In vitro: Antimicrobial profile of Lactobacillus lactis on clinical isolates of Staphylococcus aureus [+]

الفعالية المضادة لجرثومة حامض اللاكتيك على المكورات العنقودية الذهبية المعزولة سريرياً خارج الزجاج

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Abstract:

A 150 samples of ear swabs were obtained from patients suffering from Otitis media (OM), visiting the consulate and out patient's clinics of AL-Kararna hospital in Baghdad city. The period of the study was from February to the end of April 2006.

The results showed that *staphylococcus aureus* was found in 36 isolates (24%) and their was no significant differences (P >0.05) in the isolation and identification between acute cases (12.6%) and chronic cases (11.4%).

Results of antimicrobial activity of *lactobacillus lactis* on *Staphylococcus aureus* showed that their were 32 isolates inhibition (88.8%) with 18-22 mm in diameter zones of the susceptibility test between the acute or chronic cases.

المستخلص:

جمعت (١٥٠) مسحة أذن من المرضى المصابين بالتهاب الأذن الوسطى الحاد والمزمن Acute and chronic Otitis Media المراجعين للعيادة الخارجية والاستشارية لمستشفى الكرامة ببغداد ، للفترة من شباط الى نهاية نيسان ٢٠٠٦. تم الحصول على العزلات النقية للعنقوديات الذهبية بواسطة اختبار أبي (API Staph) والتي كانت (٣٦) عزلة نقية وبنسبة تواجد (٢٤%) ولم تكن هناك فروق مهمة إحصائيا في نسبة تواجد البكتريا في مسحات الأذن للحالات الحادة او المزمنة وعلى مستوى احتمالية (P<0.05). اختبرت الفعالية ضد الجرثومة لجرثومة حامض اللاكتيك (Lactobacillus Lactis) ضد عزلات الدراسة السريرية باستخدام الانتشار في الوسط ألزرعي (Agar diffusion test).

أظهرت نتائج الدراسة ان لبكتيريا حامض اللاكتيك فعالية عالية بتثبيط نمو عزلات الدراسة السريرية، حيث كانت عدد العزلات المثبطة (٣٢) عزلة وبنسبة (٨٨,٨%)، في حين تراوحت أقطار مناطق التثبيط (١٨-٢٢) ملم. كذلك لم تسجل الدراسة اية فروق مهمة إحصائيا في نسبة التثبيط للعزلات بين الحالات السريرية الحادة والمزمنة (P<0.05). أثبتت نتائج الدراسة مختبرياً النشاط الضدمايكروبي لبكتريا حامض اللاكتيك ضد البكرتيا الموجبة لصبغة كرام وإحدى المسببات المرضية لالتهاب الأذن الوسطى بنوعية الحاد والمزمن.

Introduction:

In the last decade Lactic acid bacteria (LAB) have received increasing attention because of its therapeutic value through antimicrobial activities toward a board spectrum of bacteria [1, 2, 3]

Lactobacillus lactic is one of lactic acid bacteria that produced different types of inhibitory substance against other microorganism by antimicrobial products of lactic acid, acetic acid, hydrogen peroxide, carbon dioxide, diacetyle as well as hacteriocins or bacteriocins-like substances [4, 5].

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Also this bacteria can complete others on nutritional requirements in the same area, by using alternative ways to obtain substrates which found for growth and multiplication, while other microorganisms could be inhibit or die [6, 7].

Staphylococcus aureus is one of most distributed microorganisms in the nature. It is found in about 80% of suppurative cases of patients in hospitals specially those of low body's immunity levels [8, 9].

Otitis media disease occurred when there is an inflammation of mucous membrane of ear by several pathogenic agents. There are two features of the disease, acute or chronic [10].

Because of virulence features of *Staphylococcus aureus* that could transmitted from nasal air passages to middle ear and cause the disease or complication as a secondary infection. Intra temporal of extra cranial complication such as Labyrinthine fistula, facial nerve paralysis and with other general symptoms of acute or chronic cases[11, 12, 13].

The aim of our study is to evaluate the antimicrobial activity of *Lactobacillus lactis* against clinical isolates of *Staphylococcus aureus in vitro*

Methods:

1. *Staphylococcus* isolates:

One hundred fifty samples were taken from patient's, admitted to AL-Karama hospital, suffering from Olitis *media* (OM) disease. The collection of ear swabs was from February to April 2006. Ear swabs were placed in tubes containing nutrient broth[14].

Diagnosis of the isolates was done depending on their morphological, cultural and biochemical characteristics, followed by API Staph. technique, while final identification of isolates of *Staphylococcus aureus* were obtained as pure colonies according to other testes [15, 16].

2.Test organism:

Lactobacillus lactis was taken from food Science Department, Agriculture College, University of Baghdad. To confirmation the purity for this test organism, macroscopic, microscopic and biochemical tests were done [17].

Final *Lactobacillus lactis* was cultured in De Man Rogosa Agar (MRS) and inoculated at 37°c for 24 hrs and used in the study [18].

3. Susceptibility test:

Antimicrobial activity of *Lactobacillus lactis* was detected by agar diffusion test. Mueller-Hinton agar was inoculated with a 10µl aliquot of a 48 hrs. culture of *Lactobacilli* in MRS broth by streaking the plates .After 48 hrs. incubation in 10% CO_2 environment. 1µl of Staphylococcus aureus in nutrient broth (10^3 CFU as the McFarland turbidity scale) was placed in the same plates, while walls done in agar[19].

Plates were incubated for 24 hrs. in an anaerobic environment and inhibition zones (mm) of Staphylococcus aureus growth were measured[20].

4. Data were analyzed by Chi-Squared test [21]. Analysis of variance (ANOVA) and the least significant differences (LSD) were used for the statistical analysis of the results and P- values at level ($P<0.05$) was considered to be statistically significant. These calculations were carried out according to program SPSS, version10 [21].

5. All chemicals, reagents, solutions, broth or agars and kits were used according instruction of supplied companies.

Results and Discussion:

In this study, out of *(150)* ear swabs collected from patients suffering from OM, admitted to AL-Krama hospital in Baghdad city for the period from February to end of April 2006.

The patients classified in to two groups, acute cases *(75* patients) and chronic cases (75 patients) of OM through ENT physician's investigation, according to clinical symptoms and duration of the disease, there were no significant differences ($p>0.05$) between these two groups [22, 23].

All ear swabs were subjected for culturing, on available media (Nutrient broth or agar, Mannitol Salt Agar, blood agar, Bird-Parker agar) and biochemical test which its results listed in table -1.

There were 36 isolates (24%) of *Staphylococcus aureus* through 19 (12.6%) and 17 (11.4%) isolates of acute and chronic cases. There was no significant differences in the isolation and identification between these cases ($P>0.05$).

Supiyaphum *et al,* 2000 [24] had isolated this bacteria from cases of OM with the percentage of (32%), while others indicated the same bacteria strains in high percentage in OM because of its resistance strains to different antimicrobial agents (especially beta- lactum antibiotics) and due to virulence factors and its ability to invad the infected tissues as a secondary complications [11, 12, 13].

In other samples (114 samples 76%)showed no isolation of *staph aureus* which may indicate the presence of other pathogenic microorganisms which might cause OM [*23,* 24, and *25]*.

Results of API Staph technique corroborated the isolation of the bacteria, while the final purification also insures the same results, also there were no significant differences ($p>0.05$) between results of two methods.

Furthermore all results of isolation and purification was similar to the results obtained by [22, 23, and 26].

Results of confirmation and purification of *Lactobacillus lactis* was done to obtain pure and active colonies which accompanied by biochemical test (Negative to getalinase, oxidase, catalse, production of ammonia from arginine and no growth in 45°C. but
Positive only in growing in Litmus milk medium.[16].

Spectrum of antimicrobial activity of *Lactobacillus lactis in vitro* on *Staphylococcus aureus* showed that there were 32 isolates (88.8%) inhibited with 18-22 mm in diameter zones of inhibition (Table-2).

Inhibited isolates were 17 isolates (47.2%) from acute cases and 15 isolates (41.6%) chronic cases, while no inhibitory effects against remainder isolates [1,3,5].

Also there was no significant differences reported in the inhibition zone between these two cases ($P>O.05$).

Results indicates the higher antimicrobial activity of *lactobacilli* against pathogenic agents like *Staphylococcus aureus* and these activity were not due to bactericins or bacteriocins- like substance which produced by *lactobacilli* species only but it might be due to acid production of other chemical components that could make abnormal environments to *Staphylococcal* growth in the same cultured plates which lead to kill or inhibit it [4, 7, 27].

Table-1: Laboratory tests for isolation and identification of *Staphylococcus aureus* from acute and chronic OM.

Tests	AcuteOM*	Chronic_QM*
Mannitol salt agar	+	+
Mannitol salt agar fermentation	+	+
Colonial pigmentation	Generally golden Yellow	Generally golden Yellow
Motility	-	-
Catalse	+	+
Oxidase	-	-
Coagulase	+	+
DNase	+	+
Blood haemolysis	Generally beta	Generally beta
Gelatinase	-	-
Urease	±	±
Lactose fermentation	+	+
No. of positive isolate	19(12.6%)	17(11.4%)

* 75 samples from each one.

Table -2: Antimicrobials activity *of Lactobacillus lactis* on *Staphylococcus aureus* isolates.

Isolates	Total No. of isolates	Positive isolate	No. of sensitive isolates	Range of inhibitory diameter zones (mm)	Mean ±SE
Acute cases	*75*	19	17	19-22	$20.5^{\pm 0.1}$
Chronic ases	*75*	17	15	18-22	$20^{\pm 0.2}$
Total No.	150	36	32	Average 18-22	$20^{\pm 0.2}$

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