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Study Effect of Garlic Extract on G-Ve And G+Ve Bacteria Isolation and Identification from Pizza

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Abstract:

Pizza is a type of yeasted flatbread that is frequently baked in an oven with cheese and tomato sauce on top. Typically, a variety of meats, veggies, and condiments are placed on top. The word was originally used in a Latin text from Gaeta, Central Italy, in the tenth century. Two pizza-related goods were collected for this study. Fresh pizza had a negative culture, whereas frozen pizza had a favourable one. Pie and farcing samples were included in the positive culture appearance. After obtaining 1gm from each pie and farcing and cultivating them on various medium, the results showed that the frozen pizza had 43 (86%) β-hemolytic colonies whereas the γ-hemolytic colonies were 16 (32%). Microscopically analysis In this experiment was using different stain between G-ve and G+ve bacteria. The bacteria selected in this experiment was β-hemolytic on blood agar media. The results apparent 9(21%) from 43(86%) was G+ve while the G-ve was 34(79%).

Keywords: G-Ve, G+Ve Bacteria, Garlic Extract, Isolation, Identification, Pizza

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INTRODUCTION

Pizza is a type of yeasted flatbread that is frequently baked in an oven with cheese and tomato sauce on top. Typically, a variety of meats, veggies, and condiments are placed on top. A Latin text from Gaeta, Central Italy, only applies the phrase in the tenth century (Maiden, 2003). The modern pizza and its kinds are also reported to have

originated from Naples, in Italy and quickly got to other parts of the world (Miller, 2012). Neapolitan pizza was protected in the European Union in 2009 at the request of Italy as a Traditional Speciality Guaranteed product(OJEU, 2010; ITA, 2010) The association of true Neapolitan pizza is a noncommercial organisation, formed in 1984 and located in Naples. Supports and safeguards the 'authentic Neapolitan pizza (AVPN, 2015) Pizza, a food most ordered through fast food chains in North America and Europe is available as frozen food or fresh food, whole or by portion. There are several different kinds of ovens that are used to cook them. Calzone and stromboli are two examples of comparable foods that are made with components that are frequently used to make pizza (Sanders and Sanders, 1992). Multidrug-resistant bacteria' ongoing proliferation has grown to be a major-concern for infection-control professionals worldwide and a huge threat to public health. Sanders & Sanders (1992). This situation has led to the resurgence of diseases that were previously under control, raised the expense of medication regimens, and significantly increased the incidence of opportunistic and chronic-infection-cases in developing nations. (Fernandez 2003; Dhwani 2014)

2.1.Materials

2.1.1. prodact:

A single pastry product that featured pizza that was chosen based on storage from both fresh pizza at home and frozen pizza from the market.

2.1.2. Laboratory devices and equipment:

Table (2-1) lists the laboratory apparatuses and equipment..

Table (2-1) Laboratory equipments and apparatuses used in this study

NO.	ITEM	COMPANY	COUNTRY OF ORIGIN
1.	Incubator	Memmert	Germany
2.	Water bath		
3.	Oven		
4.	Autoclave	Stermite	Japan
5.	Light microscope	Olympus	
6.	Digital camera	Sony	
7.	Micropipettes	Eppendorf	Germany
8.	Glass wares(beakers, rounded flasks)	Hysil	U.K
9.	Plastic Test tubes.	AFCO	Jordan
10.	Platinum wire loop	Himedia	India
11.	Sterile swab for streaking.	Lab.Service	S.P.A
12.	Slides	Beroslide	Germany
13.	Benson burner		
14.	Millipore filter 0.22µm	Satorins membrane filter Gm ,BH ,W. ,	
15.	pH-meter	WTW	
16.	Wooden sticks	Supreme	China
17.	Forceps	Pakistan	
18.	Tubes rack		
19.	Cork porer		china
20.	Refrigerator	Concord	Italy

2.1.3. Chemicals :

Table (2-2) lists the chemical ingredients employed in this investigation.

Table (2-2) Chemical materials used in the present study

NO;	MATERIALS	COMPANY/ ORIGIN
1	tetramethyl –ρ–para phenylene – diamine dihydrochloride	BDH / England
2	Tris-(hydroxymethyl) methylamine ($NH_2.(CH_2OH)_3$ (Tris-OH)(KOH)($BaCL_2$)(H_2SO_4)	
3	Sterile urea, Methyl red, α-naphthol	Sigma /USA
4	Bromophenol blue	Difco/USA
5	Isopropyl alcohol	Mast Diagnostic /USA
6	Gram stain set	Crescent /KSA
7	99% ethanol alcohol , ureasolution , Kovac's reagent, H2O2	Fluka chemika /Switzerland
8	Glycerol	

2.1.4. A.Culture media:

Table (2-3) lists the culture media employed in this investigation.

Table (2-3) Ready-made culture media

NO.	CULTURE MEDIA	COMPANY	COUNTRY
1	Blood agar base, Müller-Hinton agar, Nutrient agar,	Himedia	India
2	MacConkey agar	Tulip Diagnostic	Belgium
3	Nutrient broth, peptone water	Oxoid	USA
4	Peteta dextros agar		
5	Malt agar		

2.1.5. plant extracts:

Table (2-4) plant extracts used in the study

NO.	PLANT EXTRACT	CONCE.GM	COUNTRY
1	Fresh gralic	50	iraq
2	Dehydrating gralic	50	

2.2. Methods:

2.2.1. prodect collection:-

Out of them, two products—one home pizza and one market pizza—were gathered from Halla Company and stored for four months (10/10/2016–16/1/2017). Details regarding their utilisation of carlic, date, and company. Fail the pizza and pie farce. There was no continuous gralic farcing.

2.2.2. Preparation of culture media:

The manufacturer's instructions for the ready-made culture media (nutrient agar medium, nutrient broth medium, and Mueller-Hinton agar medium) were followed, and standard procedures were used to make the general culture media that are detailed below. Each was utilised in the proper tests.

2.2.3. Brain heart infusion-glycerol broth medium:-

95 millilitres of BHI broth were mixed with five millilitres of glycerol to create this medium, which was then autoclave sterilised. It was employed in a test to identify the formation of bacteriocin (Forbes et al., 2007).

2.2.4. Stain:

In the procedures outlined by Benson (2001), Gram stain was utilised to distinguish between Gram positive and Gram negative bacteria as well as to determine their shape and organisation.

2.2.5. Making an aqueous extract from garlic

We bought fresh garlic bulbs (Allium sativum L.) from AL-Hilla local markets. To extract the edible part, the cloves were split and peeled. A Waring blender was used to chop and homogenise fifty grammes of the edible component in one hundred millilitres of autoclaved water. A crude aqueous extract containing 500 mg of garlic/mL was obtained by passing the homogenate through a 25 mm pore-size filter (Millipore, St. Quentin, France). Before being utilised, this was gathered in a sterile container and kept at 4¡C.

2.2.6.Garlic and Alcoholic extract preparation

In AL-Hilla, fresh garlic bulbs (Allium sativum L.) were bought from neighbourhood markets. The edible part of the cloves was extracted by peeling and separating them. Using a Waring blender, 50 grammes of the edible portion was diced and mixed with 100 millilitres of alcohol. After passing the homogenate through a 25 mm pore-size filter (Millipore, St. Quentin, France), 500 mg of garlic/mL was obtained as a crude aqueous extract. Before being utilised, this was kept at 4¡C in a sterile container.

2.2.7.Well diffusion method.

Using this technique, a Muller-Hinton agar plate was made by evenly cutting wells (6 mm apart). The plates were then streaked throughout their surface with a cotton swab that had been dipped into a scrow tube containing bacterial suspension. The plates were then incubated for 24 hours at 37 °C after being filled with 0.1 ml of prepared concentrations for each individual antibiotic in Muller-Hinton agar wells. The inhibition zone surrounding the wells for each concentration was measured in order to assess the antibiotics' susceptibility (Norrel and Messely, 1997 and Hangnga et al., 2002).

3.1 Results:

3.1.1. Isolation and Identification of bacteria:

Two pizza-related goods were collected for this study. Fresh pizza had a negative culture, whereas frozen pizza had a favourable one. Pie and farcing samples were included in the positive culture appearance; once 1g of each was taken and cultured on various medium, the samples showed a positive culture (frozen pizza) such as the following **table (3-1)**

Table (3-1). CFU/gm of microorganisms

TYPE PIZZA	CFU/GM OF MICROORGANISMS				
	MacConky agar	**Blood agar**	**Potata dextrose agar**	**Malt agar**	**Nutrient agar**
farcing	**$6*10^2$**	**$26*10^2$**	**$26*10^2$**	**$17*10^2$**	**$16*10^2$**
pie	**$2*10^2$**	**$33*10^2$**	**$1*10^2$**	**$9*10^2$**	**$53*10^2$**

3.1.2 Blood hemolysis:

In present study the results were 43(86%) colony of β-hemolytic whereas the γ-hemolytic colonies were 16(32%) colony as the following figure (3-1)

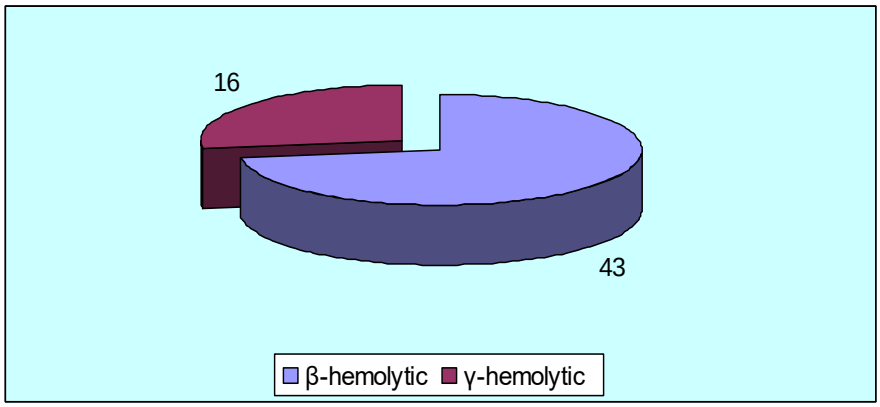

Figure (3-1) bacterial hemolysis.

3-1-3- Microscopically analysis:

In this experiment was using different stain between G-ve and G+ve bacteria. The bacteria selected in this experiment was β-hemolytic on blood agar media. The results apparent 9(21%) from 43(86%) was G+ve while the G-ve was 34(79%) such as the following **Figure** (3-2).

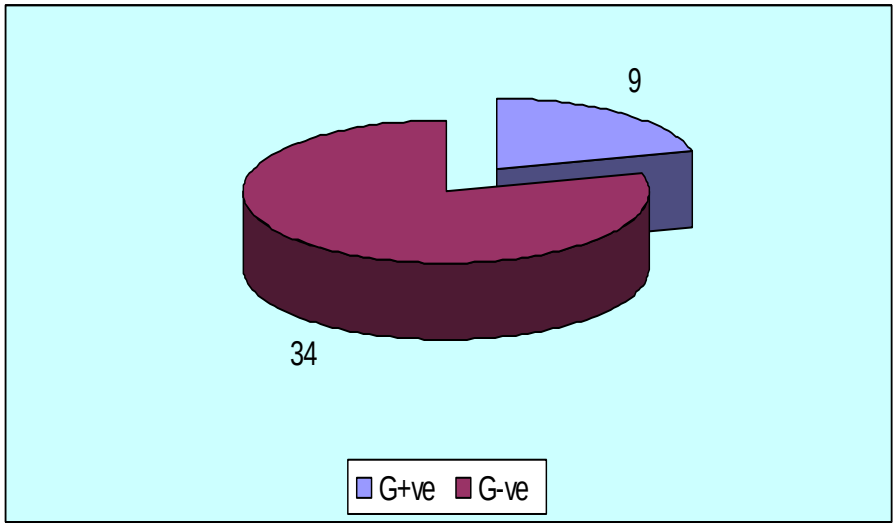

Figure (3-2) Gram stain.

For the shape and aggregation of the G+ve bacteria were according the following Table (3-2).

Table (3-2). G+ve bacteria.

NO.	SHAPE	AGGREGATION	SPORELATION
1	cocci	Dipl-cocci	-
2	Short bacilli	Streptococcus	-
3	bacilli	Diplo-bacillus	+
4	cocci	Staphylococcus	-
5	cocci	Staphylococcus	-
6	cocci	Streptococcus	-
7	bacilli	Diplo-bacillus	-
8	cocci	staphylococcus	-
9	cocci	Staphylococcus	-

3.1.2 The sensitivity of G+ve bacteria **to the plant extract(garlic) :**

In this experiment, the bacteria were sensitivity for garlic extract in diluted 10^{-2} by well diffusion method after applied extract in wells and measuring of inhibitor zone diameter as the show table (3-3) when the used fresh garlic.

Table (3-3). Inhibitor zone diameter of fresh garlic.

NO	inhibitor zone diameter(cm)		
	Watery extract	Alcoholic extract	Garlic oil
1	3.2	0	2
2	0	0	0
3	2.5	3.5	1.7
4	0	0	0
5	2.3	3.2	2.5
6	0	1	1
7	2.5	2.2	2.5
8	0	1	1
9	0	0	0

But the dehydration garlic were the results as the following table (3-4).

Table (3-4). The dehydration garlic.

NO	inhibitor zone diameter(cm)		
	Watery extract	Alcoholic extract	Garlic oil
1	1.3	5	2
2	0	5	0
3	1	1.5	1.7
4	0	0	0
5	0	0	2.5
6	0	0	1
7	0	0	2.5
8	0	0	1
9	0	0	0

3-2 Discussion:

As antibacterial agents, the methanolic extract performed less well than the aqueous extract (resuspension). This could be because fresh plants have active ingredients that can be impacted or ascribed by the solvent used, or it could be because the extract has poor diffusion capabilities in the agar.

This result was comparable to that of Iwalokun et al. (2004) on the effect of aqueous garlic extract (AGE) against 133 multiple antibiotic resistant bacterial strains; Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae, Salmonella typhi, Pseudomonas aeruginosa Bacterial susceptibility to AGE was determined using the well-diffusion and macrobroth dilution methods;

gram-positive and negative inhibition zones were 20.2Ð22.7 mm, 19.8Ð24.5 mm, respectively; MIC range concentrations were 15.6Ð48.3 mg/mL and 22.9Ð37.2 mg/mL. Compared to P. aeruginosa, the difference in the MIC values with the time points of 24 and 48 hours was again not significant (P ≥ PowerShell, the default program for choosing P values, was used; a P value of ≥ 0.05 indicates that difference is not significant).05) in these isolates. AGE also produced an anticandidal effect, thus giving a growth inhibition zone of 27.4 3.7 mm, and this was not significantly different from the inhibition zone of gentamicin (P > 0.05).05) in MIC values at both 24 and 48 h of antibiotic administration. Light microscopy revealed that the MFCs of AB/CS and AB/NCS isolates were 14.9 and 15.5 mg/mL at these incubation periods. Subsequent characterization established the nonlinear relationship between AGE concentrations and the bacterial cell kills with five different time-kill curves of the tested isolates. The findings of this research give credence to the use of garlic in any health products and the use of herbs in Nigeria.

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