STUDY THE ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACTION OF ONION (*Allium cepaL)* AGAINST *Staphylococcus aureus* ISOLATED FROM OTITIS MEDIA.

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ABSRACT

This study was aimed to determine the antibacterial activity of onion (*Allium cepaL*.) aqueous extract against *S. aureus* bacteria isolated from patients with otitis media. Twenty swabs samples were taken from patients with otitis infection; these samples were cultured on different agar media .Results revealed that half of these isolates were related to *S. aureus* and the five of these isolates were subject to sensitivity test against several antibiotic groups. Most of isolates 100% were resistance to amoxicillin, chloramphenicol, cefoxitin and pencillin, and 60 % of were showed resistance to cefotaxtime and tetracycline, where as 20 % were resistance to Ciprofloxacin. Depending on the results of antibiotic sensitivity, the two isolates(S2,S5) were chosen for determining the minimum inhibitory concentration (MIC) test, S2 isolate showed high-level of resistance at (16 and 128 µg/ml) to chloraemphenicol, amoxicillin, ampicillin and Cefotaxime respectively, while S5 at concentration (32, 64 and 128 µg\ml) were resistance to amoxicillin, ampicillin, chloraemphenicol and cefotaxtime. The MIC of onion extract against *S. aureus* was determined at two incubation periods The results were indicated that after 24 hrs, MIC of extract at 64 µg/ml was affected in inhibit the growth of S2 isolate, While 32 µg/ml was inhibited the growth of S5 isolate.However, after 72 hrs, It was found that the MIC at 32, 64 and 128 µg/ml were more effective in inhibited the growth of S2 and S5 isolates.

Keywords: antibacterial activity, *Allium cepa*, *Staphylococcus aureus*, traditional herbal, Otitis media.

دراسة التاثير المضاد البكتيري لمستخلص البصل المائي على بكتريا(*Staphylococcus aureus*) المعزولة من التهاب الاذن الوسطى

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المستخلص

هدفت هذه الدراسة الى تحديد التاثيرالمضاد البكتيري لمستخلص البصل المائي (*Allium cepaL.*) على بكتيريا المكوّرات العنقودية الذهبية المعزولة من مرضى التهاب الإذن الوسطى.حيث اخذت 20 مسحة من المرضى المصابين بلتهاب الإذن الوسطى وزرعت على اوساط زرعية محتلفة.أظهرت النتائج أن نصف العزلات تنتمي إلى المكوّرات العنقودية الذهبية. بعدها اخضعت خمسة من هذه العزلات الى اختبار الحساسية ضد مجموعات مختلفة من المضادات الحياتية. أثبتت النتائج أن معظم العزلات مقاومة للاموكسيسيلين، كلورامفينيكول، سيفوكسيتين، وبنسلين بنسبة 100% و 60% منهم أبدت مقاومة للسيفوتاكستيم وتيتراسايكلين و20% لمضاد سيبروفلوكساسين.اعتمادا على نتائج الحساسية للمضادات الحياتية ,اختيرت عزلتين(S2,S5) لتحديد التركيز المثبط الأدنى لهذه المضادات, حيث أظهرت العزلة S2 مستوى عال من المقاومة عند تركيز (16 و128مايكروغرام/ملليليتر) للكلورامفينيكول ,الاموكسيلين ,الامبسيلين والسيفوتوكسيم,بينما اظهرت العزلة S5 مقاومة للامكسوسيلين,امبيسيلين,كلورومفينيكول وسيفوتاكسيم عند التراكيز (32,64 و128 مايكروغرام/ملليليتر).حدد بعدها تاثيرتراكيزمختلفة لمستخلص البصل على نموالمكورات العنقودية الذهبية بفترات حضن مختلفة وبينت النتائج انه بعد 24 ساعة كان التركيز(64,32مايكروغرام/ملليليتر)اثرعلى نمو العزلتين, S5,S2 على التوالي بينما بعد مرور 72 ساعة وجد ان التركيز المثبط الادنى(MIC) بتراكيز(32 ,64, 128مايكروغرام/ملليليتر) اكثر فعالية في تثبيط النمو S2,S5.

الكلمات المفتاحية:مضادات حيوية,بكتريا المكورات العنقودية الذهبية,الاعشاب التقليدية,التهاب الاذن الوسطى,عزلات

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INTRODUCTION

Medicinal plants, additionally called therapeutic herbs have been found and utilized in conventional drug researches since ancient occasions. Plants synthesize several of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals. (20) Numerous photochemical with potential or established biological activity have been identified. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by research to charecterized efficacy and safety (1).Onion (*Allium cepa, L.*) considrd as essential dietary intake and have been of critical for medical approach (34). *Allium* considered as the largest and important representative genus of the *Liliaceae* family comprises 450 species. Onion *(Allium cepa)* is a bulbous plant broadly cultivated in almost every nation of the world (18).They are easily propagated, transported and stored and it has diverse biological importance like treatment of cold, heart disease, diabetes, coughs and sore throat (7).Onions has different phytochemical constituents inrichness which clarified is rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found to have antibacterial activities (12).Onion also contained proteins, carbohydrates ,sodium, potassium and phosphorus (26).Onion was expended all through Europe amide and was later thought to guard against evil spirits and the plague, all that due to strong odor (20).In folkloric medicine generally utilized onion to prevent infections and is the oldest cultivated plants utilized both as nourishment and for therapeutic applications (27).The beneficial uses of onion in reduce the risk of certain disease was also investigated for example gastric ulcers by scavenging free radicals and by preventing development of the ulcer-forming microorganism, *Heliobacter pylori*. Also, *In vitro* onion appered to have antibacterial, antiparasitic and antifungal potential (14).Anciently, Otitis media is inflammation of the middle ear , or middle ear infection. Which occurs in the area between the ear drum (the end of the outer ear) and the inner ear, including a duct known as the Eustachian tube (17). In children and adults, these disease considered as a major problem since ancient time which require specific treatment because it is leading to hearing loss in children (9)(15).

MATERIALS AND METHOD

Collection of plant materials

The plants were collected from the confined markets during September (2017), and identified previously by National Herbarium of Iraq.

Preparation the aqueous onion extract

The onion bulb was cleaned from dust by using sterile distilled water, then removing the outer cover of the bulb. (21).Peeled out manually and soaked with distill water, 200g of the onion bulb were cut into small pieces and grinded, then soak with 100 ml of distilled water for 24hrs (13). The extract was filtered with Whitman no. 1 filter paper and evaporate at 45ºC for drying. Then stored in sterile condition at 4ºC until use to prepare the required concentrations (31).

Preparation of different concentrations of onion

Five concentrations (128, 64, 32, 16 and 8 µg/ml) of onion extract were prepared according to method of Mohamed Eltaweel. 2013. (28) by resuspending the onion extract in sterile distilled water.

Bacterial isolation

Twenty ear samples were collected from patients suffering from middle ear infection (otitis) from Al-Yarmook Teaching Hospital in Baghdad from the periods (1/9/2017-1/11/2017) using sterile cotton swab these were processed for direct examination and cultivation on blood agar media, macConkey agar and brain heart agar media (Bangalore, India) and were identified initially as *Staphylococcus* species according to their morphological, physiological and biochemical properties as indicated by Baird-Parker AC. 1963 (24) by using Gram stain , colony shape, oxidase , catalase, growth on manitol salt agar, growth at 15 and 45 C^{o}.

Inoculums preparation

Nutrient broth was used to cultivate the isolate of the tested bacteria for 18 hour.Then It was standardized according to National Committee for Clinical Laboratory Standards (30) (NCCLS, 2002) by gradually adding normal

saline to compare their turbidity to McFarland standard of 0.5 which is approximately 1.0 × 10^6 cfu/ml.

Antibiotic susceptibility

Disc diffusion method was used in this study against 5 isolates of *S. aureus* depending on the Kirby-Bauer diffusion method (23) Up to 7 different groups of discs of the available antimicrobial agents (chloraemphenicol, amoxicillin, ampicillin, cefotaxtime, iprofloxacin, cefoxitin and tetracycline) were used in this study.

Minimum inhibitory concentration

In this experiment Broth micro dilution method was performed, in two fold dilutions of antibiotics were done in broth media and broth was inoculated with 10^6 CFU/ml of the tested organisms (2). After incubation for (18-24) hrs, the MIC was determined as concentration in which no visible growth was observed (4).

Antibacterial potential of the plant

The method of Karam, I. F.A. 2016 (22) was followed in assessment of antibacterial activity of plant crude extract in accordance agar-well diffusion method. After growing bacteria in a nutrient broth for 18 hour and standardized to 0.5 McFarland standards (10^6 cfu/ml). 200 µl of the standardized cell suspensions were spread on a Mueller-Hinton agar, following that wells bored into agar with 6mm. Then 50 µl of the aqueous crude extract suspensions were added at different concentrations (8, 16, 32, 64 and 128 µg/ml) and allowed to stand at room temperature for about 2 h and then incubated at 37°C. The same procedure was followed for control set by using the solvent and distilled water. Then the zones of inhibition after (24 h) was measured.

RESULTS AND DISCUSSION

The results in Table 1 indicated that resistance to antibiotics was widely distributed among isolates, however, they varies according to types of the isolates and kinds of antibiotics, All isolates (S1, S2, S3, S4 and S5) were resistance for (amoxicillin, chloramphenicol, cefoxitin, penicillin) respectively, Where as three of isolates showed resistance to cefotaxime (S2, S3 and S5),While(S1and S4) were sensitive to cefotaxime and three of them resistance to tetracyclin (S1, S2, S3) ,Also (S4,S5) were sensitive to tetracyclin, while all isolates showed sensitivity to ciprofloxacin except (S5) which was resistance to this antibiotic.

Table 1. Antibiogram of *S. aureus* isolates isolated from otitis patients

Isolate No.	CTX (10)µg	AMX (10) µg	C (30) µg	CIP (5) µg	FOX (30) µg	TE (30)µg	P (10) µg
S1	S(27mm)	R	R	S(23mm)	R	R	R
S2	R	R	R	S(30mm)	R	R	R
S3	R	R	R	S(30mm)	R	R	R
S4	S(13mm)	R	R	S(30mm)	R	S(15mm)	R
S5	R	R	R	R	R	S(12mm)	R

S: Sensitive R :Resistant CTX: Cefotaxime AMX: Amoxicillin P: Penicillin C: Chloramphinicol CIP: Ciprofloxacin FOX: cefoxitin TE: tetracycline

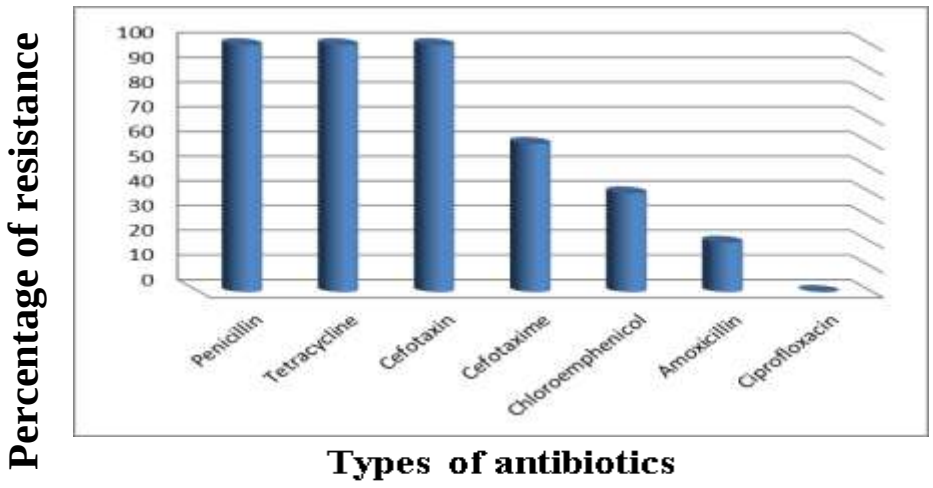

Fig 1. Percentage resistant of *S. aureus* isolated from otitis media against several antibiotics

Tow isolates were selected due to multiple antibiotic resistance therapy, in order to evaluate bacteria susceptibility to antibiotic that revealed earlier by disk diffusion test when examined against antibiotics. The results of MIC were shows in Table 2, In this Table S2 had high-level of resistance were (16µg\ml) for chloremphenicol, amoxicillin and ampicillin, while (128 µg\ml) for cefotaxime, Where as S5 were (32, 64 and 128 µg\ml) for amoxicillin, ampicillin, chloremphenicol and cefotaxime respectively.

Table 2. MIC value of some antibiotics for *S. aureus* (S2, S5) isolates

Isolate No.	C µg\ml	AMX 30µg\ml	CTX 10µg\ml	AMP 25µg\ml
S2	16	16	128	16
S5	64	32	128	32

C: Chloramphenicol, AMX: Amoxicillin, CTX: Cefotaxime., AMP; Ampicillin

The results of the antibacterial activities of the onion extracts against *S. aureus* organisms were shown in Table 3, after 24hr using Minimal inhibitory concentration was determined via macro broth dilution method. It was found that at 8µg\ml of onion extract a slightly inhibitory effect on S5 while have no effect on S2. However at 16 and 32µg\ml showed good inhibitory were observed against S2 and S5. While at 64 and 128µg\ml completely inhibit the growth of S2 and S5

Table 3. MIC value for Onion extract on *S. aureus* (S2, S5) after 24hrs

Type .of isolate	Concentration of onion µg\ml				
	8 µg\ml	16 µg\ml	32 µg\ml	64 µg\ml	128 µg\ml
S2	+++	+	+	-	-
S5	++	+	-	-	-

+: Indicates growth of *S. aureus* and – : no growth

Indicates growth of *S. aureus* and – : no growth

Table 4 reveals the results of MIC of onion extract on *S. aureus* after 72 hrs. It was found that the concentrations (32, 64 and 128 µg/ml) were more effective in inhibition growth of (S2, S5) while (8 µg/ml) showeed heavy growth of *S. aureus.*

Table 4. MIC value for Onion extract on *S. aureus* (S2, S5) after 72hrs

Type .of isolate	Concentration of onion µg\ml				
	8 µg\ml	16 µg\ml	32 µg\ml	64 µg\ml	128 µg\ml
S2	+	+	-	-	-
S5	+	-	-	-	-

+: Indicates growth of *P.aeruginosa* and – : no growth

The pH of each of the onion solution were (6.8, 7.0, 7.2, 7.4, 7.6 and 7.8). Result demonstrated that no pH effect on the onion activity has been observed. The onion extract have antibacterial activity due to the presence of flavonoids and polyphenols which has been reported to have broad spectrum of antibacterial activity (19).Also these activity belongs to they do not act directly on bacteria but create an adverse environment for them, thus causing threate their survival and they have also been found to decrease the resistant strains of microorganisms (3) .This property belongs to the onion constituent sulphur compounds, thromboxanes and by inhibiting the action of platelet-activating factor (PAF) exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited (16).The greater sensitivity with largest zone of inhibition was observed with ciprofloxacin. This result was in agreement with another by Reese, R., R. Betts, and B. Gumustop. 2000 (32) who found that ciprofloxacin was effective in the treatment of the virulent gram positive bacteria including *S. aureus*.From this result of Table (1) one could conclude that ciprofloxacin remain the first choice when most isolates were sensitive to it. Because of resistance for ciprofloxacin included effective suction pump of the antibiotic from inside to outside to escape its effect and prevent the accumulation of antibiotic inside bacterial cell (29) .Ciprofloxacin inhibit bacterial DNA gyrease, so preventing the super coiling of

DNA, a process that is necessary for compacting chromosomes into the bacterial cell (10) and this was concluded that plasmid mediated *β*-lactamase conferred high level resistance to penicillin and cephalosporin antibiotics and the level of resistance depend on the amount of *β*-lactamase produced so the mechanism (35) (36) that account for increase production of plasmid-determined *β*-lactamase include mutations or insertion elements that alter the promoter strength, microscopy plasmid and gene duplications (33) .The number of multiple antibiotic resistance strains has been increasing since resistance is mainly mediated by R- plasmids, which determined β-lactamase in Gram positive rod (25). All the R-plasmids carried the markers of resistance to chloraemphenicol, tetracycline, ampicillin, gentamycin and streptomycin (5).Results of MIC showed that *S. aureus* infections are more difficult to treat because the organism's high intrinsic resistance antimicrobial agents; this resistance is partly due to its relatively low outer membrane permeability (6). However there are other mechanisms that included decreasing the passage into or increasing the efflux of drug from bacterial cell and modification of the target site (6). Our results its matches with (11) who founded that both lower and higher concentration of petroleum ether, ethyl acetate and chloroform extracts of onion which inhibited the *Staphylococcus aureus* growth. In contrast, the Butanol, ethanol and water extracted of onion at any concentration were ineffective to control the growth of *Staphylococcus aureus*.Our results its matches with Mohamed Eltaweel. 2013 (28) who founded that the onion extract has antibacterial activity against *Staphylococcus aureus*. It has shown that dilute solutions of onion can completely inhibit the growth of *S. aureus* at the concentration of more than16 µg/ml. From our study it was concluded that *S. aureus* were highly prevalence among teenager age of otitis patients and high prevalence of antibiotic resistance was observed among *S. aureus* isolates especially to penicillin cefotaxin and tetracycline and it was found that onion extract has considerable inhibitory effects against the tested *S. aureus* isolates.

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