spp. *Campylobacter* spp. enterohaemorrhagic *E. coli Toxoplasma gondii* and *Cryptosporidium parvum.* Also (Chavoerach et al, 2004) proved contamination of chicken meat with *C. jejuni* causes human enteritis.

Figure 1, 2 and 3 showed that all tested strains showed great sensitivity toward *L.acidophilus* isolated from goat colostrum by testing using agar well diffusion test. Results match with the findings of Reid and Burton (2002) who reported that *Lactobacillus* spp. isolated from the genital tract have probiotic activities which contribute to health restoration and maintenance. Also results agree with Abd El-Moez et al, 2008 who showed high activity of *in vitro* use of *L. acidophilus* as probiotic against *E.coli* Bacillus *C.diversus E. feacalis* and *Y. enterocolitica* followed by *L. casei rhamnosus* and De Vuyst and Leroy (2007) who proved that lactic acid bacteria display numerous antimicrobial activities and the antimicrobial production by probiotic LAB plays a role during *in vivo* interactions occurring in gastrointestinal tract hence contributing to gut health. (Gupta et al, 1996) Observed that *L. acidophilus* strains showed inhibitory activity towards *S. typhi S. aureus E. coli P. vulgaris* and *Y. enterocolitica*. Our results disagree with that of (Koga et al, 1998) who reported that none of the *Lactobacillus* spp. was able to inhibit the growth of *S. enteritidis S. typhimurium E. coli* and *S. aureus*. Probiotics have shown to protect against variety of pathogens as *E. coli* (Chateau et al, 1993) and Salmonella as well as Campylobacter (Stern et al, 2001).

Table (2) revealed the incidence of diarrhea reaches 0.00% in group (1) which was used as control negative and group (2) treated with *L. acidophilus* only. While in group (3) 80.00% of the tested rats showed severe diarrhea with reisolation of *C.jejuni* from diarrhatic cases in this group. Mortality rate was 0.00% in all groups while it reaches 20.00% in group (3).On the other hand the morbidity rates due to *C. jejuni* among tested groups of rat revealed that in group (4) which was previously treated with *L.acidophilus* before challenging with *C.jejuni* one animal showed diarrhea with an incidence of 20.00% associated with the reisolation of *C.jejuni* from the fecal sample of diarrhatic rat. Results agree with (Paulius et al, 2006) who proved that the use of probiotic reduced morbidity and mortality of growing rabbits during fattening period. Our study agree with (Corr et al, 2007) who found that probiotics can significantly protect mice against infection with the invasive food borne pathogens as and protected pigs against diarrhea. Also match with Ogawa et al (2007), Casey et al (2007) who proved that probiotics were reduced the severity and duration of diarrhea in rabbit infected with *E.coli* O157. Also results agree with (Chavoerach et al 2004) who proved that *C. jejuni* should be controlled at the farm level by using orally given probiotics to prevent colonization of chicken with campylobacter.

Results in **Table (4)** revealed that the rate of isolation of *L. acidoplilus* and *C.jejuni* from feacal sample reaches 0.00±0.00 in group (1) 0.80±0.16 and0.00±0.00 in group (2) 0.00±0.00 and 0.80±0.16 in group (3) and finally 0.72±0.14 and 0.08±0.02 in group (4) respectively. While the rate of isolation from internal organs of rats after death or scarification was 0.00±0.00 in group(1) 0.20±0.04 and 0.00±0.00 in group(2) 0.00±0.00 and 0.84±0.17 in group (3) and finally 0.20±0.04 and 0.04±0.01in group (4) respectively. These findings agree with (Shu et al, 2000) who proved that probiotics reduce pathogen translocation to visceral tissues. Shah (2000) Mentioned that a number of health benefits have been claimed for probiotic bacteria such as *L. acidophilus Bifidobacterium spp.* and *L. casei*. As well as the findings of (Chateau et al 1993, Corr et al, 2007) who found that probiotics have shown to protect against variety of pathogens as *E. coli* Salmonella and Campylobacter respectively. Also results agree with (Murry et al, 2006) suggested that diets supplemented with the botanical probiotic containing *Lactobacillus* supports growth for broilers similar to the basal diet supplemented with antibiotic and coccidiostat and with lower feed to gain ratio. Also the botanical probiotic may reduce *C.perfringens* and *C. jejuni* in market-age broilers.as well as (Casey et al, 2007) characterized lactobacillus for its antimicrobial activity against *Clostridium difficile* enteropathogenic *Escherichia coli* (EPEC) verocytotoxigenic *E. coli* (VTEC) and *C. jejuni*. They added that lactobacilli displayed variations in their antimicrobial activity with few strains showing inhibitory activity against all pathogens. Also findings agree with (Shu et al, 2000) who found that supplementing lambs infected with *E. coli* O157:H7 with a mixture of probiotics including *L. acidophilus* in the diet can reduce total number of *E. coli* O157:H7 shed in the feces. Also Shah, 2000 selected Lactic acid bacteria as a competitive exclusion product that would inhibit *E.coli* O157:H7 in the intestinal tract of live cattle. Results agree with Murry et al, 2006 found that *L. acidophilus* and *L.plantarum* were have inhibitory properties against *E.coli S. aureus S. agalactiae S. uberis S. Enteritidis* and *B. pumilus*. Also results agree with Likotrafit et al, 2004 who proved the capability *of Lactobacillus* against *E.coli* and observed its ability to

decrease viability of *E.coli*. As well (Lema et al, 2001) found that supplementation of cattle with *L. acidophilus* reduce the prevalence and magnitude of fecal *E. coli* O157.

Lesions occurred in the internal organs of dead or sacrificed rats infected with *C.jejuni* alone or after administration of *L.acidophilus* showed great difference in the rate of isolation of *C.jejuni* as well as in the pathological finding. Group 4 which was challenged with *C. jejuni* only and not given probiotics showed granulomatus reaction in the lung tissue infiltration of inflammatory cells in the lung tissue thickening of wall of blood vessels with alveolar emphysema **(fig.7)** Kidney tissue showed congestion and hemorrhages of renal blood capillaries **(fig.10)** and coagulative necrosis of the renal tissue **(fig.11)** degeneration and necrosis of hepatocytes and proliferation of fibrous tissue in the liver tissue was shown **(fig.14)**. Macroscopically the livers showed hepatomegaly and sever congestion kidney showed large abscesses while the lungs showed sever pneumonia. These results agree with Brashears et al, 2003 who described the histopathological changes occurred in the internal organs of experimentally infected guinea pigs with *Campylobacters* revealing that the majority of hepatic cells showed degenerative changes mainly cloudy swelling and hydropic degeneration as well as focal coagulative necrosis of hepatocytes. Hepatic sinusoids contain fatty changes with intensive lymphocytic aggregation seen inside the hepatic sinusoids and blood vessels. This picture was previously described by Marsalkova et al, 2004 who inoculated ducks with *C.jejuni* and resulted in swollen hepatic cells with partial occlusion of hepatic sinusoids some of them contain vacuoles of sharp borders (fatty changes) Intensive leukocyte aggregation mainly lymphocytes in hepatic sinusoids. Hepatic cells were seen with cloudy swelling. Brashears et al, 2003 illustrates that the lung tissues showed interstitial mononuclear cells infiltration that revealed foccal areas of interstitial pneumonia associated with thickening of interalveolar septa and diffuse interstitial haemorrhage lung tissues showed perivascular mononuclear cell infiltration and perialveolar blood capillaries that were engorged with blood. Also renal tubules revealed mononuclear cellular infiltration with destruction of epithelial lining of renal tubules; some renal tubules showed necrobiotic changes and cast formation. Moreover degenerative changes of epithelial lining of tubules. Severe congestion of blood vessels and blood capillaries with haemorrhages degenerative changes in renal tubules showing mononuclear cellular infiltration with destruction of epithelial lining. On the contrary group 4 which was pretreated with *L.acidophilus* before being challenged with *C.jejuni* lesions were less severe in comparing with group 3; lesions were in the form of interstitial pneumonia and inflammatory cellular infiltration in the lung **(Fig.8)** swelling and degeneration of renal epithelium in the kidney **(fig.12)** and hepatocytic degeneration with infiltration of inflammatory cells in the liver **(fig.15)**. While group 2 given only *L.acidophilus* showed that lung **(fig.9)** and kidney **(fig.13)** tissues appears within normal limits while liver **(fig.16)** showed diffused vacuolar degeneration in hepatocytes. Our study agrees with Corr et al, 2007 who found that probiotics can significantly protect mice against infection with the invasive food borne pathogens as *L. monocytogenes* and *S. typhimurium*. Also results match with the findings of Atassi et al, 2006 challenged mice with *E.coli* O115 and O119 after being fed on 10% skim milk containing *L. acidophilus* ($1.5x10^{8}$ CFU) for 1 week they found 100% survival rate whereas controlled unprotected mice showed 53.3% and 73.3% survival rate respectively. These results confirmed the probiotic effect of *L. acidophilus* against colonization of *E. coli* in the animal tissues as well as enhancing their immune response.

Table (1) Rate of isolation of pathogenic bacteria from poultry carcasses.

Isolated strains	**Positive no**	**Positive %**	**Mean ±SE**
Campylobacter spp.	2	6.67	0.06±0.01
C. jejuni	19	63.30	0.63±0.12
Total Campylobacter spp.	**21**	**70.00**	**0.70±0.13**
E.coli	15	50.00	0.50±0.09
E.coli O157	2	6.70	0.67±0.01
Total *E.coli*	**17**	**56.70**	**0.57±0.10**
Salmonella spp.	6	20.00	0.20±0.04
S. typhimurium	4	13.30	0.13±0.02
Total Salmonella spp.	**10**	**33.30**	**0.33±0.06**
Total bacteria isolated	**48**	**160.00**	**1.60±0.29**

Total number of Samples=30

Table (2): Antibiotic agar diffusion test against pathogenic strains isolated from poultry carcasses.

Isolates / Antibiotic	Campylobacter (2)		*C. jejuni* (9)		*E.coli* (15)		*E.coli* O157 (2)		Salmonella (6)		*S. Typhimurium* (4)		Total (38)	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Amoxicillin /Clavulinic acid 30 µg/ml	1	50.00	5	55.6	4	26.67	1	50.00	2	20.00	2	50.00	15	39.47
Erythromycin 15 µg/ml	1	50.00	2	22.22	2	13.33	0	0.00	1	16.67	0	0.00	6	15.79
Garamycin 10 µg/ml	2	100.00	9	100.00	9	60.00	1	50.00	3	50.00	1	25.00	25	65.79
Ofloxacin 5 µg/ml	2	100.00	9	100.00	15	100.00	2	100.00	6	100.00	4	100.00	38	100.00
Tetracycline 30 µg/ml	1	50.00	4	44.44	2	13.33	0	0.00	1	16.67	1	25.00	9	23.68
Tobramycin 10 µg/ml	2	100.00	9	100.00	15	100.00	2	100.00	6	100.00	3	75.00	37	97.37

No between brackets showed total no of tested isolates
No and % illustrate the sensitive strain toward different antibiotics

Table (3): Incidence of Diarrhea among different groups of rats

	Morbidity rate			Mortality rate		
Rats Groups	**No**	**%**	**Mean ± SE**	**No**	**%**	**Mean ± SE**
Group 1	0/5	0.00	0.0±0.00	0/5	0.00	0.0±0.00
Group 2	0/5	0.00	0.0±0.00	0/5	0.00	0.0±0.00
Group 3	4/5	80.00	0.8±0.36	1/5	20.00	0.2 ±0.09
Group 4	1/5	20.00	0.2 ±0.09	0/5	0.00	0.0±0.00

Group 1 control negative
Group 2 *L.acidophilus* alone from day 1 to day 14 daily oral doses
Group 3 *C.jejuni* alone at day 7 one oral dose
Group 4 *L. acidophilus* followed by *C.jejuni* at day 7 one oral dose

Table (4) Reisolation of *L.acidophilus* and *C.jejuni* from feacal swabs at different intervals and from internal organs after scarification.

Tested Isolates	Fecal sample (day of sampling)					Mean ± SE	Internal organs (Site)					Mean ± SE
	Day 9	Day 12	Day 15	Day 18	Day 21		Liver	Kidney	Spleen	Lung	Intestine	
L.acidoplilus												
Group 1	0/5	0/5	0/5	0/5	0/5	**0.00±0.00**	0/5	0/5	0/5	0/5	0/5	**0.00±0.00**
Group 2	1/5	4/5	5/5	5/5	5/5	**0.80±0.16**	0/5	0/5	0/5	0/5	5/5	**0.20±0.04**
Group 3	0/5	0/5	0/5	0/5	0/5	**0.00±0.00**	0/5	0/5	0/5	0/5	0/5	**0.00±0.00**
Group 4	1/5	4/5	4/5	4/5	5/5	**0.72±0.14**	0/5	0/5	0/5	0/5	5/5	**0.20±0.04**
C.jejuni												
Group 1	0/5	0/5	0/5	0/5	0/5	**0.00±0.00**	0/5	0/5	0/5	0/5	0/5	**0.00±0.00**
Group 2	0/5	0/5	0/5	0/5	0/5	**0.00±0.00**	0/5	0/5	0/5	0/5	0/5	**0.00±0.00**
Group 3	2/5	4/5	5/5	5/5	4/4	**0.80±0.16**	5/5	5/5	2/5	4/5	5/5	**0.84±0.17**
Group 4	0/5	0/5	0/5	1/5	1/5	**0.08±0.02**	0/5	0/5	0/5	0/5	1/5	**0.04±0.01**

NB: Total no of each group is 5 *One animal in group 3 was found dead on the day 19 of the experiment

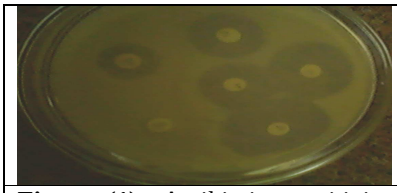
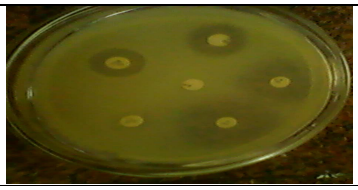
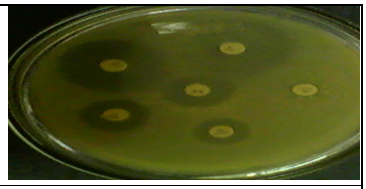
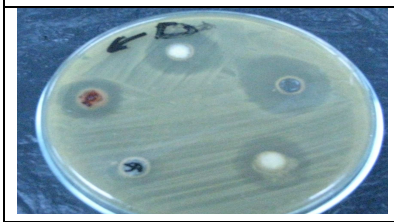
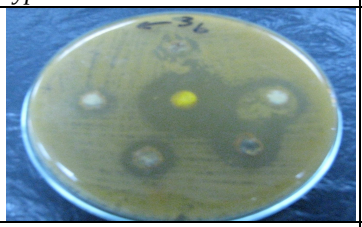
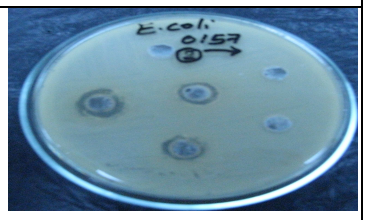
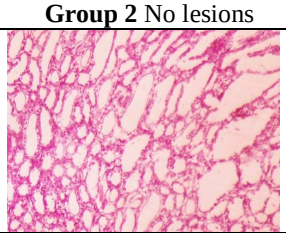
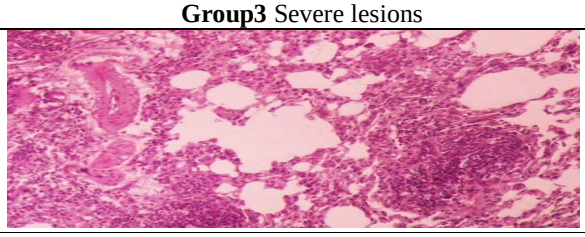
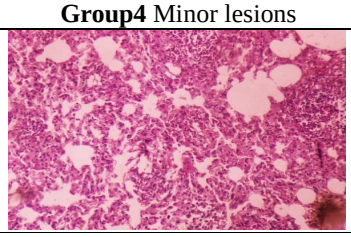
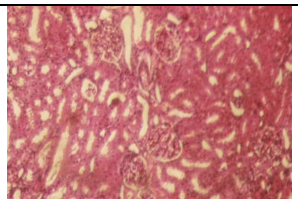
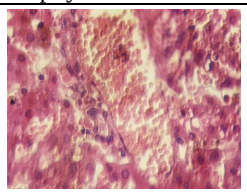
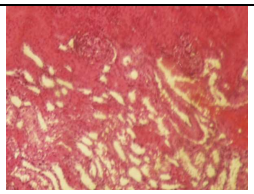
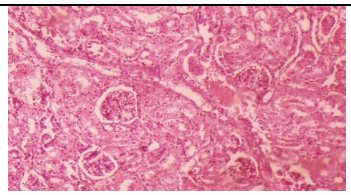
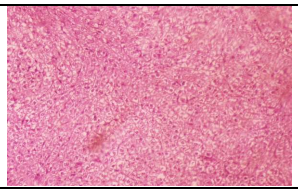
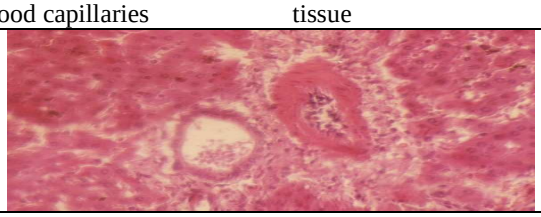
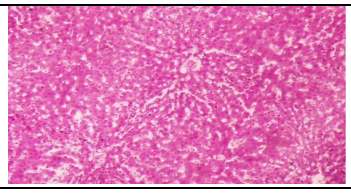
		

Figure (1): Antibiotic sensitivity test against *C.jejuni*	**Figure (2):** Antibiotic sensitivity test against *S. typhimurium*	**Figure (3):** Antibiotic sensitivity test against *E.coli* O157
		
Figure(4): Probiotic activity of *L.acidophilus* isolated from milk of different species against *C.jejuni*	**Figure(5):** Probiotic activity of *L.acidophilus* isolated from milk of different species against *S. typhimurium*	**Figure (6):** Probiotic activity of *L.acidophilus* isolated from milk of different species against *E.coli* O157

The widest zone of inhibition shown by *L. acidophilus* isolated from mare then from goat colostrums as shown in plates. The widest zone of inhibition was shown toward *C. jejuni* against *L.acidophilus* isolated from colostrums of mare.

Histopathological changes in the three treated groups

	Group 2 No lesions	**Group3** Severe lesions	**Group4** Minor lesions
Lung			
	Figure (9): lung tissue within normal limits	**Figure (7):** Granulomatus reaction in the lung tissue infiltration of inflammatory cells in the lung tissue thickening of wall of blood vessels with alveolar emphysema	**Figure (8):** Interstitial pneumonia and inflammatory cellular infiltration
Kidney		 	
	Figure (13): kidney tissue within normal limit	**Figure (10):** Congestion and hemorrhages of renal blood capillaries **Figure (11):** Coagulative necrosis of the renal tissue	**Figure (12):** Swelling and degeneration of renal epithilium
Liver			
	Figure (16): Diffused vacuolar degeneration in hepatocytes	**Figure (14):** Degeneration and necrosis of hepatocytes and proliferation of fibrous tissue	**Figure (15):** Hepatocytic degeneration with infiltration of inflammatory cells

5. Conclusions

Overall the present results indicated the ability *L.acidophilus* to survive and to colonize the digestive tract of rats during its application to increase lactic acid bacteria population and to decrease the population of *C.jejuni* in faeces and internal organs and decrease its pathogenic effect on different body organs. Moreover the applied strain did not induce any stress in group taking *L.acidophilus* only and as well no pathological lesions were found in internal organs of this group except in the liver which show diffused vacuolar degeneration in hepatocytes. Therefore *L.acidophilus* may have the potential to enhance intestinal health in Lab. animals after their applications as well as it help in their ability to overcome *C.jejuni* infection.

6. Recommendations

Researchers suggest the need of probiotic as safe method for competing food borne pathogens in poultry products to hinder the spread of food borne pathogens instead of using antibiotics which leave residues in poultry carcasses causing human consuming the animal by products to take small doses of different antibiotics and in turn develop acquired multiple drug resistance bacterial flora toward different antibiotics.

7. References

1-Younus M, Hartwick E, Siddiqi A A, Wilkins M, Davies DH, Rahbar M, Funk J and Saeed M (2007): The role of neighborhood level socioeconomic characteristics in Salmonella infections in Michigan (1997–2007): Assessment using geographic information system. Intern. J. of Health Geographics **6**:56

2-Oliver S P, Patel D A, Callaway TR and Torrence M E (**2009):** ASAS Centennial Paper: Developments and future outlook for preharvest food safety J. Anim Sci. **87**:419-437.

3-Lengsfeld C, Faller G and Hensel A (2007): Okra polysaccharides inhibit adhesion of Campylobacter jejuni to mucosa isolated from poultry in vitro but not in vivo Animal Feed Science and Technology. **135**: 113-125.

4- **Sivapalasingam S**, **Friedman CR**, **Cohen L** and **Tauxe RV (2004):** Fresh produce: a growing cause of outbreaks of foodborne illness in the United States 1973 through 1997. J Food Prot.; **10**: 2342-53.

5-Musa HH, Wu SL, Zhu CH, Seri HI and Zhu GQ (2009): The Potential Benefits of Probiotics in Animal Production and Health. J. of Animal and Veterinary Advances. **8**:313-321.

6- Quinn P J, Markey B K, Carter M E, Donnelly W J C and Leonard F C (2002): Veterinary Microbiology and Microbial Diseases. Black well Scientific Publications Oxford London.

7- Cruickshank R, Dugid TP, Marmion BP and Swain RH (1975): Med. Micro. The Practice of Medical Microbiology. 12th Ed. vol. 11 Edinburgh London and New York.

8- Oxoid Manual (1982): The Oxoid Manual Culture Media and Other Laboratory Service 5th edition Oxoid Limited Hampshier R G 240 PW England.

9- Collins CH and Lyne PM (1976): Microbiological Methods. 4th ed. Butterworths London U.K.

10- Konman EW, Allen SD, Dowell VR and Sommers HM (1983): Colour Atlas and Text Book of Diagnostic Microbiology 2nd edition. Lippincott Co. N. Y. London.

11- Sneath PH, Mair NS, Sharpe ME and Holt JG (1986): Bergey's Manual of Systemic Bacteriology9th ed the Williams and Wilkins Company. Baltimore UK.

12- Sgouras D, Maragkoudakis P, Petraki K, Martinez-Gonzalez B, Eriotou E, Michopoulos S, Kalantzopoulos G, Tsakalidou E and Mentis A (2004): *In vitro* and *in vivo* inhibition of *Helicobacter pylori* by *Lactobacillus casei* strain *Shirota*. Appl. Environ. Microbiol. **70**: 518-526.

13- Strompfova V; Marcinakova M, Gancarcikova S, Jonecova Z, Scirankova L, Guba P, Koscova J, Boldizarova K, Laukova A (2005): New probiotic strain Lactobacillus fermentum AD1 and its effect in Japanese quail. Vet. Med. – Czech; **50:** 415–420.

14- Snedecor GR and Cochran RG (1980): Statistical methods. 7th ed. Iwoa State Univ. Press USA.

11…15- Schlundt J, Toyofuku H, Jansen J and Herbst SA(2004): Emerging food-borne zoonoses. Rev Sci Tech. ; **23:** 513-533.

16- Chavoerach P, Keuzernkamp DA, Lipman LJ and Vant Knapen F (2004): Effect of organic acid in drinking water for young broilers on Campylobacter infection volatile fatty acid production gut microflora and histological cell changes. Poult.Sci. **83:**330-334.

17- Reid G and Burton J (2002): Use of *Lactobacillus* to prevent infection by pathogenic bacteria. Microbes and infection **4**: 319-324.

18- Abd El-Moez SI, Ahmed WM, El-Jakee JA and El- Seedy FR (2008): Isolation of Lactobacillus from the vagina of buffalo-cows and its in vitro probiotic activity. Global Veterenaria; **2**: 15-21.

19- De Vuyst L and Leroy F (2007): Bacteriocins from lactic acid bacteria: production purification and food applications. J Mol Microbiol Biotechnol.; **4:194**-199.

20- Gupta PK, **Mital BK and Garg SK (1996):** Antagonistic activity of *Lactobacillus acidophilus* fermented milk against different pathogenic bacteria. Indian J. Exp. Biol. **34**: 1245-1247.

21- Koga T, Mizobe T and Takumi K (1998): Antibacterial activity of *Lactobacillus* species against *Vibrio* species. Microbiol. Res. **153**: 271-275.

22- Chateau N, Castellanous I and deschamps AM (1993): Distribution of pathogen inhibition in Lactobacillus isolates of a commercial probiotics consortium. J.Applied Bacteriol.**74:** 36-40.

23- Stern N J, Cox N A, Baily J S, Berrang M E and Muegrove M T (2001): Comparison of mucosal

competitive exclusion and competitive exclsion treatment to reduce Salmonella and Campylobacter sp colonization in broiler chickens. Poult. Sci. **80:**156-160.
24- Paulius M, LinaA, AnsZ, AndrzejG, Manfred OL and Areta H (2006): Effect of probiotics BIOPLUS 2B on performance of growing rabbit. Arija Zooyechnika T. **36:**54-59.
25- Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C and Garhan CGM (2007): Bacteriocin production as mechanism for anti-infective activity of Lactobacillus salivarius UCC118. PNAS. **104:**7617-7621
26- Ogawa M, Shimizu K, Nomoto K, Takahasi M; Watanuki M, Tanaka R, Hamabata T, Yamasaki S. and Takeda Y (2001): Protective effect of Lactobacillus casei strain shirota on shiga toxin-producing E. coli O157:H7 infection in infant rabbits. Infect. Immun. **69:**1101-1108.
27- Casey PG, Gardiner E, Casey, Bradshow, Lawlor G, Lynch PB, Leonard C, Stanton, Rose P, itzgerald F and Hill C (2007): A 5-Strain probiotics combination reduces pathogenic shedding and alters disease signs inpigs challenged with *S. enterica serovar Typhimurium.* Applied Envirn. Microb..**73:**185-186.
28- Shu Q, Lin H, Rutherfurd KJ, Fenwick SG, PrasadJ, Gopal PK and Gill HS (2000): Diatery Bifidobacterium lactis (HN019) enhances resistance to oral Salmonella typhimurium infection in mice. Microbiol. Immuno. **144:** 213-222.
29- Shah NP (2000): Probiotic bacteria: Selective enumeration and survival in dairy food J.Dairy Sci. **83:**894-907.
30- Murry AC, Hinton A and Buhr RJ (2006): Effect of Botanical Probiotic Containing Lactobacilli on Growth Performance and Populations of Bacteria in the Ceca Cloaca and Carcass Rinse of Broiler Chickens. International Journal of Poultry Science **5 (4):** 344-350.
31- Likotrafit E, Manderson KS, Fava F, Tuohy KM, Gibson GR and Rastall RA (2004): Molecular identification and anti-pathogenic activities of putative probiotic bacteria isolated from faeces of healthy elderly individuals. Microbial Ecology in Health and Disease **162:** 105 – 112.
32- Lema M, Williams L and Rao DR (2001): Reduction of faecal shedding of enterohemorrhagic *Escherichia coli O157:H7* in lambs by feeding microbial feed supplement. Small Rumin. Res.; **39:** 31-39.
33- Brashears MM, Galyean ML, Loneragan GH, Mann JE and Killinger-Mann K (2003): Prevalence of *Escherichia coli O157:H7* and performance by beef feedlot cattle given Lactobacillus direct-fed microbials. J. Food Prot.; **66:** 748-754.
34- Marsalkova S, Cizek M, Vasil' M, Bomba A, Nad' P, Datelinka I, Jonecova Z, Rimkova S, Kalinacova V, Styriak I, Bugarsky A and Greserova G (2004): Testing two *Lactobacillus plantarum* and Lactobacillus acidophilus strains for their suitability as a lipoid probiotic. Berl Munch Tierarztl Wochenschr. **117:** 145-147.
35- Atassi F, Brassart D, Grob P, Graf F and Servin AL (2006): Vaginal Lactobacillus isolates inhibit uropathogenic *E.coli*. FEMS Microbiology Letters **257:** 132-138.

Submission date: 2/1/2010