



ISOLATION AND IDENTIFICATION OF DENTAL CARIES BACTERIA AND STUDY OF THEIR SENSITIVITY TO SOME ANTIBIOTICS AND NANOPARTICLES

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Article history:	Abstract:
<p>Received: December 8th 2021 Accepted: January 10th 2022 Published: February 19th 2022</p>	<p>The current study aimed to know the bacterial causes of dental caries, and for this purpose, 850 samples were collected from people with caries Tooth decay for the period from the beginning of November 2020 to the end of March 2021, for both sexes, at different ages ranging from (8-50) years, Then they were collected and transported directly to the laboratory and the samples were planted by plotting on plates of nutritious agar and then acaral blood and mkonki And in solid saline mannitol medium under aerobic conditions , 800 samples gave a positive growth, i.e. 94.11%, which included bacterial species <i>Streptococcus mutans</i> By 357 isolates, or 44.62%, the type <i>Staphylococcus aureus</i> appeared at 248 isolates, or 31%, while <i>Streptococcus pyogenes</i> made up 98 isolates, or 12.25%, while the type <i>Klebsiella pneumonia</i> appeared in 49 isolates, or 6.12 %, and type <i>Escherichia coli</i>, Appeared by 48 isolates, or only 6%.</p> <p>The sensitivity of the bacteria isolates under study was tested against 3 types of antibiotics Clindomycin, Metronidazole, Amoxicillin. Where wasolates of bacteria showed-<i>S. aureus</i> Resistance against all antibiotics. For isolates of <i>Streptococcus</i> It showed a difference in the response to antibiotics in different races. <i>S. pyogenes</i> showed a mixed response to antibiotics Amoxicillin, Clindamycin, and Metronidazole showed no effect ,while she was <i>S. muntans</i> More responsive to antibiotics Amoxicillin, Clindamycin, Metronidazole, The isolates of bacteria showed-<i>E. coli</i> resistance against Clindamycin, Metronidazole, and the sensitivity of the bacterial isolates under study was tested towards the synergism of the three types of antibiotics used, and the results of the study showed a different response.</p>

Keywords: Dental Caries, Metronidazole

INTRODUCTION:

Tooth decay topic Dental caries, the interest of many researchers and specialists in the field of dentistry, so It is a very difficult problem, OK that van Tooth decay accompany him Severe pain is much stronger than other diseases, in addition to the fact that tooth decay remains the factor responsible for losing most teeth at all ages without other causes (Alkarimi et al., 2012) . prepare only Tooth decay is the most common chronic injury infection chronic occurs and progresses slowly over the years, and bacteria that are already present in the oral cavity may cause the normal flora Normal flora occurs, so the immune system of the human body cannot identify these infections and form immunity against them over time., And in the absence of appropriate treatment, this leads to harmful

developments that lead to damage to the tooth and surrounding tissues. Farsi, 2010), Causes dental plaque bacteria Dental Plaque Bacteria, to the formation of caries lesion Caries lesion, If it is not treated, it causes the formation of holes, and then the enamel layer begins Enamel necrosis due to acids that lead to the dissolution of metals (Taubman and Nash., 2006) , Another problem that the teeth are exposed to is gingivitis gingivitis , and inflammation around the tooth periodontitis , Studies have indicated that 60% of those aged 45-55 years complain of these infections, and nearly 80% suffer from infections when they are over 65 years old (Tiwari et al., 2008). More than 390 types of germs live in the human mouth (Doran et al., 2004) so These germs are found attached to the surface of the tongue, tonsils, plaque



on the teeth and gums, and in saliva *Streptococcus* spp. Among the most important bacterial species that cause dental caries in humans, other bacterial genera are *Staphylococcus*, *Lactobacillus*, *Bacteroids*, *Actinomyces*, *Eubacterium*, *Peptococcus*, *Bifidobacterium*, among other caries causes (Stenudd et al., 2001).

Many people resort to the use of antibiotics to eliminate the germs that cause oral diseases (Nascimento et al., 2000). Some people also resort to brushing, sticks and flossing the teeth in order to get rid of germs in the mouth or eliminate them because they cause many diseases, but these methods do not lead to the disposal of germs completely because these germs are beginning to show a high resistance to antibiotics, especially antibiotics Beta-lactams, as well as because the toothbrush and floss cannot reach all places in the oral cavity, so the use of mouthwash was resorted to because it can reach all places in the oral cavity ((Watanabe et al., 2015).

MATERIALS AND WORKING METHODS:

Sample collection Collection of samples:

Collected 850 smears from persons with dental caries of both sexes and From Different ages, as these swabs

were taken from patients arriving at the private dental clinics for the period from the beginning of October 2020 until the end of May 2021.

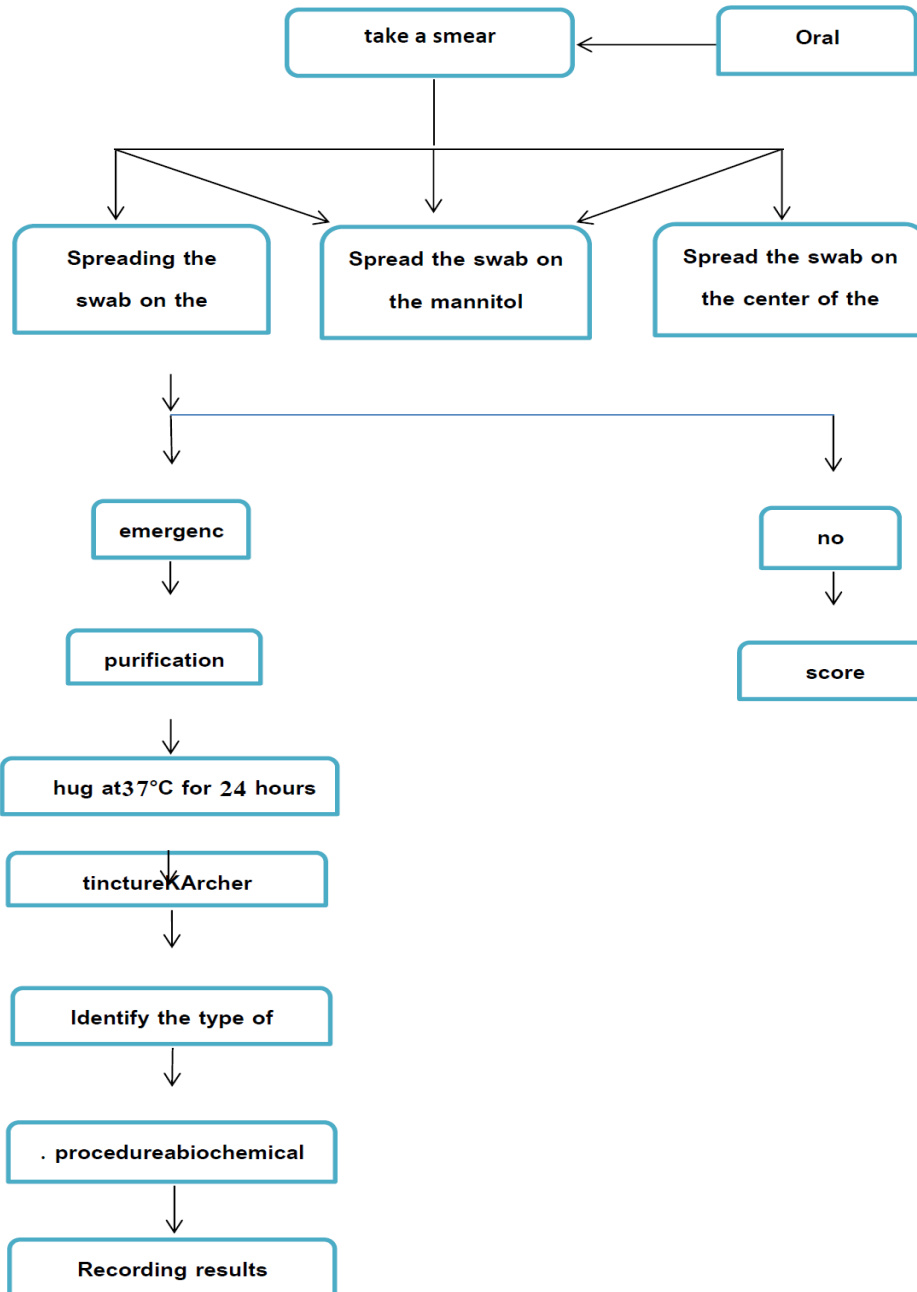
Most of the study period was during the fall and winter seasons. The information related to the research was recorded in a questionnaire prepared for this purpose, and the samples were transferred to the public health laboratory using the swab containing the medium. transport media swab with to maintain sample viability and avoid external contamination.

The samples were planted on blood agar medium and incubated at 37°C for 24 hours in order to identify the bacterial growth and how or the type of hemolysis caused by the bacteria grown on the medium. .

and use Swab as a transport tool for samples, then blood dishes were incubated under aerobic conditions at a temperature of 37 °C for a period of 48-24 hours.

The initial diagnosis of the isolated bacteria was made according to the colony's shape, size, color and the type of decomposition it caused on the blood agar plates.

The pure isolates were preserved on a highly nutritious agar liquid 4 pm in order to conduct phenotypic and biochemical tests for it.



PHASES OF BACTERIAL ISOLATION AND DIAGNOSIS

Antibiotic sensitivity test

An antibiotic susceptibility test was carried out on the isolates under study by the disc diffusion method according to the method The Baure method - Kiby modified and described by the World Health Organization (Vandepitte, 1991), and 10 types of antibiotic tablets were used using Muller Hinton's

medium. Suspended bacterial isolates were prepared and a number of pure colonies were transferred to tubes containing Nutrient broth medium and the tubes were incubated. at a temperature of 35 °C for 18–24 hours, and then the growth in the tubes was compared with the tube containing MacFarland's solution, which is approximately 1.5 * 10 cells / ml, Using a sterile cotton swab, the bacteria were spread on the medium of Muller-Hinton acar and left for a period of time 15 minutes for the dishes to dry,



then the antibiotic tablets were distributed by sterile forceps on the surface of the medium, and the dishes were incubated for 24 hours at a temperature of 37 °C. Resistant, sensitive and moderately sensitive bacteria to antibiotics.

RESULTS AND DISCUSSION:

Bacterial isolation and diagnosis:

The results of the isolation showed 850 samples which were collected from people with tooth decay, that 50 samples did not show any growth that's 5.88% while growth has been observed in a 800 sample i.e. 94.11%.

Includes isolates types of bacteria chromium cation it constituted 87.8% of 703 isolates. The isolates were distributed according to the type *Streptococcus mutans* which appeared in 357 isolates, or 44.62%, and the form of the species *Staphylococcus aureus* which appeared in 248 isolates, or 31%, while the type *Streptococcus pyogenes* which appeared in 98 isolates, or 12.25%. As shown in Table (1).

As for the negative bacteria isolates it appeared in a rate of 12.12%, of which 97 isolates were distributed among the species *Klebsiella pneumonia* which appeared in 49 isolates, or 6.12%, while the type appeared *Escherichia coli* 8 isolates, or 6% .

I showed results: The genus Streptococci is the most isolated species that causes dental caries, especially the bacteria- *Streptococcus mutans* and this is the result is consistent with what was found Al-Jumaili et al , (2019) and a contradiction with Jalal et al., (2017)) who received that bacteria Staphylococcus it was the sex that caused the most tooth decay .

It was completed diagnosis of 248 isolates of *Staphylococcus aureus aureus* Staphylococcus in the

rate of 31% This percentage is higher than the percentage obtained researcher Fazza (2018) which was 15.56% And the Zaidi researcher (2019) received the Rate 14.8% It is less than the percentage that we obtained, and it is a result that is close to what was obtained by the researcher, Effat (2018), whose percentage was 16.2% , While researcher Das et al. (2019) got 62.5% of isolates were from staph golden *Streptococcus aureus* And this higher than the percentage we obtained, as well as the researcher Al-Shammari (2019) obtained a percentage of 45.23%.

It was completed diagnosis of 455 Streptococcus pyogenesis isolated from Streptococcus pyogenes Less percentage 12.25% of isolates, in a whereas, Streptococcus mutans represents the highest isolate rate of 44.6%. as shown in the Table) 1).

This result is in agreement with (2018) Al-mohammadawy et al. Which accounted for approximately 51% of the isolates were Streptococcus mutans, and the beautiful researcher and others (2018) mentioned that Streptococci bacteria play an important role in dental caries and most oral infections, which are called carious types .

Additional till then that Genus Streptococci prepare from species that cause opportunistic diseases (Opportunistic infection); It is also the main cause of acute infection in hospitals (Hetem et al., 2017) because they have resistance mechanisms as well for being a quick propagation by conjugation and transformation by plasmids, in addition to surface antigens and enzymes Analyst which helps it to enter the various tissues of the body as well as the case in bacteria cluster (Von et al., 2016) .

The table (1)

Accounting adrand types of bacteria chromium cation isolated from dental caries and their percentages

The ratio Centennial %	a Counting ad isolates	the isolates bacterial chromium cation
44.62	357	<i>S. mutans</i>
31	248	<i>S. aureus</i>
12.25	98	<i>S. pyogenes</i>
87.8	703	Total

On the other hand, negative bacteria were less than that of Kras-positive bacteria. According to numerous studies, most of the Gram-negative bacteria are found to be gram-negative being source infections the device respiratory or gastrointestinal tract (Huffnagle et al., 2017)) .

The negative isolates were divided into 48 isolation from E. coli, 6%, and 49 isolates of Klebsiella pulmonary *K. pneumonia* any percentage .126% As shown in Table (2) .

researcher got Jamy Lee et al. (2019) found only 5 isolates of Escherichia coli. He pointed out that as many bacteria as possible that they are found singly or as a group present in dental caries of which *E. coli*, *K. pneumonia* (Yadav, 2016).



The table (2)

Accounting and types of bacteriogram negative isolated from dental caries and their percentages

The ratio Centennial %	a Counting and isolates	the isolates bacterial gram negative
6.12	49	<i>K. pneumonia</i>
6	48	<i>E. coli</i>
12.12	97	Total

EXAMINATION MICROSCOPIC MICROSCOPICAL EXAMINATION

Diagnosis of gram positive species Identification of Gram Positive Bacteria :

The bacteria were diagnosed based on the bacteria's response to the gram stain, as well as the characteristics phenotypic for bacterial colonies on agricultural dishes plus biochemical tests .

Kinds appeared Staphylococcus when dyed with gram dye which stained in purple appeared in shapes Spherical in the form of non-clustered clusters regular while The bacteria showed growth on beta-acid blood, i.e. partial dissolution of blood cells in a progressive manner paternal uncle It is white, shiny, with a smooth surface, with an oily texture and size It ranges (3-2) mm as shown in the picture (1) And the rosaries also appeared Streptococci B The form of binary groupings or in the form of short chain or stick-shaped Yeh, and when planted on agar blood The colonies showed alpha-type growth bacteria, partial lysis, where bright streaks appear, spherical, Smaller size, convex the edge It is dark green in color as shown in the picture (2). after planting Microbial Swab

medium of the Maconkey, it was observed that some bacterial colonies appeared It was pink or red in color and was fermented Some of them appeared in the form of unfermented yellow colonies of the sugar lactose *S. aureus*, On the other hand, it was checked results Catalysts for all *S. aureus* isolates gram positive for catalase assay and this matches what mentioned Researcher (2011) Mahon et al. that bacteria *S. aureus* Positive assay for catalase by TCO finish bubbles aerobic The result of the transformation of H_2O_2 to oxygen and water by the enzyme catalase and this test is necessary to distinguish them about races Streptococcus SPP negative for this examination, On the other hand, when performing the oxidase test, it gave a negative result for all isolates of Gram-positive bacteria, As for checking Coagulase All isolates of Gram-positive *S. aureus* showed a positive response to this assay, as these bacteria contain the enzyme Coagulase, which has the ability to convert fibrinogen into fibrin (Saleh et al., 2015).

the Table (3)

the exams Biochemistry used to diagnose Gram-positive bacteria

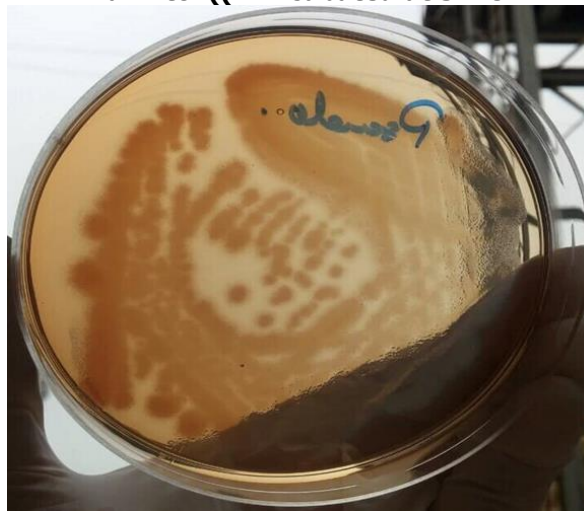
<i>S. muntans</i>	<i>S. pyogenes</i>	<i>S. aureus</i>	type of bacteria the exams
+	+	-	Lactose ferment
Alpha hemolysis	Alpha hemolysis	Beta hemolysis	Hemolysis
-	-	-	Oxidase
-	-	+	Catalase
		+	Coagulase

(+): positive for the test, (-): negative for the test



AB

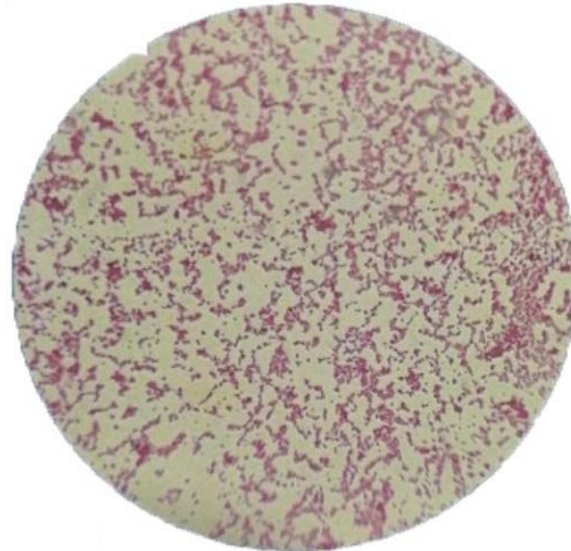
Picture (1): Bacteria *Staph.aureus* on the middle of the blood agar ((A and amid the acre of saline mannitol ((B Incubated at 37°C°



Picture (2): Bacteria *S. pyogenes* On the middle of the blood agar, incubated at 37°C°

Diagnostics of the speciesnegative cream dye Identification of Gram negative Bacteria :

Mother Isolates of gram-negative species appeared in the form of colonies pink appeared in shapes solo or in pairs or short chains as shown in the picture (3).



Picture (3):bacteria *K.pneumonia* Under a light microscope, magnification100X

When culturing bacteria *E.coli* on agar blood M I showed growth Gray flat circular colonies, while bacteria *K.pneumonia* It appeared in colonies pale big mucous on agar blood, In return, sleep and bacteria *E.coli* on Center Mkonki pink color picture (4), And *K.pneumonia* Appears in large continuous, mucous, Dark pink in color as a result of lactose fermentation picture (5).



Figure 4: Bacteria *E. coli* On the medium of the konki akar and incubated at a temperature of 37 °C°

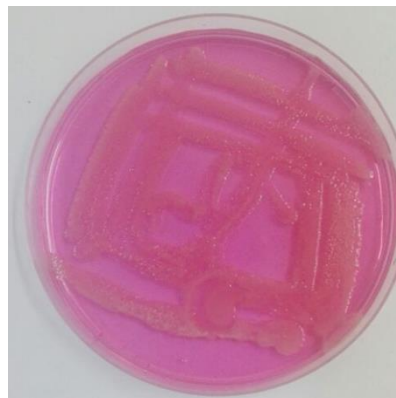


Figure (5): Bacteria *K.pneumonia* On the medium of the konki akar and incubated at a temperature of 37 °C°



A Several biochemical assays were carried out on gram-negative isolates Andisolated from tooth decay and the result was as in the table (4) When doing a catalase test Indole and methyl were the results of the examination Positive for all isolates *K.pneumoniae* *E.coli*, . while checking Citrate gave negative results for all isolates, WhileTake an oxidase testAll isolates of bacteria gave- *E. coli*calendar Negative for this test On the other hand, all isolates are bacteria *K.pneumoniae* gave a result positive aftercuddling

periodAs forcheck upVoges-proskauer showed *E. coli*, the color of the medium changed from yellow to red. As for *K.pneumoniae* isolates, it gave a negative result for the examination, while the medium remained in yellow.

These checks were doneIMVIC (Indole, methyl red, Voges-Proskauer and Citrate) because it is important in the diagnosis of the family of enterobacteriaceae.*Enterobacteriaceae*(2016Abdallah et al.,).

theTable (4)
the examsBiochemistry used to diagnose gram-negative bacteria

<i>K. pneumoniae</i>	<i>E. coli</i>	type of bacteria the testat
+	+	Lactose ferment
Alpha hemolysis	Alpha hemolysis	Hemolysis
-	+	Oxidase
+	+	Catalase
+	+	Indol
+	+	methyl red
+	-	Voges proskaur
-	-	Simmon citrate

(+): positive for the test, (-): negative for the test

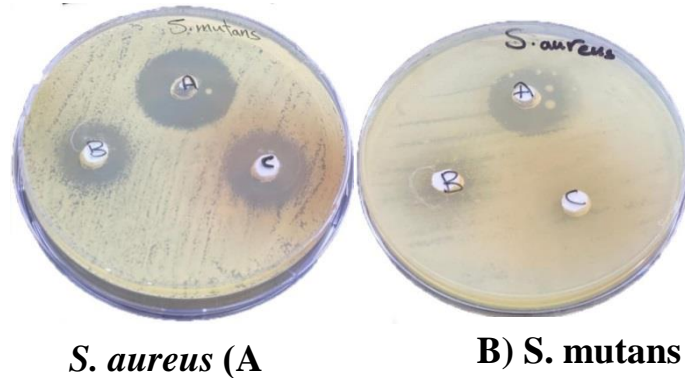
Sensitivity of bacterial isolates to antibiotics allergicBc isolatesterryCram-positive for antibiotics :

The results of the study showedVariable response of Gram-positive bacteria isolates to a number of antibiotics used in this studywhich included(Clindomycin, Metronidazole, (Amoxicillin as described intable (5)).

soBacterial isolates showed-S. aureus resistanttoallSpeciesAntibiotics And this The resultagreedwith (2011) Gousia et al.,The result of this study varies with what The researcher found it(Seifi et al., 2012) where it was mentioned that bacterial resistancecationfor antibioticsClindamycin was only 20%,The researcher scored) (Akanbi et al., 2017 Bacterial resistance to- Clindamycin 80%,While the researcher(Deyno et al., 2017) A test of *S. aureus* bacteria on different types of antibiotics showed different results depending on the type of antibiotic Where it was stated that the resistance of bacteria to-Amoxicillin (77% while resistance to- Gentamycin is 26% , asThe cause of resistance is due to a genetic change, i.e. a genetic mutation, such as deleting or replacing a specific gene or transferring a gene within

the same sex or from another sex (*et al.*, 2018 (Bitrus ,The researcher saidMustafa et al., 2008) (This antibiotic Metronidazole No It has the ability to inhibit aerobic bacteria .

Sex sensitivity test results *pyogenes Streptococcus* was resistant toMetronidazole, and showed sensitivity with an inhibitory diameter25mmforamoxicillinAnd13mm. but regarding *S. muntans*Allergies to all types offor anti-used under study in diametersinhibitionYeh(mm, 20mm, 22mm30 (For each of the(amoxicillin, clindamycin, metronidazole) respectively ,These results are close to what the researcher found(Lim, 2018) who conducted a test for antibiotics (amoxicillin, clindamycin, metronidazole) and obtained results (13.50 mm, 34.67 mm, 32.67 mm) respectively,The result obtained by the researcher.*et a/* Olajuyigbe (2014) found that (amoxicillin) is highly antibacterial on *S. pyogenes*. ,for- Clindamycin is an antibiotic that has an effect on many gram-positive bacteria, including staphylococci, Streptococci Vassallo, 2020)),WorksClindamycin inhibits protein production at the level of the 50S ribosome and reduces the production of toxins (Olajuyigbe et al., 2014). .



**Picture (6): Inhibitory activity of antibiotics on a group of Gram-positive bacterial isolates .A-Amoxicillin
 B-Clindamycin C-Metronidazole**

**Table (5)
 Inhibitory activity of antibiotics on a group of Gram-positive bacterial isolates**

C-Metronidazole	B-Clindamycin	A-Amoxicillin	bacteria
-	-	-	<i>S.aureus</i>
-	13mm	25mm	<i>Strep.pyogenes</i>
22mm	20mm	30mm	<i>Strep.muntans</i>

Allergic isolates of weptyer Gram-negative for antibiotics :

Where bacterial isolates appeared *E.coli* resistance against clindamycin, metronidazole and sensitive of Amoxicillin inhibitory diameter 12mm, And this is the result differ with the researcher (2014) (Olufunmiso et al., where He indicated that of *E.coli* She was sensitive for antibiotics Metronidazole, while isolates *K.pneumonia* It was resistant to Metronidazole and sensitive for antibiotics (amoxicillin, clindamycin) measuring ((20mm, 12mm respectively , Excessive use of antibiotics leads to bacterial mutations I became more resistant to antibiotics so that *K.pneumoniae* Produces extended-spectrum β -lactamases

(ESBL) and β -arrestin that increase bacterial resistance to many antibiotics, including penicillin, amoxicillin, and clindamycin (Wei et al., 2018). The result was obtained by the researcher (2016) Yadav that the bacteria *S. mutans*, *K. pneumoniae*, *S. albus*, *P. vulgaris*, *P. aeruginosa* and *S. mutans* isolated from dental caries are more sensitive to the antibiotic ciprofloxacin, gentamycin (norfloxacin, male researcher 2012) Soares et al.) that the antibiotic Metronidazole was initially used against protozoa and then showed impact in the treatment of bacteria to Anaerobes that cause tooth decay and this explains why gram-negative bacteria do not respond to this antibiotic.

**Table (5)
 Inhibitory activity of antibiotics on a group of Gram-negative bacterial isolates**

C-Metronidazole	B-Clindamycin	A-Amoxicillin	bacteria
-	-	12mm	<i>E.coli</i>
-	12mm	20mm	<i>K.pneumonia</i>

That Bacteria resistance problem For antibiotics longer A real problem that threatens health and the environment, so antibiotics must be constantly developed Some antibiotics succeed in overcoming gram-negative bacteria by inhibiting the action of the gram-negative enzyme- β -lactamase (Breijyeh et al., 2020).

Studies have indicated that the type *E.coli* γ Pregnancy enzyme extended spectrum beta-lactamases In the plasmid, this enzyme has antibiotic resistance -lactam β except cephamycins This enzyme is inhibited by inhibitors β -lactamase The plasmid carries other genes that cause resistance to other types of antibiotics, such as Aminoglycosides (Rogers et al., 2011) .

The effect of aZari for antibiotics For both positive and negative isolates of the gram stain:

It was completed Sensitivity test of isolates of gram-positive bacteria and gram-negative bacteria under study, the direction of synergy of 3 types of antigens (A-Amoxicillin, B-Clindamycin, C-Metronidazole). The results showed the variation in the response of the isolates to the effect of aZari for antibiotics. Vitality and its sensitivity and resistance differ from one isolation to another according to the difference in the isolation area and the region. According to the use person to

antihistamines advance as well as excessive use of it, has been tested sensitive isolates for antibiotics depending on the method of diffusion from the disc Kirby-Bauer method by measuring the area of inhibition around antibiotic tablets. The bacteria showed a different response to the antibiotics, as shown in Table (7), where it was completely resistant to all kinds of synergy among these types used under study before *S.aureus*, as for the *Strep.pyogenes*, *K.pneumoniae*. It was resistant and became sensitive after mixing B+Cas for the *E.coli*. It was sensitive and became resistant after mixing A+Z.

Table (7)

the influence of zero antibiotics on a group of gram-positive and gram-negative bacterial isolates

+	Clindamycin	Amoxicillin + Clindamycin + Metronidazole	Amoxicillin + Clindamycin	anti biotic Type bacteria
-	-	-	-	<i>S.aureus</i>
25	25	25	27	<i>Strep.pyogenes</i>
20	25	25	25	<i>Strep.mutans</i>
-	-	-	-	<i>E.coli</i>
20	20	20	20	<i>K.pneumoniae</i>

The purpose of the impact examination is aZari antibiotics to increase efficiency for quick treatment and get rid of the problem of bacteria resistance to antibiotics, as well as to reduce the concentration used, so it becomes less toxic (2019). (Olutumbi et al.,



Picture (4): **Inhibitory activity of synergy antibiotics on bacteria *S. pyogenes* using Crane dye**

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