



Study Antibacterial Activity of *Bifidobacterium* spp. against some pathogenic bacteria isolated from patients with Cardiac Catheterization *in vitro*

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Abstract : Evaluation *in vitro* antibacterial activity of *Bifidobacterium* spp liquid against some pathogenic bacteria isolated from patients with diagnostic and therapeutic cardiac catheterization. A total of 45 samples were randomly collected from different imported and domestic dairy products source, 5(11.11%) positive sample of *Bifidobacterium* spp were obtained from yoghurt product, The study was included getting 89 patient from patients cardiac catheterization unit in Imam Hussain teaching Hospital in holy city Karbala, and from both sex (male and female) of different ages ranged between (29-75) years, due to the absence of a local study on pathogens isolated from cardiac catheterization patients. Perform test blood culture before and after the catheterization. These tests included diagnostic catheterization samples 66 (74.16%) has given 14 (58.33%), and 15 (62.5%) infected, and therapeutic catheterization samples 15 (16.85%) has given 6 (25%) infected for blood culture before and after the catheterization, while both the diagnostic and therapeutic catheterization samples 8 (8.99%) the results were given 4 (16.67%), 3 (12.5%) infected respectively. It became clear from the results after the sample cultivations on the enrichment and differential media that 89 patients gave 24 (26.97%) positive sample for bacterial cultivation, and two of them showed two types of bacteria to become 26 isolated bacterial, isolates were diagnosed 11 (24.31%) gram positive, 15 (57.69%) gram negative. and estimate the inhibition activity of *Bifidobacterium* spp against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *E coli*, *Klebsiella pneumoniae*, *Serratia marcescens* isolated from cardiac catheterization by wells in agar. All of which showed notable sensitivity to the *Bifidobacterium* spp liquid bacteria where inhibition diameters ranged between (24, 25, 21, 24, 22, 23,) mm respectively.

Keywords : *Bifidobacterium* spp, antibacterial activity, cardiac catheterization, *in vitro*.

Introduction

Many studies have shown a high count operations for the bacteria *Bifidobacterium* spp, and lower cases of stomach and intestinal inflammation in children, breast-feeding infants than bottle feed infants^[1,2,3]. That many doctors recommend using these bacteria as probiotics either pill or capsule or cosmetics lyophilized, so the choice of probiotics as an alternative to the treatment proved his role in several studies^[4]. *Bifidobacterium* spp bacteria contribute to the compilation of the commercial probiotic, as well as the genus is the most secure, since many of the food and drug companies are trying to find new and innovative methods to get the

Bifidobacterium spp within reach of humans, and a few of the companies used in chocolate have launched probiotic for infant formula line ^[5]. Atherosclerosis (arterial lipoidosis) is the term used to describe a condition in which there are pool and the accumulation of fatty material along the walls of the arteries, these materials with the time become dense and strong, with the potential for causing narrow or blockage of the arteries leads to weakness vitality and function of this organ ^[6]. Latent autoimmune disease against endothelial cell in adult early in the course of the disease process was critical because high risk of infections agent, it has been found that most of viruses and bacteria have play important role in these cases ^[7]. The biofilm tends to protect the bacteria from the action of antimicrobial agent and makes treatment difficult ^[8]. Cardiac catheterization was used to visualization the coronary arteries, the great vessel and the chambers of the heart. Although a number of diagnostic and therapeutic tools have been used in the treatment of heart and blood vessels disease, one of these tools are the cardiac catheterization mediated by a rubber tube inserted inside the blood vessels to reach to the right or left side of the heart to diagnose the condition or to fix the found problem, cardiac catheters use to diagnosis and treatment various cardiovascular cases ^[9]. The catheter is inserted into the groin or arm guided under fluoroscopy in to the heart in addition photo camera aid placed at the top of the catheter to viewed desired position ^[10]. Our study aimed inhibition of *Bifidobacterium* spp bacteria for other pathogenic bacteria species.

Materials and methods:

1) bacterial isolates :

A) bacteria *Bifidobacterium* spp :

In this study was to isolate and diagnose the bacteria *Bifidobacterium* spp of imported yoghurt products, total of 45 samples were randomly collected from deferent imported and domestic dairy products source, 5(11.11%) positive sample of *Bifidobacterium* spp were obtained from yoghurt product (Kaller seven dairy product) supplied from Islamic Republic of Iran, several methods were used in the diagnosis of bacterium represented as microscopic and biochemical tests, in addition its compared with standard isolate whey from local markets in the holy city of Karbala. Then cultured all of the samples after transported to the laboratory by taking part of the product by sterile carrier and cultured directly over L- Cysteine MRS-agar by planning manner and incubated dishes conditions Anaerobic using Anaerobic Jar and Gas generating kit (gas pak) for 48 hours and at a temperature of 37 °C. After the appearance of grown colonies on the L- Cysteine MRS agar, has noted the size and form the developing colonies, color, strength, and stained by gram stain and diagnosed by morphological, microscopic and biochemical tests depend on identification methods as approved by an encyclopedia ^[11,12,13].

B) Isolate pathogenic bacteria

A total of (89) samples of cardiac catheterization were selected from patient who attended the Imam Hussein teaching hospital in holy city Karbala, during 7 months (January 2014 to July 2014), cardiac catheterization of study population was classified into three types, diagnostic catheterization, therapeutic catheterization and both of them to gather the, ages of those patient ranged from (29-75) years, from both sex and different residency. Blood culture samples were collected from patient, tincture of iodine (2% solution of iodine) is used to prepare the skin prior to blood culture, because tincture of iodine can be irritating to the skin it should be removed with 70% ethanol. About 8 ml of blood was withdraw from patient in one arm. So it also 8 ml was drained from other arm. Blood samples for culturing are taken first to the laboratory by special transport media within hour after collection the blood was immersed in the BactALERT 3D (BioMerieux /France) for 1-7 days. Positive culture from blood agar examination was indicated wherever there is any special suspicion colonies. Generally, The present study also evaluated the diagnostic efficiency macroscopic, microscopic and biochemical test for each species according to the ^[13,14,15]. API system designed for confirmation of infections agent have become available in this study for routine diagnostic use such as: API Staph, API Strep, API 20E. all of these characterized by easy, speed and available of reagent. Chosen six isolates of pathogenic bacteria isolated from patients with cardiac catheterization, to conduct which included. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *E coli*, *Klebsiella pneumoniae* and *Serratia marcescens*.

2) Estimate the inhibitory effectiveness to liquid culture of Bifidobacterium spp against pathogenic bacteria in vitro:

Prepared the of L-Cysteine -MRS broth inoculum (1×10^8) cells / ml, of *Bifidobacterium* spp incubated at a temperature of 37° C for 48 hours under anaerobic conditions, compared turbidity solution by Macfarland tube , Then cultured at the dishes contained Mueller Hinton agar

Then left to dry at room temperature for 15 minutes, use a sterile cork borer piercing to make of several wells, filled each wells with 0.5 ml of cultivated bacteria *Bifidobacterium* spp and incubated 4° C for 6 hours to facilitate spreading in media then incubated at 37°C for 48 hours , measured inhibition zones around the wells were compared with control that contain MRS broth media without bacterial inoculum ^(16,17).

Results :

A total of 45 samples were randomly collected from different imported and domestic dairy products source, 5(11.11%) positive sample of *Bifidobacterium* spp was obtained from yoghurt product (Kaller seven dairy product) supplied from Islamic Republic of Iran, several methods were used in the diagnosis of bacterium represented as microscopic and biochemical tests , as approved by an encyclopedia ^[11], in addition its compared with standard isolate .

Also conducted blood culture test was performed to make sure the two previous methods had two stages: -

First stage: - It was done before a catheterization that diagnostic catheterization samples gave the 14 (58.33%) patients, and therapeutic catheterization was gave 6 (25%) patients either two together has given 4 (16.67%) patients was the number of people infected 24 (26.97%) patients.

Second stage: - It was done before a catheterization that diagnostic catheterization samples gave the (15/2.5 %) patients, and treatment catheterization was gave 6(25 %) patients either two together has given 3(12.5 %) patients was the number of people infected 24 (26.97%) patients (Table 1)

Table 1: Percentages for the appearance of pathogens from cardiac catheterization samples by blood culture test .

Blood culture						
Type of sample type of catheterization	Sample number	percentage	Sample positive number	percentage	Sample positive number	percentage
			Before		After	
			Diagnostic	66	74.16%	14
Therapeutic	15	16.85%	6	25%	6	25%
Diagnostic and Therapeutic	8	8.99%	4	16.67%	3	12.5%
Total	89	100%	24	100%	24	100%

The study showed after samples culturing on enrichment and differential media , that out of 89 patient's gave 24 positive bacterial culture , and two of these samples showed the presence of two types of pathogens, 26 bacterial isolated, as 11(24.31 %) isolates for gram positive and 15 (57.69 %) isolates for gram negative in the (Table 2 ,3) .

Table 2 :Percentages pathogens bacteria isolates from cardiac catheterization .

Types of pathogenic agent	Isolates number	percentage
positive bacteria gram	11	24.31%
negative bacteria gram	15	57.69%
Total	26	100%

Table 3: Percentages pathogenic of bacterial and fungal isolates from cardiac catheterization patients.

Types of germ	Isolates number	percentage
<i>Bacillus spp.</i>	2	7.69 %
<i>Staphylococcus aureus</i>	4	15.38 %
<i>Staphylococcus epidermidis</i>	2	7.69 %
<i>Streptococcus pneumoniae</i>	2	7.69 %
<i>Streptococcus pyogenes</i>	1	3.85 %
<i>Escherichia coli</i>	2	7.69 %
<i>Klebsiellapneumoniae</i>	3	11.54 %
<i>Proteus mirabilis</i>	2	7.69 %
<i>Pseudomonas fluorescense</i>	1	3.85 %
<i>Pseudomonas aeruginosa</i>	2	7.69 %
<i>Pantoeaspp</i>	1	3.85 %
<i>Salmonella spp</i>	1	3.85 %
<i>Aeromonashydrophila</i>	1	3.85 %
<i>Enterobacter cloacae</i>	1	3.85 %
<i>Serratiamarcescens</i>	1	3.85 %
Total	26	100 %

Genera that were bacterial isolates included highest rate of *Staphylococcus aureus* as (15.38 %), followed by *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Bacillus spp* (7.69 %) and *Streptococcus pyogenes* (3.85 %). To second gram positive bacteria which causes bacteremia in our result was *Streptococcus pneumoniae* (7.69 %). The high reported incidence of gram negative bacteria was *Klebsiella pneumoniae* as (11.54 %), followed by *E.coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* as (7.69 %). While the lowest reported rate in our study *Pseudomonas fluorescense*, *Pantoeaspp*, *Salmonella spp*, *Aeromonashydrophila*, *Enterobacter cloacae* and *Serratiamarcescens* as (3.85 %).

Generally, the present study also evaluated the diagnostic efficiency macroscopic, microscopic and biochemical test for each species according to the [13,14,15]. API system designed for confirmation of infections agent have become available in this study for routine diagnostic use such as: API Staph, API Strep, API 20 E all of these characterized by easy, speed and available of reagent.

Results of morphological, microscopic and biochemical tests, which were identical to those contained in the classification systems based on both [12,18].

Results of the study also showed the clear inhibitory effectiveness of the broth culture against bacteria test, bacteria was selected *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, *K. Pneumoniae* and *Serratia marcescens* (Table4).

Table 4: diameters of inhibition zones for bacteria *Bifidobacterium spp* against test bacteria:

Bacterial species	Diameters of Inhibition / mm
<i>Staphylococcus aureus</i>	24
<i>Staphylococcus epidermidis</i>	25
<i>Pseudomonas aeruginosa</i>	21
<i>E coli</i>	24
<i>Klebsiellapneumoniae</i>	22
<i>Serratiamarcescens</i>	23



Figure 1: antibacterial activity of *Bifidobacterium* spp. against *Pseudomonas aeruginosa*.

Discussion

The presence of these bacteria in large numbers from cardiac catheterization patient indicates the patient may be previously infected with these species, so occasionally it is possible to continue or recurrent bacteria due to the ability of these bacteria to resist antibiotics and harsh extreme environment^[19]. Infections affecting the heart valves include endocarditis primarily affecting the aortic and mitral valve when "viridans" streptococci are involved, however with IV drug users, *Staphylococcus aureus* commonly encountered and usually right side valves are affected^[20].

Pneumococci are normal inhabitants of the upper respiratory tract of 4-5% of humans and can cause pneumonia, meningitis, bronchitis and bacteremia, among the factors that probably lower or loss patient resistant immune cell and thus predispose to pneumococcal infection was viral or other respiratory tract infection and abnormal circulatory dynamics as (pulmonary congestion or heart failure) especially in the cardiac catheterization, have a significant role in causing bacteremia children^[21]. Hospitalized patients in some cases demonstrated increased susceptibility to infection of *pseudomonas aeruginosa* as a consequence of debilitation associated with concurrent illness and in some cases the administration of immunosuppressive drugs, also wide spread use of antibiotics promote over growth of antibiotic resistant strain of gram negative bacilli such as *pseudomonas aeruginosa* these organisms are often resistant to many antibiotics, in addition to potential lipopolysaccharide responsible to cystic fibrosis^[22]. *Klebsiella pneumoniae* and gram negative rods, may be implicated this condition may occur at any age but is typically uncommon in cardiac catheterization patients and late life there are approximately more than one pathogenic factor and virulence factor that causes significant disease in humans during infection by *klebsiella pneumoniae*, one of them capsular activity possesses sophisticated virulence strategy that are designed to overcome phagocytosis, by prevent opsonization process^[23]. Surprisingly, these bacteria have ability to resist of some antimicrobial agent, further more, pathogens that cause intestinal disorders and often nosocomial infections through hospital acquired infection in the genitourinary tract surgical wounds and lung all these are the most common sites of nosocomial infections^[24]. The bacteria *P.aeruginosa* is the second of negative bacterial causes possibility of moving through the hands of the medical staff and patients^[25]. The most of the gram negative bacteria isolate are Enterobacteriaceae, which access to the bloodstream through the lining of the intestines^[26]. The finding of this study is similar to these studies^[27], where it was found that gram negative bacterium transmission more than the gram positive bacteria and anaerobic bacteria, as well as, it being resistant to antibiotics due to lack of permeability of the outer membrane to most antibiotics, on the other hand, the incidence of bacteremia may be result from repeated intravenous injection and fluids which prevent dehydration^[28]. On the other hand its possession of virulence factors such as adhesion cells of the host and secretion of analytic enzymes and destroy the cells of the host when an immune system weakened of the host, which led to overcome the defense factors of the host and thus the invasion of host cells^[29].

The results of this study approximate with the results of converged⁽³⁰⁾, that the milk containing the bacteria *Bifidobacterium Lactis* has inhibitory effect on bacteria *H.Pylori*, as well as the effectiveness of bacteria *B.infantis*, *B.themophilum* and *B.longum* against *E.coli* bacteria and many of the *invitro* pathological bacteria⁽³¹⁾ mentioned using the deployment agar test.

Explains⁽³²⁾ that these bacteria capable of producing anti-bacterial compound to include bacteriocins and nonbacteriocins Peptides and production Peroxides (H₂O₂). As mentioned⁽³³⁾ that the ability of *Bifidobacteria* isolated from the feces of infant inhibition of intestinal bleeding caused by enterohemorrhagic *Escherichia Coli* Serotype 0157: H7 *invitro* and reduce adhesion to human cells, such as intestinal Caco-2 cells, this effect depends on the concentration Bifidobacterial cells.

Explains⁽³⁴⁾ inhibitory effective of strains *Bifidobacterium* against pathogenic *E .coli* of the gut with a common extension to lower the pH and increase the concentrations of acetate. Many studies showed the antibacterial mechanisms to bacteria *Lactobacillus* spp, it may be due to a number of factors such as Symbiosis with potential pathogens, and the production of bio-sink which inhibits adhesion pathogens, reducing the level of PH, competition for materials, production of H₂O₂, Lactic acid and pesticide bactericidal or bacteriostatic include dual diacetyl⁽³⁵⁾. As well as the existence of antimicrobial effective produces Probiotic contribute to the anti-microbial potential of these bacteria such as Biosurfactant⁽³⁶⁾. Conducted proficiency test antagonism to bacteria *Bifidobacterium*spp to make sure the possession and production of inhibitory substances inhibitory effect.

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