

## Isolation & Identification of aerobic bacteria Causing Urinary Tract Infection in Pregnant Women in Al-Diwaniya city and its sensitivity to some antibiotics

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### ABSTRACT

( 250 ) urine specimens were collected from women contacting the Educational Hospital for children and & Delivery in Al Diwaniya city that suffering from urinary tract infection , ( 200 ) from pregnant women and ( 50 ) from non pregnant women .Isolated and diagnosed bacteria from pregnant women were :

*Escherichia coli*( % 26.49 ) , *Pseudomonas aeruginosa* ( % 24.50 ) , *Staphylococcus aureus* ( % 14.56 ) , , *proteus vulgaris* ( % 11.29 ) , *proteus mirabilis* ( % 7.28 ) , *Klebsiella pneumoniae* ( % 5.96 ) , Coagulase negative Staphylococci ( % 4.63 ) , *Klebsiella oxytoca* ( % 3.31 ) , *Enterococcus cloacae* ( % 1.32 ) . While *Escherichia coli* , *Pseudomonas aeruginosa* , *Staphylococcus aureus* , *proteus vulgaris* , Coagulase negative Staphylococci were ( % 37.5 ) , ( % 25 ) , ( % 18.75 ) , ( % 12.5 ) , ( % 6.25 ) respectively were isolated from non pregnant women.

As for the effect of antibiotics on bacteria it was found the most powerfull antibiotic on *Escherichia coli* , *Pseudomonas aeruginosa* , *Staphylococcus aureus* and *proteus spp* was Amikacin with percentage ( % 89.23 ) , ( % 84.54 ) , ( % 90.57 ) , ( % 88.12 ) while the isolates of *Pseudomonas aeruginosa* resisted Ampicillin and Amoxicillin with percentage ( % 100 ) and *Staphylococcus aureus* resisted Ampicillin and with percentage ( % 100 ) .

### INTRODUCTION

An urinary tract infection in women of the most common problems faced by doctors in all countries of the world, especially during pregnancy suffer as 10 - 20% of pregnant women from this disease [13] and urinary tract infection known as inflammatory Urological. Result of the invasion and colonization by pathogenic microorganisms and the presence of these pathogens may be facilities or other facilities for the appearance of clinical symptoms of inflammation of the bladder cystitis , pyelonephritis and Asymptomatic bacteria [ 8 ]and it knows that doubled the bacteria inside the urinary tract and arrival of preparing more than  $10^5$  cell / ml one of the mid stream urine [14 ] , it is also knows that the presence of pathogens in urine neighborhoods up to  $10^5$  cell / ml accompanied by clinical symptoms, whether or not accompanied by [ 23 ] also known as hungarian urinary infection pathogenic organisms ranging prepared between  $10^4 - 10^6$  cell / ml of the Mid stream urine [ 20 ] .

The two type of inflammation of the urinary tract infection :

A - Uncomplicated U.T.I.

Occurs in the normal urinary tract (not abnormal) on the anatomical and functional and unaccompanied disorders lead to a shortage in the immune defense mechanisms, on kidney and caused by single strain of bacteria solitary.

#### B – Complicated U.T.I.

The three cases are important:

1 - oddity urinary tract because it contains obstruction, calculi, vesico – ureteric reflux , neurological abnormalities , analgesic nephropathy , renal scarring , Indwelling catheterization .

2 – Impaired function .

3- The presence of turbulence inhibition defense mechanism, such as disruption of diabetes, Medication or treatment to discourage resistance, such as cancer medicines and steroids and organ transplant medications [ 18 ]. The clinical symptoms that appear on the infected women, they are urinary tract infection, abdominal pain, Dysuria, Hematuria, Urinary hesitancy, Incontinence, Nocturia, Frequency, Suprapubic pain, Lower back pain [ 18 ].

Enterobacteriaceae is the leading cause of the urinary tract infection, which includes :

*Escherichia coli* , *Klebsiella spp* , *proteus spp* , *Enterobacter* [ 11 ] , It is worth noting that a *E.coli* cause 80 – 90 % of acute complicated urinary tract infection when adult women [ 25 ] It is most common in this type of infection among pregnant women[ 20 ] and possession of the virulence factors that help them to attack the urinary tract , such as the presence of somatic antigen and fimbria and production of certain proteins such as aerobactin and haemolysin , various other recipes [ 34 ]. There are also other possible bacterial species that cause a urinary tract infection such as *Pseudomonas aeruginosa* which is one of the opportunistic pathogen that cause inflammation of the urethra frequent syndrome in hospitals [ 27] , *Staphylococcus spp* is most of the causes of bacterial involved in the urinary tract infection within the gram positive bacteria , *Staphylococcus aureus* have a capacity to produce toxins and enzymes that contribute to pathogenesis [ 25 ] , *Enterococcus spp* include *Streptococcus pyogenes* and *Streptococcus faecalis*[ 36 ]. There are also other pathogenic organisms require special growth conditions, including : *Neisseria gonorrhoea* , *Leptospira spp* , *Mycobacterium tuberculosis* *Salmonella spp* , *Candida spp* [ 21 ] , and also *Peptostreptococci* which rarely accompany urinary tract infection in special cases [ 37 ] .The objective of this study as follows :

1 - Isolation and identification of the bacteria that cause of the urinary tract infection in pregnant women and non-pregnant

2 - Determine the effect of antibiotics on the prevailing bacteria isolated from samples of urine

#### METHODS & MATERIALS

1- Collection of Samples :( 200 ) samples of urine were collected from pregnant women contacting the Educational Hospital for children and & Delivery in Al Diwaniya city , mid stream urine sample was taken for each woman in sterilized test tubes , ( 50 ) urine samples of non-pregnant women were taken as a control sample [ 26]

2 – Isolation of bacteria : culture media used are Macconkey agar & Blood agar , the standard loop was sterilized and dive in vertical way after cooled in urine samples transport carrier full of culture media and streaks to duplicate each , the dishes placed in incubator at the an aerobic conditions of growth at a 37 °C for 24 hours [ 16 ]

3 – Identification of bacterial isolates

Culture media were prepare for the purpose of purification, conservation, revitalization, identify qualities appearance and cultural recipes and making biochemical test of the bacterial isolates also attended the dyes and solvents and reagents to complete biochemical test and studied following the completion of diagnosis :

A - Characteristics manifestation of the developing colonies:

Observed qualities manifestation of the colonies developing on culture media form color, the surface of the colony, strength, transparency, smell, lactose fermentation on Macconkey agar [ 12 , 16 ]

B - Microscopic characteristics of the developing colonies : Swabs were the work of the pure colonies segments of slides the colored character gram stain observed forms of cells ,arranged, responding to gram stain [ 16 ]

C – Biochemical Tests : Tests conducted a production of the enzyme Catalase , Oxidase , Coagulase Voges proskauer , Methyl red as stated in [ 9 ] Motility test , Urease , Indol , H<sub>2</sub>S Production , Citrate utilization , Sugar fermentation as stated in [ 16 ] Haemolysis by the way [ 17 ]

4 - Sensitivity to antibiotics : from the use of nine types of antibiotics are:

Amikacin ( AN ) , Chloramphenicol ( C ) , Trimethoprim ( W ) , Ampicillin ( Am ) , Nalidixic acid ( AN ) , Gentamycin ( GN ) , Nitrofurantion ( F ) , Cefotaxime ( CTX ) , Amoxicillin ( AMX ) . determining sensitive and resistant bacteria to antibiotics by measuring the diameter of inhibition zone by mm and then compared with the standard diameters that installed in the standard scales [ 10 , 29 ]

**Results & Discussion**

(151) isolated of bacteria was isolation of the total (200) sample of pregnant women with percentage ( % 75.5 ) and ( 16 ) isolates with percentage ( % 32 ) for non-pregnant women infected with urinary tract infection , the purification and diagnosis of developing isolates was remained dependent on [ 12 , 24 ] Identifying characteristics appearance and culture media, recipes cells under the microscope and results of biochemical tests as shown in table (1) the bacteria contain : *Staphylococcus aureus* and coagulase negative Staphylococci as gram positive ,the gram negative included *Escherichia coli* , *Pseudomonas aeruginosa* , *proteus vulgaris* , *proteus mirabilis* , *Klebsiella pneumoniae* , *Klebsiella oxytoca* , *Enterococcus cloacae* as shown in table ( 2 ) .

**Table ( 1 ) biochemical tests of gram positive bacteria isolated from samples of urine and some attributes appearance and microscopic**

<b>Type of bacteria</b> <b>Test</b>	<i>Staphylococcus aureus</i>	<b>Coagulase negative Staphylococci</b>
<b>Coagulase</b>	+	-
<b>Haemolysis</b>	+	-
<b>Catalase</b>	+	+
<b>Oxidase</b>	-	-
<b>Qualities appearance</b>	<b>colonies of medium-to large-sized karimi-yellowish color, smooth ,β –haemolysis</b>	<b>small colonies of white-analysed blood</b>
<b>Attributes microscopic</b>	<b>spherical form of the grapes , G +</b>	<b>spherical form of the grapes ,G +</b>

Table ( 2 ) biochemical tests of gram negative bacteria isolated from samples of urine and some attributes appearance and microscopic

Tests Type of bacteria	oxidase	V P	Indol	Methylred	Motility	Urease	Simmons citrate	H2S production	Kliglar agar	Maltose	Lactose	Fructose	Mannitol
<i>Escherichia coli</i>	-	-	+	+	+	-	-	-	A/A	+	+	-	+
<i>Pseudomonas aeruginosa</i>	+	-	-	-	+	V	+	-	K/A	-	-	+	V
<i>Proteus vulgaris</i>	-	-	+	+	+	+	V	+	K/A	+	-	-	-
<i>proteus mirabilis</i>	-	V	-	+	+	+	+	+	K/A	+	-	-	-
<i>Klebsiella pneumoniae</i>	-	+	-	-	-	+	+	-	A/A	+	+	-	+
<i>Klebsiella oxytoca</i>	-	+	+	V	-	+	+	-	A/A	+	+	-	+
<i>Enterococcus cloacae</i>	-	+	-	-	+	V	+	-	A/A	+	+	-	+

**Abbreviation**

A : Acid , K : Alkaline , V : Variable , + : Positive , - : Negative  
 K/A: Alkaline / Acid , A /A : Acid / Acid

We shall note from the table (3) the arrangement bacteria isolated from samples of urine , it was a bacteria *Escherichia coli* in the first place among other bacterial causes in pregnant women, it was isolated (40) of the total bacterial isolates with percentage ( % 26.49 ) , it also occupied the same location in non-pregnant women it was isolated (6) isolates with percentage ( % 37.5 ) , the results were similar to the [ 6 ] she has isolated the bacteria with percentage ( % 36 .6 ) of pregnant women as isolation [ 5 ] of pregnant women by ( % 37.03) . It is believed that the rule of *Escherichia coli* infection in the urinary stream were resulting of their presence in large numbers in feces making it a source of autoinfection leaves are native to the natural bowel and easily transmitted to the urinary tract causing infection cases [ 32]. It also owns a number of factors pathogenicity that the most important of which fimbria to help them adhesion to specific receptors molecules in epithelial cells of the urinary tract [ 16 ] , it also

topped *Pseudomonas aeruginosa* second place causing of the urinary tract infection it was isolated ( 37 ) isolates of pregnant women with percentage ( % 24.50 ) and( 4 ) isolates with percentage ( % 25 ) from non-pregnant women as isolated in [ 5 ] of pregnant women by ( % 15.22 ) and not pregnant by ( % 22.22 ). These bacteria from opportunistic and systemic pathogen they cause a number of injuries including urinary tract infection , it is also resistant to most antibiotics used and that are difficult to treat [ 31 ] . *Staphylococcus aureus* was third causative , it was isolated by ( % 14.56 ) by ( 22 ) isolates of pregnant women and(3) isolates with ( % 18.75 ) in non-pregnant women , these bacteria isolated by[ 5 ] with percentage ( % 11.93 ) from pregnant women and ( % 11.11 ) ) from non pregnant women and by [6] with percentage ( % 6.6 )from pregnant women and [ 19 ] with percentage ( % 10 ) because of role in the occurrence of urinary tract infection in pregnant women [ 28 ] .

It also was isolated *Proteus vulgaris* with percentage ( % 11.92 ) and *proteus mirabilis* with percentage ( % 7.28 ) from pregnant women respectively ,it also was isolated by [ 5 ] with percentage ( % 8.64 ) and ( % 5.34 ) respectively from pregnant women , these types of opportunistic bacteria of Enterobacteriaceae caused injuries satisfactory to urological, and comes after important of *Escherichia coli* [ 33 ].It also was isolated other causes of unsatisfactory in the low rates such as Coagulase negative Staphylococci ( % 4.63 ) , *Klebsiella pneumoniae* ( % 5.96 ), *Klebsiella oxytoca* ( % 3.31 ) , *Enterococcus cloacae* ( % 1.32 ) .

It is worth noting that there are( 49 ) samples with percentage ( % 24.50 ) did not show bacterial growth was due to the possibility the presence of other causing of infection such as anaerobic bacteria and certain parasites as there are fungal and viral causes of the urinary tract [38] , or to deal patient antibiotics as treatment thus discouraging the growth of bacteria during sampling it also shows symptoms similar to urinary tract infection, such as Dysuria and urinary hesitancy this is a result of injury parts of the outer genital system especially among women [ 5 ] .

**Table ( 3 ) aerobic bacteria which had been isolated and diagnosed from urine samples of pregnant women and non pregnant women infected by urinary tract infection**

Source of samples Type of bacteria	Pregnant women		Non pregnant women	
	No. of isolates	Percentage %	No. of isolates	Percentage %
<i>Escherichia coli</i>	40	26.49	6	37.5
<i>PS. aeruginosa</i>	37	24.50	4	25
<i>Staphylococcus aureus</i>	22	14.56	3	18.75
Coagulase negative Staphylococci	7	4.63	1	6.25
<i>Proteus vulgaris</i>	18	11.92	2	12.5
<i>proteus mirabilis</i>	11	7.28	-	-
<i>Klebsiella pneumoniae</i>	9	5.96	-	-
<i>Klebsiella oxytoca</i>	5	3.31	-	-
<i>Enterococcus cloacae</i>	2	1.32	-	-
<b>Total</b>	<b>151</b>	<b>100</b>	<b>16</b>	<b>100</b>

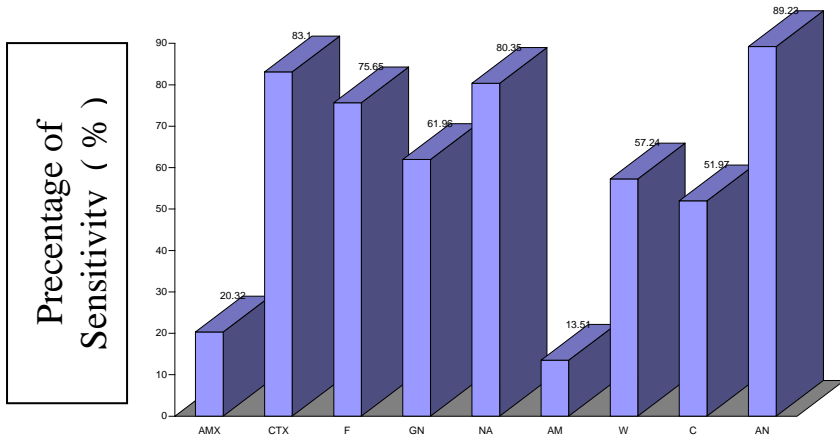
Shown in Figure (1) the sensitivity of *Escherichia coli* isolates to antibiotics used showed the highest sensitivity to Amikacin with percentage ( % 89.23 ) followed by Cefotaxime with percentage ( % 83.10 ) while the proportion was less sensitive to Ampicillin with percentage ( % 13.51 ) it is touching to the [ 5 ] reaching sensitivity to Amikacin ( % 90.53 ) and to Ampicillin ( % 10 ), as has reached at [ 2 ] ( % 87.35 ) to Amikacin and ( % 12.6 ) to Ampicillin while sensitive to the rate of [ 3 ] ( % 80.39 ) to Amikacin and ( % 7.72 ) to Ampicillin , it notes results the highest proportion of resistance was to  $\beta$  – Lactam antibiotics attributed the cause these resistant bacteria to  $\beta$  – Lactam antibiotics to possess coded genes on conjugated plasmids which helps to produce the enzyme  $\beta$  –

Lactamase it found that ( % 64 ) of the bacterial isolates producer of this enzyme can transfer the resistance by conjugation [ 35]. As shown in figure ( 2 ) the highest sensitivity record *Ps. aeruginosa* was to Amikacin with percentage ( % 84.54 ) followed by Cefotaxime with percentage ( % 78.75 ) , it is similar to reached [ 5 ] the rate of sensitivity *Ps. aeruginosa* has isolated ( % 85.12 ) to Amikacin , and [ 2 ] was the proportion of sensitivity ( % 83.2 ) while the highest than recorded [ 1 ] ( % 57 ) , it also notes that the bacteria resisted Ampicillin and Amoxicillin with percentage ( % 100 ) , the result was consistent with the findings of the [ 3 ] as the proportion of resistance to Ampicillin ( % 100 ) ,and [ 1 ] it was the proportion of resistance to Amoxicillin ( % 100 ) ,the reason is due to a high resistance to antibiotics to genetic mutations from the production of enzymes capable of modifying antibiotic molecule controlled by the R factor or the resistance be the result of change the permeability of germ reduce the quantity of lipopolysaccharides and lipids [ 25 ] . As figure ( 3 ) shows that the highest sensitivity of *Staphylococcus aureus* was to Amikacin with percentage ( % 90.57 ) followed by Cefotaxime with percentage ( % 89.97 ) it also isolates showed resistance to fully to Ampicillin with percentage ( % 100 ) , the results were similar to results of [ 5 ] as the ratio of sensitivity of *Staphylococcus aureus* has isolated ( % 92.23 ) to Amikacin and [ 2 ] ( % 85.7 ) to Amikacin while the highest than recorded [ 1 ] as the ratio of sensitivity of *Staphylococcus aureus* has isolated ( % 81.25 ) while in [ 4 ] resistance by ( % 100 ) to Cefotaxime and Ampicillin respectively . As shown in Figure( 4 ) the highest sensitivity of *proteus spp* was to Amikacin with percentage ( % 88.12 ) followed by Cefotaxime with percentage ( % 86.85 ) while the proportion was less sensitive to Ampicillin with percentage ( 10.52 ) the results were approaching to [ 5 ] as the ratio of sensitivity ( % 89.85 ) to Amikacin and [ 2 ] ( % 92.85 ) , ( % 85.7 ) to Amikacin and Cefotaxime respectively, as the proportion of sensitivity to Ampicillin ( % 5.3 ) in [ 5 ] and ( % 9.5 ) in [ 2 ] .

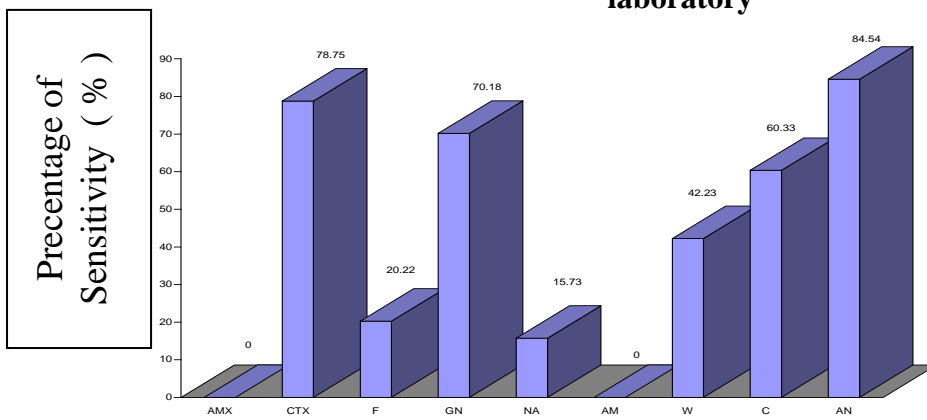
According to the results the high sensitivity of bacteria isolated the causative of urinary tract infection to Amikacin it derives semi synthetic to Kanamycin the reason for this is due to the limited use of pregnant women and that because of its effects on the fetus therefore, the bacteria had not been able to adapt to the resistance is also more stable to modified enzymes of the effectiveness of Aminoglycosides with little absorption into the digestive ,therefore gives a substitute for treatment Gentamycin and Tobramycin [ 7 ] .

As can be seen from results high resistance of bacterial isolates to Ampicillin and Amoxicillin , the reason is due to a production of the enzyme  $\beta$ -Lactamase that analyst for the  $\beta$ -Lactam ring in the antibiotic and is transformed into composite ineffective [ 22 ] and the resistance is due to the change in the permeability of cell membranes of bacteria[ 15 ] the bacteria show different mechanisms of antibiotic resistance such qualities autopsy for example, the components of plasma membrane role in receiving antibiotics and transfer from outside to inside the cell and access to objective, which affect on it, the absence of such as a result of obtaining genetic mutation lead to resistant bacteria to antibiotic ,while plasmids have an important role in the transfer of resistance because the presence of genes that code for them when it moves to does not contain bacteria become resistant after it was sensitive for those antibiotics this is achieved either through transformation , conjugation , transduction and other mechanisms [ 30 ] .

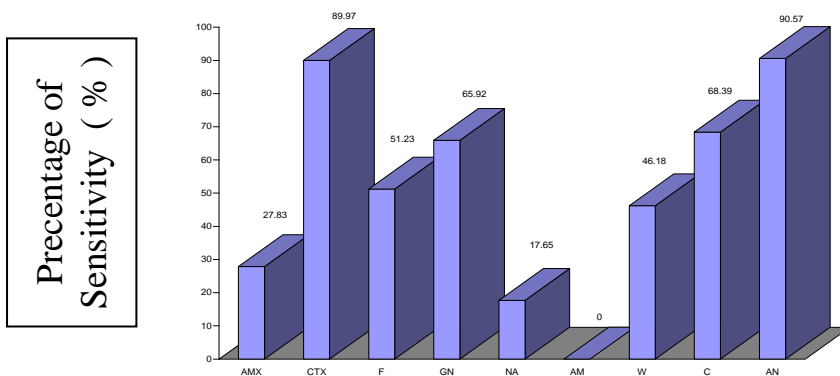




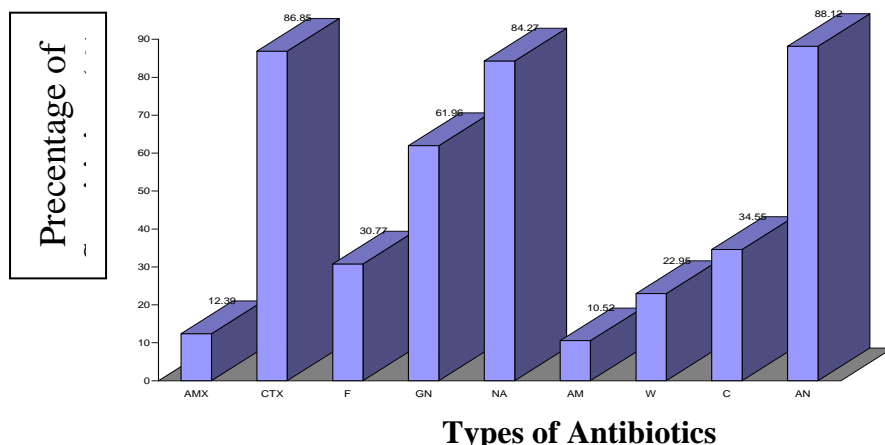
**Types of Antibiotics**  
**Form (1) the sensitivity of *Escherichia coli* for some types of antibiotics laboratory**



**Types of Antibiotics**  
**Form (2) the sensitivity of *PS. aeruginosa* for some types of antibiotics laboratory**



**Types of Antibiotics**  
**Form (3) the sensitivity of *S.aureus* for some types of antibiotics laboratory**



Form ( 4) the sensitivity of *Proteus spp* for some types of antibiotics laboratory

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عزل وتشخيص البكتريا الهوائية المسببة لالتهاب المجاري البولية عند النساء الحوامل في مدينة الديوانية وحساسيتها لبعض انواع المضادات الحيوية

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**الخلاصة** تم جمع 250 عينة إدرار من النساء المراجعات إلى مستشفى الولادة والأطفال التعليمي في مدينة الديوانية واللواتي عانين من التهاب المجاري البولية وبواقع 200 عينة من النساء الحوامل و 50 من غير الحوامل ، تم عزل الأنواع البكتيرية التالية من النساء الحوامل  
*Escherichia coli* ( % 26.49 ) , *Pseudomonas aeruginosa* ( % 24.50 ) , *Staphylococcus aureus* ( % 14.56 ) , , *proteus vulgaris* ( % 11.29 ) , *proteus mirabilis* ( % 7.28 ) , *Klebsiella pneumoniae* ( % 5.96 ) , *Coagulase negative Staphylococci* ( % 4.63 ) , *Klebsiella oxytoca* ( % 3.31 ) , *Enterococcus cloacae* التالية :

:  
*Escherichia coli* , *Pseudomonas aeruginosa* , *Staphylococcus aureus* , *proteus vulgaris* , *Coagulase negative Staphylococci* were ( % 37.5 ) , ( % 25 ) , ( % 18.75 ) , ( % 12.5 ) , ( % 6.25 ) .

كما أظهرت النتائج أن أكثر المضادات الحيوية تأثيرا على الأنواع البكتيرية *Escherichia coli* , *proteus* , *Pseudomonas aeruginosa* , *Staphylococcus aureus* , *spp*

على التوالي كما أظهرت عزلات بكتريا ( % 89.23 ) , ( % 84.54 ) , ( % 90.57 ) , ( % 88.12 )

مقاومة تامة لمضاد *Pseudomonas aeruginosa*

كما أظهرت بكتريا *Amoxicillin* و *Ampicillin*

*ampicillin* . مقاومة تامة لمضاد *Staphylococcus aureus*