Biochem. Cell. Arch. Vol. 22, No. 1, pp. 263-267, 2022	www.connectjournals.com/bca	*ISSN 0972-5075*
DocID: https://connectjournals.com/03896.2022.22.263		*eISSN 0976-1772*

STUDY THE EFFECTIVENESS OF GARLIC EXTRACT ON THE GROWTH OF PATHOGENIC BACTERIA CAUSES TONSILLITIS IN CHILDREN

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(Received 10 July 2021, Revised 14 September 2021, Accepted 20 September 2021)

ABSTRACT : Tonsillitis is a common disease that affects children of all ages and frequently during childhood. The present study aimed to demonstrate the effectiveness of garlic extract on different types of bacteria which cause tonsillitis. The study was carried out in Ibn Al-Baladi Hospital and Central Children Hospital in Baghdad City. 190 tonsillar swabs were collected from children and 178 samples gave positive growth while 12 samples did not give any growth, the age group between 2-16 years. The bacterial isolates were diagnosed according to standard bacteriological methods and the examination of their sensitivity to different antibiotics was carried out according to Kirby – Bauer methods. The antimicrobial activity of different concentrations of garlic extract was measured by the agar well diffusion method. Our study revealed that the isolation rate was 57% Male and 43% Female. The results appeared that the dominant causative agent was *Streptococcus pyogens* (38%) followed by *Staphylococcus aureus* (25%), *Haemophilus influenza* (17%), *Streptococcus pneumonae* (11%), *E. coli* (6%) and *Klebsiella pneumonae* (3%). The bacterial isolates showed multiple resistance to the antibiotics used. The garlic extract was effective as an antibacterial agent against all tested bacterial isolates and the maximum inhibitory effect was against *Streptococcus pyogens* (29.00 ± 0.57 mm), while minimum inhibitory effect against *E. coli* (6.32 ± 1.52mm). Garlic extract is considered a safe alternative to antibiotics in the treatment of tonsillitis.

Key words : Tonsillitis in children, garlic, antibiotic treatment, bacterial tonsillitis.

How to cite : Khalida J. Ibraheem (2022) Study the effectiveness of garlic extract on the growth of pathogenic bacteria causes tonsillitis in children. *Biochem. Cell. Arch.* **22**, 263-267. DocID: https://connectjournals.com/03896.2022.22.263

INTRODUCTION

Tonsillitis is a common disease in various countries of the world, as the incidence of it increases among children in the age groups of 5-9 years and continues until adolescence, as it almost disappears in 50 years (Ayda *et al*, 2021). The disease appears in two forms, the first is the acute form, which occurs most often when the initial infection with the germs of the disease occurs, and when this infection recurs or continues, the disease seventh is chronic and it is the second that may be accompanied by other diseases such as Otitis media an Arthritis (Girish *et al*, 2019). The disease occurs as a result of infection of the tonsils from the secondary lymphoid organs with germs entering the oral cavity from opportunistic aerobic and non-aerobic germs. The beta-hemolytic streptococcus has been located as the precursor to these bacteria (Rokhsana *et al*, 2020). Because the increasing, continuous and indiscriminate demand for antibiotics has led to the emergence of bacterial isolates resistant to antibiotics, which has led to the search for natural sources to produce life-saving antibiotics against resistant bacteria. The use of medicinal plants goes back to ancient historical times because they contain active compounds such as glycoside, phenols, alkaloids, and resins (Chen *et al*, 2020). The garlic plant is classified within the Liliaceae family. The Scientific name is "*Allium Sativum*". This plant was used in the treatment of pulmonary tuberculosis and bronchitis and has a broad antibiotic spectrum against bacterial and viral infections. The therapeutic uses of garlic are because it contains several biologically active chemical compounds, such as allicin, which plays an important role in the antimicrobial activity of garlic (El-Saber *et al*, 2020). The current study aims to find alternative materials for antibiotics to treat tonsillitis in children.

MATERIALS AND METHODS

Sample collection

A total number of 190 tonsillar swabs were obtained from children attending the Central Children Hospital and Ibn Al-Baladi Hospital in Baghdad city, 178 samples gave

positive growth while 12 samples did not give any growth (102 male and 76 female). The age group ranged from 2 – 16 years.

Bacterial isolation

Tonsillar swaps were cultured on blood agar, MacConkey's agar and chocolate agar. Then incubated at 37°C for 24 hrs. The isolates were diagnosed according to the standard bacteriological methods (Forbes *et al*, 2007).

Preparing of aqueous garlic extract

Fresh garlic was collected from local markets of Baghdad city. A hundred grams of washed garlic are put in an electric mixer and add the same proportion of distilled water (1:1 ratio, weight: volume). Then the mixture was filtered (Venugopal *et al*, 2003). This filtrate was considered 100% of the aqueous garlic extract. The other concentration (75, 50 and 25%) were prepared by adding the appropriate volumes of distilled water.

Antimicrobial activity

The agar well diffusion method was used to study the antimicrobial activity of aqueous garlic extract (Fisgin *et al*, 2009).

Agar well diffusion method : The selected bacterial colonies from each isolate were inoculated into 10 ml of nutrient broth and then incubated at 37°C for 18 to 24 h. Inoculums were cultured on the agar plates by using a sterile cotton swab. Using a sterilized Cork borer with a diameter (6mm), the agar wells were prepared. 100 ml of aqueous garlic extract was added to different wells in the plates. Plates were incubated for 24 h. at 37°C. The diameter of the inhibition zone (in mm) was determined.

Antibiotic Susceptibility test : The antimicrobial susceptibility test of bacterial isolates to different antibiotics (Ciprofloxacin, Penicillin G, Cefotaxime, Gentamycin, Erythromycin, Amikacin, Streptomycin, Amoxicillin, Vancomycin, Tetracycline) was performed by disk diffusion method according to Kirby – Bauer method (Bauer *et al*, 1966). The cultures of each bacterial isolate were transferred to nutrient broth adjusted to 0.5 Macfarland turbidity standards (1.5×10^8 CFU/ml). Inoculums were cultured on the plates of Muller-Hinton agar followed by the application of selected antibiotic disc by sterile forceps and then incubated at 37°C for 24 h. The inhibition zones were determined according to the standards outlined by the Clinical and Laboratory Standards Institute (CLSI) (Johnson *et al*, 2010).

Agglutination assay

The serotype of hemolytic *Streptococci,* which are cultured in bile media10%were diagnosed with a sensitivity to bacitracin and streptococcal agglutination assay using the Aviapath-strep kit from Omega diagnostic- UK (Kawakami *et al*, 2003).

RESULTS AND DISCUSSION

The results of the current study showed that out of a total of 190 samples collected from patients with tonsillitis, that 178 samples gave bacterial growth while 12 samples did not give any growth, the reason may be attributed to the presence of the virus or bacterial pathogens requiring special culture media or that the patients received treatment while they were in the period of recovery and convalescence. The results in Table 1 revealed that among 178 isolates, 102 (57%) were male and 76 (43%) were female. The highest percentage for males was 32% for the age group 6-8 years and the lowest for males 6% for the age group 14-16 years. As for a female, the highest percentage was 30% for the age group 6–8 years and the lowest percentage for female was 5% for the age group 2-4 years, this is consistent with Ross (1985), who also indicated that children are frequently infected with tonsillitis at the age of 7–9 years due to the weak immune system on the one hand and the behavior of children of this age on the other hand that increases the chances of infection.

Table 2 showed that the beta-hemolytic Streptococcus (BHS), which is *Streptococcus pyogenes* was the dominant one, as it constituted (38%) of the total isolates and that coincides with the findings of Omurazakova *et al* (2008) and Vieira *et al* (2006), who indicated that the

Table 1 : Infection rate by age group and gender.

Age group	Male N (%)	Female N (%)	Total N (%)
2 – 4	8 (8%)	4 (5%)	12 (7%)
4 – 6	11 (10%)	14 (18%)	25 (14%)
6 – 8	33 (32%)	23 (30%)	56 (32%)
8 – 10	20 (20%)	12 (16%)	32 (18%)
10 – 12	17 (17%)	10 (13%)	27 (15%)
12 – 14	7 (7%)	8 (11%)	15 (8%)
14 – 16	6 (6%)	5 (7%)	11 (6%)
Total	102 (57%)	76 (43%)	178 (100%)

Table 2 : The percentage of the bacterial isolates from tonsillitis.

Bacterial isolates	Number	Percentage
Streptococcus pyogenes	68	38%
Streptococcus pneumoniae	19	11%
Haemophilus influenza	31	17%
Staphylococcus aureus	45	25%
Escherichia coli	10	6%
Kiaelebsiella pneumon	5	3%
Total	178	100%

Table 3 : Serotype group of *Streptococcus pyogenes*.

Serotype group	Number	Percentage (%)
A	46	68%
B	11	16%
C	0	0%
D	5	7%
G	5	7%
F	0	0%
Non – type able	1	2%
Total	68	100%

thousand children annually in the United States of America. It was followed by (GDS) 7%and(GGS)7% while one isolate showed no interaction with any of the antibodies prepared in the examination kit, forming a percentage of 2%. The tonsil region is considered a suitable medium for adhesion (GBS) because it contains the secretory component of IgA fibronectin, which is important for GGS, GDS and GAS adhesion (Lindmark *et al*, 1996).

Table 4 : The percentage of resistance of bacterial isolates to different types of antibiotics.

Antibiotics	*S.pyogenes* No. (%)	*S.pneumoniea* No. (%)	*S.heamophilus* No. (%)	*S. aureus* No. (%)	*E. coil* No. (%)	*K.pneumoniea* No. (%)	Total No. (%)
CIP	3(4.4)	2(10.5)	7(22.5)	6(13.3)	1(10)	1(20)	20(11)
TE	30(44)	9(47.3)	15(48.3)	22(48.8)	7(70)	3(60)	86(48.3)
UA	0(0)	0(0)	25(80.5)	10(22.2)	0(0)	5(100)	40(22.4)
CTX	26(38.8)	3(15.7)	5(16.1)	16(35.5)	2(20)	1(20)	53(29.7)
GN	38(55.8)	12(63.1)	10(32.2)	19(42.2)	7(70)	2(40)	88(49.4)
E	23(33.8)	9(47.3)	22(70.9)	21(46.6)	6(60)	1(20)	82(46)
AK	40(58.8)	15(78.9)	13(41.9)	30(66.6)	3(3)	4(80)	105(58.9)
S	10(14.7)	3(15.7)	29(93.5)	14(31.1)	0(0)	2(40)	58(32.5)
AMC	51(75)	9(47.3)	27(87)	40(88.8)	8(8)	5(100)	140(78.6)
P	44(64.7)	11(57.8)	20(64.5)	35(77.7)	10(100)	10(100)	125(70.2)

CIP (Ciprofloxacin), TE (Tetracycline), VA (Vancomucine), CTX (Cefotaxime), GN (Gentamycine), E (Erythromycin), AK (Amikacin), S (Streptomycin), AMC (Amoxicillin), P (Penicilln G)

(BHS) bacteria is one of the main causes of tonsillitis. *Streptococcus pneumoniae*, *Haemophilus influenza*, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, with isolation rates of 11%, 17%, 25%, 6% and 3%, respectively. This is consistent with their findings of Brow (2001) as they indicated the presence of other bacterial causes of tonsillitis and that the isolation rate for *Haemophilus influenzae* was 16% and *Staphlococcus aureus* 26%.

The serological examination for beta-hemolytic *Streptococci* (Table 3) revealed the presence of five serotypes (A, B, D, G, non–typeable). The percentage of the group A *streptococcus* (GAS) is 68% of the total isolates and this is consistent with what was found (Carapetis *et al*, 2005), which stated that the percentage of Streptococcus group A (GAS) in the mouth of people with pulmonary fever is 29% and it is the most frequent type and the results of the research (De-Miguel and Ramos-Macias, 1998) reported that the percentage of bacteria (GAS) isolated from patients with recurrent tonsillitis was 25% of the total isolates. The results of this study indicated that the percentage of group B *streptococcus* (GBS) is 16% of the total isolates and this is consistent with the findings of (Steel,1993) indicating that this type of bacteria causes the death of a

The bacterial isolates showed a variation in their resistance to the antibiotics used (Table 4), as the cause of resistance is due to the random and increasing use of antibiotics (Dumre *et al*, 2009) as well as the variation insensitivity. Most of the isolates were resistant to the penicillin antibiotic, which is consistent with what was found (Miguet *et al*, 2009), which indicated a high percentage of penicillin-resistant isolates due to secretion of the penicillinase enzyme. The result also showed that most of the bacterial isolates were resistant to Erythromycin and this is consistent with what was found (Tayel and Al-Tras, 2009), which indicated an increase in the resistance of the pathological isolates to the antibiotic Erythromycin, while the resistant isolates to aminoglycoside antibiotics through the presence of the enzyme Aminoglycoside transferase as in *S. aureus*, or through the change in cell membrane permeation as in *Klebsiella* (Iram and Anjum, 2008) and for resistance to *Streptococcus pneumoniae* comes through the production of the enzyme encoding chromosomal phosphotransferase (Bere *et al*, 2009). While the lowest resistance was to Cephalosporin antibiotics, which are more effective for children under the age of 12 years and chronic recurrent infections of tonsillitis (Casey and Pichichero, 2004).

Table 5 : Antimicrobial activity of aqueous garlic extract.

Bacterial isolates	Inhibition zone (mm) different concentration of garlic extract (%)			
	25	50	75	100
S. pyogenes	18.00+0.00	23.00±0.57	23.33±0.15	29.00±0.57
S. pneumonia	15.33±0.00	19.68±0.47	16.31±0.00	25.00±0.57
S. Heamophilus	19.12±0.00	11.22±0.00	12.33±0.00	17.23±0.00
S. aureus	13.37±0.15	13.00±0.57	12.11±0.00	14.55±1.72
E. coli	14.51±0.57	9.66±1.72	6.32±1.52	20.00±1.54
K. pneumonia	17.23±0.00	16.77±1.15	18.65±0.00	13.11±1.53

Data are mean of double replicates.

The result in Table 5 showed the effect of the aqueous garlic extract against different bacterial species using the good diffusion method. The diameter of inhibition zones (mm) for different concentrations of aqueous garlic extract ranged from 29.00 ± 0.57 to 6.32 ± 1.52 and the maximum inhibitory effect was against *Streptococcus pyogenes* while minimum inhibitory effect against *E. coli*. These results are consistent with the findings from several studies (Silva and Fernandes, 2010; Daka, 2011). In this study, water was used to prepare garlic extract in various concentrations to prevent the release of active organic substances that might affect the results. If ethanol is used as a solvent this will lead to show higher inhibition zones of the tested bacterial isolates (Shobana *et al*, 2009). Garlic possesses anionic substances including sulfates, nitrates, chlorides and other compounds that contain antimicrobial properties. Allicin is the most effective element in garlic and it works as an antimicrobial agent by preventing the synthesis of DNA and protein moderately and completely preventing the synthesis of RNA as a primary target (Rahiman *et al*, 2011).

CONCLUSION

Garlic extract is a safe compound that could be utilized in the treatment of tonsillitis, which contains a wide spectrum of antibacterial activity. However, further studies on the synergistic effects between garlic and antibiotics against multi-drug resistant bacteria are recommended.

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