



Research Article

STUDY THE INHIBITION ACTIVITY OF *BIFIDOBACTERIUM SPP.* FILTRATE AGAINST SOME PATHOGENIC BACTERIA ISOLATED FROM PATIENTS WITH CARDIAC CATHETERIZATION *IN-VITRO*

Batool Shakir Abed AL- Mjalawi ¹, Ali Raheem Handhal AL-Hamil ^{*2}, Hiyame Abdul Ridha Kareem AL-Awade ¹

1. College of Education for Pure Sciences, University of Karbala, Karbala, Iraq.

2. AL-Imam AL-Hussain Teaching Hospital, Karbala, Iraq.

*Corresponding author's Email: aliraheem_69@yahoo.com

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ABSTRACT

Evaluation in vitro antibacterial activity of Bifidobacterium spp filtrate against some pathogenic bacteria isolated from patients with diagnostic and therapeutic cardiac catheterization. A total of 45 samples were collected from dairy products to isolated Bifidobacterium spp and sample collection of cardiac catheterization and estimate the inhibition activity of Bifidobacterium spp filtrate against staphylococcus aureus, Staphylococcus epidermidis, E coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Serratia marcescens isolated from cardiac catheterization by wells in agar. All of which showed notable sensitivity to the Bifidobacterium spp bacteria filtrate where inhibition diameters ranged between (24,25,21,24,22,32) mm, E.coli bacteria were more affected compared to other species. Bifidobacterium spp. Filtrate have antibacterial activity against the bacteria S.aureus, S.epidermidis, K.pneumoniae, E.coli, P.aeruginosa, P.mirabilis, Enterobacter cloacae and Serratia marcescens, isolated from cardiac catheterization.

Keywords: inhibition activity, Bifidobacterium spp bacteria filtrate, 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 has launched probiotic for infant formula line [5]. Atherosclerosis (arterial lipoidosis) are 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 is 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 in to 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 filtrate for other bacteria species.

MATERIALS AND METHODS

1) Bacterial isolates

A) *Bifidobacterium spp* bacteria

In this study was to isolate and diagnose the bacteria *Bifidobacterium spp* of imported yoghurt products, a 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) Isolates pathogenic bacteria

A total of (89) samples of cardiac catheterization were selected from patient who attended the Imam Hussein

teaching hospital in holy Karbala province, 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 prion 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 withdrawing 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 Bact ALERt 3D (BioMerieux /France) for 1-7 days. Positive culture from blood agar examination was indicated wherever there are any special suspicion colonies. Generally, the present study also evaluated the diagnostic efficiency macroscopic, microscopic and biochemical test for each species (table 7&8) according to the [14, 15, 16]. 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 E20 and API Candida all of these characterized by easy , speed and available of reagent .Chosen 8 isolates of pathogenic bacteria isolated from patients with cardiac catheterization, to conduct which included. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Serratia marcescens*.

2) Estimate the inhibition activity of *Bifidobacterium spp* filtrate against some pathogenic bacteria isolated from patients with cardiac catheterization in vitro Followed the method described by [17], filtrate liquid culture was attended by development of bacteria *Bifidobacterium spp* in the test tubes contain on De-Man Rogosa shrape (MRS) broth medium with a pH 6, the tubes were incubated anaerobically (anaerobic jar and gas pack) at 37°C for 48 hours, the pH measured of the growth medium in the tube, and harvested by cool centrifugation (10000 rpm for 10 min . 4 °C .) to get a cells free Liquid of bacterial cultivated was nominated through the accurate filters (Millipore filters) diameter (0.22) µm , Lyophilizer used to dry the filtrate by the freeze and saved at 4°C. until use .Diffusion method in

wells was used (well-diffusion) ^[18], for the detection on inhibitory effectiveness to bacterial filtrate of *Bifidobacterium spp*, where the dishes contained at the Muller Hinton agar media (0.1) mL were cultured from of pathogenic bacteria isolates inoculums during the test (105 cells / ml), and the using the sterile glass spreader, and allowed to solidify at room temperature ,and wells 5 mm were hollowed out in agar using a sterile cork borer .Eight of bacteria was chosen isolates of pathogenic bacteria isolated from patients with cardiac catheterization to test the antibacterial activity test of *Bifidobacterium spp* filtrate against some pathogenic isolated from patients with cardiac catheterization in vitro, which included. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Serratia marcescens*.

RESULTS

A 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 , as approved by an encyclopedia ^[11], in addition its compared with standard isolate.

First stage: - It was done before a catheterization that diagnostic catheterization samples gave the 14 (21.21%) patients, and therapeutic catheterization was gave 6 (40%) patients either two together has given 4 (50%) patients been the number of people infected 24 (26.93%) patients.

Second stage: - It was done before a catheterization that diagnostic catheterization samples gave the 15 (% 22.73) patients, and treatment catheterization was gave 6 (40%) patients either two together has given 3(37.5 %) patients was the number of people infected 24 (26.93%) patients (Table 1)

The study showed after samples culturing on enrichment and differential media, that out of 89 patient s (a common sample) gave 25 positive bacterial culture, and two of these samples showed the presence of two types of pathogens, 27 bacterial isolated, as 11(70.74%) isolates for gram positive and 15 (55.56%) isolates for gram negative and 1(3.703) yeast, in addition 64 (71.91%) Sample no gave any growth in the (Table 2, 3).

Genera that were bacterial isolates included highest rate of *Staphylococcus aureus* as (14.81 %), followed by *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Bacillus spp* (7.40 %) and *Streptococcus pyogenes* (3.70 %). To second gram positive bacteria which causes bacteremia in our result was *Streptococcus pneumonia* (7.41%).

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

Type of Sample	Sample number	percentage	Sample positive		Sample positive	
			number	percentage	number	percentage
Type of catheterization			Before		After	
Diagonistic	66	%74.16	14	%21.21	15	%22.73
Therapeutic	15	%16.85	6	%40	6	%40
Diagonistic and Therapeutic	8	%8.99	4	%50	3	%37.50
Total	89	%100	24	%26.97	24	%26.97

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

Table 2 : Percentages pathogens isolates from cardiac catheterization.

Types of pathogenic agent	Isolates number	percentage
gram Positive bacteria	11	% 40.74
gram negative bacteria	15	% 55.56
Yeasts	1	% 3.70
Total	27	% 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	% 7.41
<i>Staphylococcus aureus</i>	4	% 14.81
<i>Staphylococcus epidermidis</i>	2	% 7.41
<i>Streptococcus pneumoniae</i>	2	% 7.41
<i>Streptococcus pyogenes</i>	1	% 3.70
<i>Escherichia coli</i>	2	% 7.41
<i>Klebsiella pneumoniae</i>	3	% 11.11
<i>Proteus mirabilis</i>	2	% 7.41
<i>Pseudomonas fluorescense</i>	1	% 3.70
<i>Pseudomonas aeruginosa</i>	2	% 7.41
<i>Pantoea spp</i>	1	% 3.70
<i>Salmonella spp</i>	1	% 3.70
<i>Aeromonas hydrophila</i>	1	% 3.70
<i>Enterobacter cloacae</i>	1	% 3.70
<i>Serratia marcescens</i>	1	% 3.70
<i>Candida albicans</i>	1	% 3.70
total	27	% 100

Table 4 : diameters of inhibition zones for Bifidobacterium spp filtrate against the growth of testing bacteria.

Bacterial species	Diameters of Inhibition mm
<i>S. aureus</i>	29
<i>S. epidermidis</i>	30
<i>E. coli</i>	32
<i>K. pneumoniae</i>	27
<i>P. mirabilis</i>	26
<i>P. aeruginosa</i>	24
<i>E. cloacae</i>	25
<i>S. marcescens</i>	26

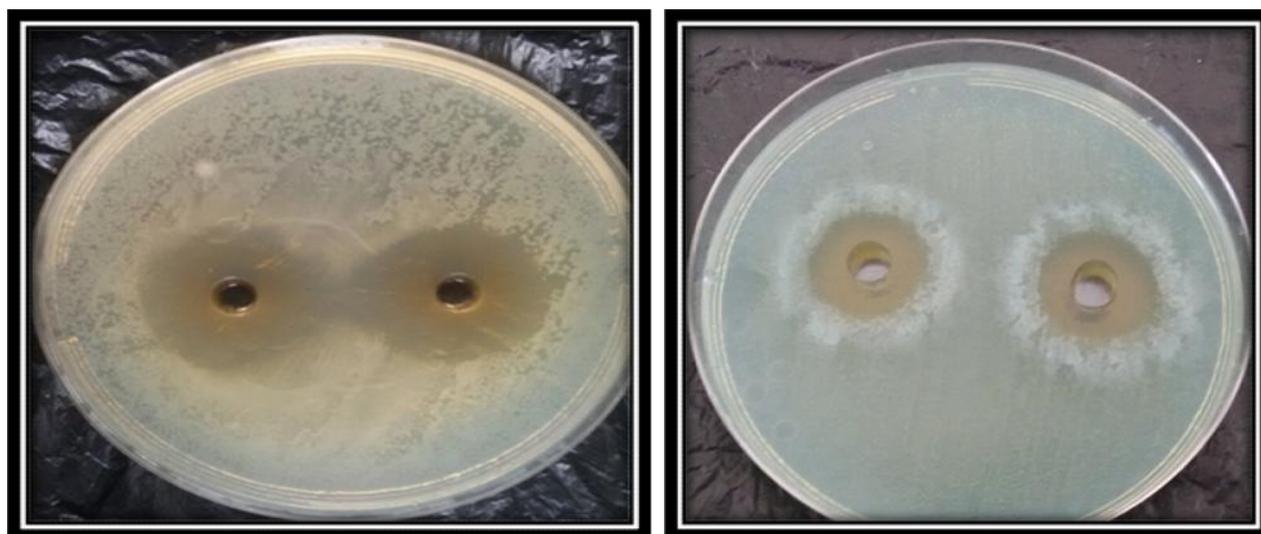


Figure 1: inhibitory effectiveness of *Bifidobacterium* spp bacteria in the growth of testing bacteria.

The high reported incidence of gram negative bacteria was *Klebsiella pneumoniae* as 11.11%, followed by *E.coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* as (7.40%), while the lowest reported rate in our study *Pseudomonas fluorescens*, *Pantoea* spp, *Salmonella* spp, *Aeromonas hydrophila*, *Enterobacter cloacae* and *Serratia marcescens* as (3.70%). Strain of *Candida albicans* have been isolated as single isolation (3.70%).

Generally, the present study also evaluated the diagnostic efficiency macroscopic, microscopic and biochemical test for each species according to the [14, 15, 16]. 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 E20 and API Candida all of these characterized by easy , speed and available of reagent. Results of morphological, microscopic and biochemical tests as in the (Table 2,3) which were identical to those contained in the classification systems based on both [12,19] .

Test was conducted form inhibitory effective of bacterial filtrate in growth by the test bacteria diffusion in agar method by wells , all of which showed notable sensitivity to the *Bifidobacterium* spp bacteria filtrate where inhibition diameters ranged between (24-32) mm, *E. coli* bacteria were more affected compared to other species, reaching inhibition diameter for this bacteria was 32 mm, and lowest effect bacteria *P. aeruginosa* inhibition diameter where reached 24 mm (Fig. 1) (Table 4) .

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 resisted antibiotics and harsh extreme environment [20]. Infections affecting the heart valves include endocarditis's primarily affecting the aortic and mitral valve when "viridian's" streptococci are involved, however with I V drug users, *Staphylococcus aureus* is commonly encountered and usually right side valves are affected [21].

Pneumococci are normal inhabitants of the upper respiratory tract of 4-5% of humans and can cause pneumonia, meningitis, bronchitis and bacteremia, among the factor 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) apically in the cardiac catheterization , have a significant role in causing bacteremia children [22] .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 [23]. Klebsilla pneumonia 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 Klebsilla pneumonia, one of them capsular activity posses sophisticated virulence strategyie that are designed to overcome phagocytosis, by prevent opsonization process [24]. Surprisingly, these bacterium have ability to resistant of some antimicrobial agent, farther 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 [25]. The bacteria P.aeruginosa is the second of negative bacterial cause's possibility of moving through the hands of the medical staff and patients [26]. The most of the gram negative bacteria isolates are Enterobateriaceae, which access to the bloodstream through the lining of the intestines [27]. The finding of this study is similar to these studies [28]. 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 [29]. 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 a immune system weakened of the host, which led to overcome the defense factors of the host and thus the invasion of host cells [30]. The pathogen was grew as a parasitic yeast in the host and exogenously as a saprophytic pseudo hypha this apportumstic fungus can become a pathogen from oral pharyngeal mucosa, rectal mucosa and vaginal mucosa, other infections are also possible under certain conditions such as therapy with corticosteroid and /or immune suppressive agent that suppress lymphocytopoiesis during cardiac catheterization [31]. Candida has become one of the most common blood isolates as well as one of the leading

causes of nosocomial blood stream infections [32]. germ tube is an important virulence factor of Candida albicans because it helps the yeast to invade tissue and growth as fungal false filaments [33]. This is consistent with [34] that Bifidobacterium spp bacteria filtrate at the MRS broth medium was highly inhibitory effectiveness against gram positive bacteria, Staphylococcus aureus, Bacillus subtilis, and gram negative bacteria to E.coli, K.pneumoniae and inhibitory zones ranged between (13-19) mm. This indicates that the filtrate has a high intimacy effectiveness and correlation with the of living organisms, which increases the antagonism effectiveness against bacteria [35]. Observed Bifidobacterium spp bacteria possess inhibitory effectiveness against bacteria due to the ability of these bacteria to produce inhibitory substances such as organic acids (lactic acid and acetic), which works to reduce the pH of the media [36]. The mechanical of antimicrobial activity of Bifidobacterium spp bacteria linked reduce the pH and the presence short chain of fatty acids on the one hand and its ability to produce materials have a wide-range inhibitory effect to the pathogens on the other hand, bacteria Bifidobacterium can produce and other inhibitory materials inorganic acids such as Bacteriocin which already possesses a similar antibiotics [37]. Confirmed [38] that Bacteriocin produced from bacteria Bifidobacterium spp has the ability to inhibit bacteria Listeria monocytogenes. Filtrates of bacteria strains Bifidobacterium spp characterized as being inhibiting effectiveness in for growth gram positive and negative bacteria as well as its ability in the inhibition of yeasts [39] Confirmed [40] that Bifidobacterium spp bacteria filtrate has inhibiting effectiveness against the bacteria E.coli and Bacillus Cereus.

CONCLUSION

Bifidobacterium spp. Filtrate have antibacterial activity against the bacteria S.aureus, S. epidermidis, K. pneumoniae, E.coli, P.aeruginosa, Pr.mirabilis, Enterobacter cloacae and Serratia marcescens, isolated from cardiac catheterization.

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