



Antibacterial Activity Test To Ethanol And Aqueous Extracts Of Fruit Cucurbita Pepo On Multi-Antibiotic Resistance Bacterial Strains Isolated From Human Urinary Tract Infections

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Abstract

Bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Proteus mirabilis*) identified biochemically from human urinary tract infections were used to investigate the antibacterial activity of aqueous and ethanolic extracts of the *Cucurbita pepo* fruit. The in vitro examination of antibacterial activity was performed using the Kirby-Bauer diffusion technique. For the first time, high to moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was found in the ethanolic extracts of the *Cucurbita pepo* fruit. The aqueous extract, on the other hand, had a weak effect on *Staphylococcus aureus*. When tested on *Klebsiella pneumoniae* and *Proteus mirabilis*, the extracts showed only a moderate amount of antibiotic activity. According to the results, treating bacterial UTIs with an extract from the *Cucurbita pepo* fruit may offer a novel source that may be utilized as an adjuvant to antibiotics. The aqueous and Ethanolic extracts of *Cucurbita pepo* fruit, were used as antimicrobial activity against multi-antibiotic resistance strains (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Proteus mirabilis*) which were isolated biochemically from human urinary tract infections. The in vitro antimicrobial activity was performed by using Kirby-Bauer diffusion. The ethanolic extracts of *Cucurbita pepo* fruit showed for the first time a high to moderate antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* respectively whereas the aqueous extract showed a moderate effect against *Staphylococcus aureus* only. Weak antimicrobial activity was seen on *Klebsiella pneumoniae*, and *Proteus mirabilis* using both extract. The results concluded that the application of *Cucurbita pepo* fruit extract against human multiresistant urinary tract pathogens may represent a new source as adjuvant to antibiotics for effective treatment of bacterial urinary tract infections..

Keywords: Antimicrobial activity, ethanolic extract, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Cucurbita pepo*.

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1. Introduction

The pumpkin is a member of the genus *Cucurbita*, which is part of the family Cucurbitaceae. Its native range is North America, and it is usually identified as a member of the *Cucurbita pepo*, *Cucurbitamixta*, *Cucurbita maxima*, or *Cucurbita moschata* species. Their thick shells are either orange or yellow from their inherent pigmentation. Pumpkins have several purposes ranging from culinary to decorative to decorative, which is why they are farmed commercially on such a large scale. In India, it is among the most popular veggies. The fact that pumpkins, which are mostly water, are nevertheless considered to be fruits is a bit of a mystery. Pumpkins are an excellent source of the antioxidant beta-carotene, which can help to reduce the risk of getting cancer and cardiovascular disease. In Australia, winter squash is more usually referred to as pumpkin. The word pumpkin was originally derived from the Greek word "pepon," which means "large melon." It is possible to find pumpkins weighing anything from 9 to 18 pounds (4 to 8 kg) all the way up to 75 pounds (34 kg) (34 kg) Research has shown that this is the case (Okon, O. G., & James, U. S. 2014). According to Bergantin et al., the nutritional and therapeutic significance of pumpkin fruit is mostly due to the high overall amount of carotenoids, including more than 80% beta-carotene (2018). According to (Kurz, C., Carle, R., & Schieber, A. 2008) . pectin and non-pectin polysaccharides, minerals (potassium, phosphorus, magnesium, iron, and selenium), vitamins (C, E, K, thiamine (B1) and riboflavin (B2) and piridoxine (B6)), dietary fiber, phenolic compounds (flavonoids, phenolic acids), and other substances beneficial to human health are present in apples (Sharma, S., & Rao, 2012). (Kim, M. Y. et al,2012). *Cucurbita pepo* also contains oleic acid, which is significantly more concentrated than linoleic and palmitic acids. There is also palmitic acid. Only magnesium and manganese showed remarkably stable quantities across seed samples of different origins; all other endogenous mineral concentrations showed significant variation (Idouraine, A., Kohlhepp

et al ,1996). Triglyceride fatty acid combination, tetrahydro-thiophene, linoleic acid, calotropoleanly ester, and cholesterol oleanen-3-ol are only a few of the many important components found in *Cucurbita pepo* fruit extracts after chromatographic purification. Each element shown antimicrobial, antiviral, and anticancer activities (Badr, S. E et al,2011). Scientists have taken an interest in pumpkin because of its long history of use in traditional medicine for a wide range of medical issues (including as an anti-diabetic, anti-hypertensive, anti-tumor, immunomodulatory, anti-bacterial, anti-hypercholesterolemic, intestinal antiparasitic, anti-inflammatory, and antalgic agent) (Jafarian, A., Zolfaghari, B., & Parnianifard, M, 2012). Pumpkin extracts, which may contain chemical and pharmacological components, have been linked to a number of potential health advantages. Several investigations have shown that these compounds have antimicrobial effects (Nawirska-Olszaska et al, 2013). Over the past few decades, there has been a steady increase in the prevalence of harmful multidrug-resistant bacterial strains that are resistant to a wide variety of antibiotics. There is an urgent need for the discovery of new antimicrobial drugs to combat this increasing problem. In providing us with a wide variety of antimicrobial chemicals, plants are by far the most important sources of antibiotics. Plants make what are called secondary metabolites, which are beneficial therapeutic chemicals. There are many different types of these, such as terpenoides, xanthones, benzophenones, coumarins, alkaloids, saponins, tannins, flavonoids, glycolipids, and galactolipids (Belguith, H., Kthiri et al,2010).

1.1 *Cucurbita pepo* Plants Phytochemical Composition:

Carotenoids, including -carotene, -carotene, and -carotene, and neoxanthin, violaxanthin, lutein, zeaxanthin, taraxanthin, luteoxanthin, auroxanthine, neurosporene, flavoxanthin, flavoxanthin, 5,6,50,60-diepoxy-beta-cryptoxanthin, and flavo Specifically, (Azevedo-Meleiro, C. H., & Rodriguez-

Amaya, D. B., 2007). The total carotenoid concentration in *C. moschata* fruit ranged from 234.21 g/g to 404.98 g/g (de Carvalho et al, 2012), whereas in *C. pepo* fruit it was between 171.9 g/g and 461.9 g/g. Carotenoids in Cucurbita species such as Cucurbita moschata, Cucurbita pepo, and Cucurbita maxima have been the subject of several research studies and publications. To cite: (Gutierrez, R. P. 2016). Vitamin E is plentiful in edible Cucurbita seeds (49.49 g/g to 92.59 g/g), with -tocopherol predominating over α -tocopherol (Chandrika et al ,2010). (Chandrika et al, 2010) Studying the work of Yang et al (Kim, M. Y., Kim et al,2012). Flavonoid levels were undetectable in both immature and mature *C. maxima* fruit (0.05 mg/100 g). The plant's shoots and buds were the only portions that showed any signs of improvement. Total phenolic content of *C. maxima* was determined to be 46.43 mg gallic acid equivalent (GAE) per 100 g by Yang, R. Y., Lin, S., and Kuo, G. (2008), based on research conducted by Sreeramulu and Raghunath. Another study looked at the flavonoid content of *C. maxima* and found Researchers found that p-hydroxybenzoic acid was the most common phenolic acid, making up 34.72 percent, 67.38 percent, and 51.80 percent of the total phenolic acid content in the whole dehulled seed, kernels, and hulls, respectively. Apart from p-hydroxybenzoic acid. Here is a rundown of the most abundant phenolic compounds, from most abundant to least: Caecic, ferulic, and vanillic acids can be found in small amounts in dehulled, whole seeds. Trans-synaptic, protocatechuic, and p-hydroxybenzaldehyde were all present in high concentrations in the pumpkin seeds of the hulled variety, whereas p-hydroxybenzaldehyde, vanillic, and protocatechuic acids were found in high concentrations in the pumpkin hulls.

2. Materials and methods

2.1 . Materials

Chemical Substances

Ethanol (BDH England) 99%, Methanol (BDH England) 99%, 1- butanol (BDH England) 99%, Chloroform (BDH England) 99%, Ethyl acetate (BDH England) 99%, ferric Chloride (BDH England) 99%,

that kaempferol, at a concentration of 371.0 mg/kg of dry weight, was the only flavonoid present in this species (Sreeramulu, D., & Raghunath, M.) (2010). (2010). *C. pepo* was found to have a negligible polyphenol content. The fresh fruit of this plant was found by Mongkolsilp et al. to have just 0.02 mg GAE per 100 mg sample (Miean, K. H., & Mohamed, S. 2001). However, a group led by Iswaldi and colleagues (Mongkolsilp, S., Pongbupakit et al, 2004) has reported for the first time a list of 34 polyphenols in the fruit of *C. pepo.*, including a variety of flavonoids and maybe other unidentified polar compounds. Additionally, *C. pepo* flowers may store a substantial amount of phenolic compounds. Andjelkovic et al. (Iswaldi, I., Gómez-Caravaca et al., 2013) investigated the phenolic content of six different pumpkin (*C. pepo*) seed oils, and they detected tyrosol, vanillic acid, vanillin, ferulic acid, and luteolin. The average tyrosol content was 17.7 milligrams per kilogram, and it ranged from 1.6 to 17.7 milligrams per kilogram. Percin et al. looked at the phenolic acid levels in *C. pepo* seeds (Andjelkovic, M.,et al,2010). Hydrochloric acid 1%, Potassium iodide, lead acetatehydrat, Sodium Hydroxid.

2.2 Instruments Equipments:

Rotary evaporator, Oven 2004 Japan – Hirayama, Ultrasonic bath (England), UV-VisibleSpectrophotometer,double+90Plus(Japan), Vortex mixer, Hot plate stirrer, Drying Oven, 1H NMR Bruker 500 MHz, Water bath (Shimadzu), 1H NMR Bruker 500 MHz, Water bath, 1H NMR Bruker 500 MHz, 1H NMR Bruker Spectrometer de masse (Japan) Spectrophotometer made by MS; model number: 5975C VL MSD with tripe FTIR Affinity Spectrometer Manufactured by Shimadzu of Japan.

3. Experimental set-up

3.1 Extraction

The Baghdad area of northern Iraq was scoured for specimens of the Cucurbita pepo plant. After being weighed at room temperature for four days, slicing the fruit of Cucurbita pepo into fine pieces, and then powdering it, the total weight was 130 grams, and 100 milliliters of a mixture of ethanol and

water was used to extract the fruit. For six hours at 40 degrees Celsius, the crude solution was regularly stirred in an ultrasonic bath to complete the 70/30 (vol/vol) extraction.

A rotary evaporator was used to remove the solvent and yield the dried crude methanolic extract. After that, 1-butanol, hexane, chloroform, and ethyl acetate were progressively partitioned with the methanolic extract to get fractions in those solvents. Finally, distilled water was used to separate the fractions in a separatory funnel. A residual watery fraction was discovered after this procedure was completed. Solvents were removed using a rotary evaporator operating at a pressure of around 10 mbar to obtain dry fractions..

3.2 Preliminary qualitative phytochemical analysis

1- The test for alkaloids began by adding a few drops of diluted hydrochloric acid to 2 milliliters of each extract in order to acidify it. The next step involved adding 1 milliliter of Dragendorff's reagent. The presence of alkaloids can be determined by the color of the precipitate, which ranges from orange to red (Herborne, J. B. 1973).

2- The tannin content was determined by adding a few drops of lead acetate at a concentration of 10% to 2 milliliters of each extract. Tannins are present when there is a visible white precipitate, which confirms their presence (Herborne, J. B. 1973).

3- Perform an analysis of the saponin content by adding 9 milliliters of distilled water to 1 milliliter of extract that has been placed in a measuring jar. After vigorously shaking the mixture for 15 seconds, the extract was let to sit for 10 minutes.

The presence of saponins can be determined by the formation of a stable foam (1 cm) (Herborne, J. B. 1973).

4- The flavonoid test consisted of treating 4 milliliters of the extract solution with 1.5

milliliters of the methanol solution. After heating the solution, magnesium metal was added to it. This solution was then heated. After the addition of 5 or 6 drops of Con. HCl acid, a color was seen for the flavonoids, and an orange color was seen for the flavones (Herborne, J. B. 1973)

5- Glycoside content was determined by adding a few drops of glacial acetic acid and ferric chloride, together with three to four drops of concentrated sulphuric acid, to one milliliter of each extract. The presence of glycosides can be identified by the coloration of a bluish-green hue (Herborne, J. B. 1973).

6- The Phenol Test: A few drops of lead acetate with a concentration of 10% were added to 2 milliliters of each extract. Tannins are present when there is a visible white precipitate, which confirms their presence (Herborne, J. B. 1973).

7- Test for DPPH (Leaves, L., & Leaves, L. 2014).

3.3. Put the microorganisms to the test:

E. coli, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Proteus mirabilis* are the four multi-resistant bacterial strains that were isolated and identified biochemically from urine specimens of in-patients and out-patients at the General Hospital of AL-Yarmook in Iraq. These strains were used for antimicrobial activity cultures, and their identification was carried out by using standard techniques (Udo, E. E., Al-Sweih, et al, 2008) As tested bacteria, we used multi-resistant strains of bacteria that were resistant to a total of fourteen antibiotics, including two multiresistant strains of *Escherichia coli* that were each resistant to thirteen antibiotics, one multiresistant strain of *Proteus mirabilis* that was resistant to twelve antibiotics, and two strains of *Klebsiella pneumonia* that were resistant to both twelve and thirteen antibiotics (Table 3.2).

3.4. Test for the Activity of Antimicrobials

The Kirby-Bauer diffusion technique was utilized in order to carry out the antimicrobial

susceptibility testing. The tests were conducted using the diffusion method using Muller Hilton agar, and the antibacterial action was measured based on the diameter of the inhibitory area. It was decided to flood the surface of the Muller Hilton agar with two standardized overnight cultures of each strain, each of which contained 108 colony-forming units per milliliter (CFU/ml). The antibiotic

discs impregnated in 20 mg/ml of extract fruit and the controls (Amoxicillin 10 mcg and Gentamycin 10 mcg) were then placed aseptically on the inoculated plates at a fair equidistance apart and let to stand for one hour. After that, the plates were left to incubate at 37 degrees Celsius for 18 hours (Ehinmidu, J. O. ,2003)

4. Results and Discussion

Table 1 phytochemical test of Cucurbita pepo fruit extract

Compound	Detector	Detection Guide	Eethanol extract	aqueous Extract
Alkaloids	Dragendorff's	turbid	+	+
Phenols	Lead acetate	Bluish green color	+	-
Flavonoid	KoH+ Ethanol	yellow color	+	-
Tannines	Lead acetate	White precipitate	+	-
Coumarin	UV	blue color	+	+
Glycosides	Molisch	Red deposit	+	+
Saponins	HgCl2 (1%)	White precipitate		+
Steriod	H2SO4+ CHCl3	Red deposit		+

presence(+) Chemical presence(+) Chemical no presence(-)

The eethanol extract from the Cucurbita pepo fruit plant was tested as an antioxidant using the DPPH method. The findings were given in figure (1), and the overall inhibition was 79.74%. This is in comparison to the 96% inhibition seen in figure (2) for ascorbic acid. According to the findings, both an alcoholic and aqueous extract of the fruit of Cucurbita pepo had a significant impact on the growth of Staphylococcus aureus. In comparison to the control group, the alcoholic extract of fruit demonstrated a greater antibacterial activity

with a high inhibition zone (16 mm), but the aqueous extract of fruit shown only a moderate antibacterial effect (10 mm) (18mm). For Escherichia coli, the effect that it had on this pathogen was mild (Table4.3). When utilizing Aex, a greater effect was observed (16mm),. Aqex and Aex both demonstrated a modest antimicrobial action when tested with Escherichia coli, with just 2mm of inhibitory zone for each extract (Wex and Aex) as compared with the control (Figure 1).

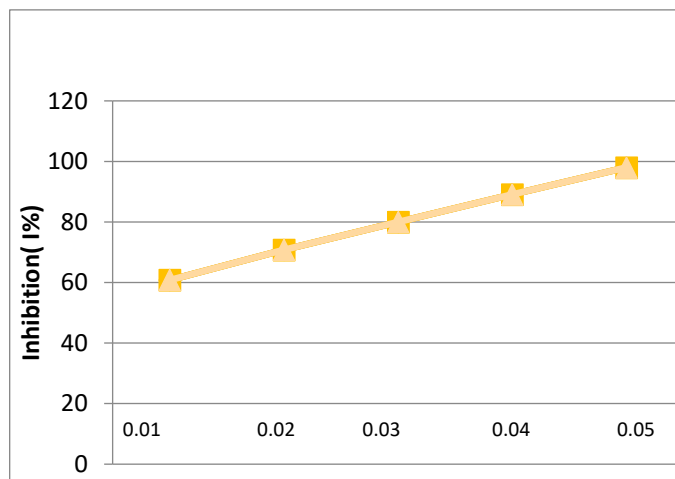


Figure. (1) : Eethanol extract from the Cucurbita pepo fruit plant in DPPH free radical inhibition compared to; ascorbic acid

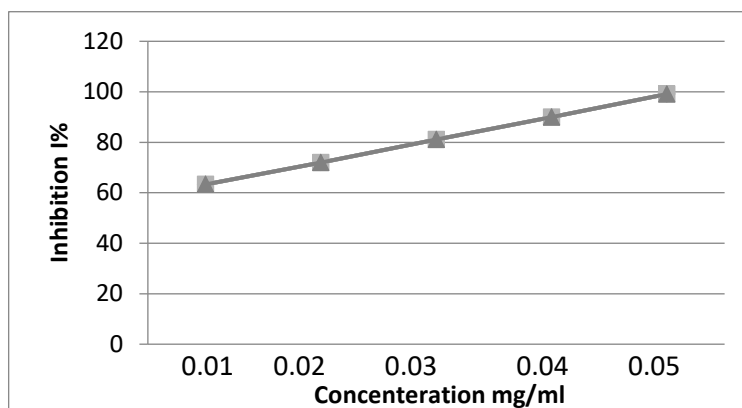


Figure. (2): ascorbic acid inhibition with DPPH free radical inhibition.

These results lead us to conclude that gram-positive *Staphylococcus aureus* can be killed by the fruit extract (cxt) of *Cucurbita pepo*. This suggests that these components may have

a specialized reaction with the murine layer in gram-positive bacteria. The results showed that these ingredients had little and weak impact on gram-negative bacteria.

Table 2: Inhibition zone (mm) of fruit extracts.

Bacteria	fruit		Control	
	Aqex	Aex	Amo	Cn
<i>Staph. aureus</i>	6	8	18	
<i>E. coli</i>	2	2		20
<i>Proteus mirabilis</i>	----	----		18
<i>Klebsiella pneumonia</i>	-----	-----		18

Aqex=aqueous extract, Aex= Alcoholic extract Amoxicillin (Amo), Gentamycin (Cn).

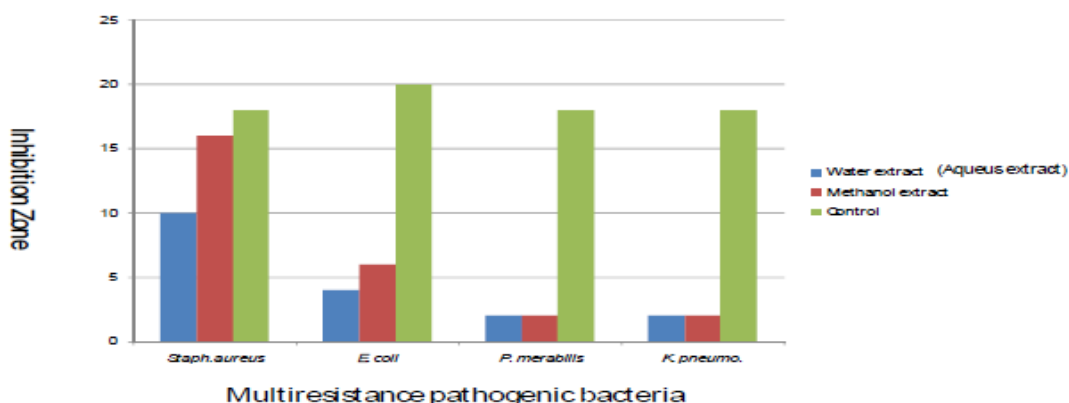


Figure (3): Inhibition zone in millimeters for antimicrobial activity of *Cucurbita pepo* fruit extract in both water and ethanol against multidrug-resistant pathogens.

An earlier study found that two separate oil extractions of *Cucurbita pepo* produced a zone of inhibition on *Staphylococcus aureus* and *Escherichia coli* that was 60% larger than with the control. The results were gathered as stated. Extracts of *Cucurbita pepo* have been

analyzed phytochemically, and they have been found to include bioactive components such as tannins, flavonoids, saponins, cyanogenic glycosides, and cardiac glycosides. It has been suggested that these extracts could be used as sources of antibacterial agents that are both

effective and inexpensive in the treatment of bacterial illnesses (Obi, R. K., Nwanebu, F. C., Ndubuisi, U. U., and Orji, N. M.). This would be in addition to their roles as food additives and supplements (2009).The research involved collecting samples. There were 109 cases of CAUTIs reported, and 35.85% of the isolates grew bacteria when placed in a culture. This was true no matter the age or gender of the patient (14-85 years old). All of the samples taken from patients

with indwelling catheters between January 1, 2019, and December 31, 2020, at three hospitals in Baghdad province (Baghdad Teaching Hospital/The Medicine City, Al-Yarmook Teaching Hospital, and General Al-Karama Hospital) under the supervision of intensivists. Table displays all of the bacterial isolates found using traditional biochemical and microbiological techniques (1). Patil & Patil's research (n.d.) revealed that 27.70% of patients experienced CAUTIs.

Table (3): standard biochemical microbiological tests

Bacteria / Tests	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus mirabilis</i>	<i>Proteus vulgaris</i>
Gram stain	(Rod) -Ve	(Rod) -Ve	(Cocci) +Ve	(Rod) -Ve	(Rod) -Ve	(Rod) -Ve
Indol	(+)	(-)	(-)	(-)	(-)	(+)
Methyle red	(+)	(-/+)	(+)	(-)	(+)	(+)
Vogus-proskauer	(-)	(+)	(+)	(-)	(-)	(-)
Citrate utilization	(-)	(+)	(+)	(+)	(-/+)	(-)
catalase	(+)	(+)	(+)	(+)	(+)	(+)
oxidase	(-)	(-)	(-)	(+)	(-)	(-)
urease	(-)	(+)	(+)	(-)	(+)	(+)
TSI	A\A	A\A	A\A	k\k	K\A	k\A
	H ₂ S -	H ₂ S -	H ₂ S -	H ₂ S -	H ₂ S +	H ₂ S +
	Gas +	Gas +	Gas -	Gas -	Gas +	Gas +
Coagulase	(-)	(-)	(+)	(-)	(-)	(-)
Lactose fermentation	(+)	(+)	(+)	(-)	(-)	(-)
Hemolysis on blood agar	γ\α	γ\α	β	β	γ	γ

The distributions of all samples were as follow: 171/304 (56.25%) of 14-85 years old all females were admitted into intensive care units (ICUs), 133/304 (43.75%) of 14-85 years old all males were admitted into ICUs,

109/304 (35.86%) of positive growth with CAUTIs from all patients included in this study over 1 year, 55/171 (32.16%) isolates of 14-85 years old all females.

	Total	Female	%	Male	%
Total Foley Catheter (n)	304	171	56.25	133	43.75
CaUTI (n)	109	55	50.46	54	49.54
%	35.86%	32.16%		40.60%	

Table (4): The distributions of all samples

(40.60%) of the CAUTI bacterial isolates were found in patients aged between 14 and 85 years old, and all of these patients were male. The distributions of CAUTI's bacterial isolates were as follows: isolates of *Escherichia coli* numbered 43/109 (39.45%),

isolates of *Klebsiella pneumoniae* numbered 21/109 (19.27%), isolates of *Staphylococcus aureus* numbered 17/109 (15.6%), isolates of *Pseudomonas aeruginosa* numbered 15/109 (13.76%), isolates of *Proteus mirabilis* numbered 10/109 (9.17%), and isolates of

Prote (1). *Escherichia coli* was found to be the most common (65.3%), according to Rahman, M.M. and coworkers, followed by species of *Klebsiella* (12%), *Pseudomonas* (9.3%), and *Proteus* (4%). *E. coli* was found in catheterized patients 48.4% of the time, *Klebsiella* spp. 31% of the time, *Staphylococcus aureus* 1.5% of the time, *Pseudomonas aeruginosa* 3.1% of the time, and *Proteus mirabilis* 14% of the time, according to Das R N and colleagues. *Escherichia coli* was found in 31.8% of catheterized patients, *Proteus mirabilis* was found in 18.2% of catheterized patients, *Klebsiella pneumonia* was found in 14.5% of

catheterized patients, and *Pseudomonas aeruginosa* was found in 12.7% of catheterized patients. *Escherichia coli* was found in 54/146 (36.99%) of the isolates from CAUTIs patients, *Klebsiella pneumonia* was found in 24/146 (16.40%) of the isolates, and *Pseudomonas aeruginosa* was found in 14/146 (9.58%) of the isolates. *Escherichia coli* isolates from catheterized patients were found to be 35/135 (31.8%), *Klebsiella pneumonia* isolates were 16/135 (14.5%), *Pseudomonas aeruginosa* isolates were 14/135 (12.7%), and *Proteus mirabilis* isolates were 20/135 (18.2%), as reported by Hanan and colleagues..

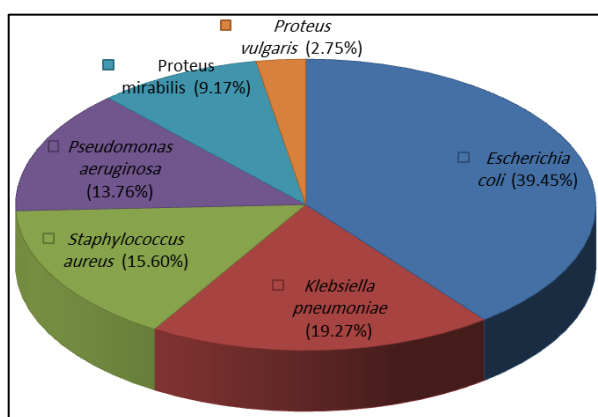


Figure (4) : The distributions of bacterial isolates.

Bacterial isolates were collected from both sexes, and the total number of isolates was then sorted into groups based on age, from 14 to 85 years old (Table 1). (4). Figure from the study shows that CAUTI infection is equally likely to affect males and females, with females making up 55/109 (50.46%) and males making up 54/109 (49.54%). (2). Males made up 3/54 (5.56%) of the bacterial isolates in the 14-20 year old age range, while females made up 1/55 (1.82%) of the isolates in this age range. To compare, only 4/55 (7.27%) of the cases involve women between the ages of 21 and 30, whereas 8/54 (14.81%) involve men in same age range. There are 11.11 percent of men in the 31–40 age range who are 6/54 and 9.09 percent of women. Males (13/54, or 24.07%) and females (21/55, or 38.18%) were evenly distributed across the age range of (41-50) years. For men between the ages of 11 and 54 (20.37%) and women

between the ages of 9 and 55 (16.36%), ages 51 to 60. 7/54 males (or 12.96%) and 6/55 females (10.91%) fell into the 61-70 age bracket. 4/54 (7.41%) males and 5/55 (9.09%) females fall within the (71-80) year old age bracket. Two men out of every fifty-four (3.70%) and four women out of every fifty-five (7.27%) make up the group of people older than 80 years old in the data shown in Table. (5). Catheter-related urinary tract infection Worldwide, UTIs account for the largest proportion of healthcare-associated infections. Urinary catheterization is commonly used in hospitals and nursing homes, however it is often done poorly, leading to infections. Hospitals spend a lot of time and money on extras in an attempt to reduce the number of occurrences of catheter-associated urinary tract infections (CaUTIs). Based on research by Melzer and Welch (2013).

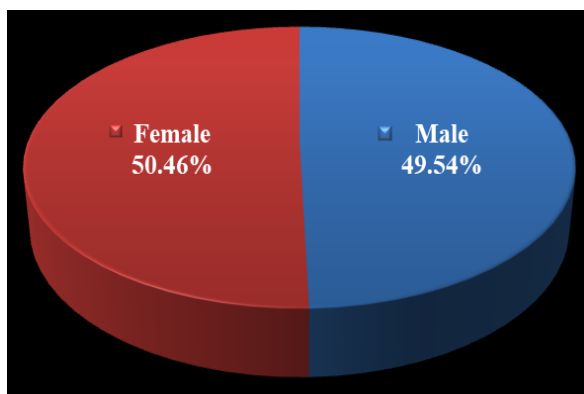


Figure (5): The distributions of bacterial isolates up to sex.

Table (5): The distributions of bacterial isolates up to age groups

Age group	Sex of the patients			
	Male		Female	
	Number	%	Number	%
14-20	3	5.56	1	1.82
21-30	8	14.81	4	7.27
31-40	6	11.11	5	9.09
41-50	13	24.07	21	38.18
51-60	11	20.37	9	16.36
61-70	7	12.96	6	10.91
71-80	4	7.41	5	9.09
>80	2	3.7	4	7.27
Total	54	100	55	100
	54/109 (49.54%)		55/109 (50.46%)	

4.1 Antibiotic susceptibility test (AST)

In this study, the sensitivity of bacteria was tested using various antibiotic classes and generations, including Amoxicillin/clavulanic acid (20/10 g) and piperacillin (100 g), all of which are members of the penicillin family. Four generations of cephalosporins are represented here: cefoxitin (30 g), the second generation; cefotaxime (30 g), the third generation; ceftazidime (30 g), the fourth generation; and cefepime (30 g), the fourth generation. As a member of the monobactam class, aztreonam (30 g) is a useful antibiotic. Carbapenems like meropenem and imipenem, both at 10 milligrams. Doxycycline, a member of the tetracycline family (30 micrograms). The aminoglycosides include the antibiotics amikacin (30 g) and gentamicin (10 g). Quinolones of the first, second, and third

generations are ciprofloxacin (5.0 g), norfloxacin (10.0 g), and levofloxacin (5.0 g), respectively.

Therefore, the results showed that the percentage of E. coli isolates resistant to antibiotics among those isolated from catheterized patients across all ages and both sexes was as follows: 38/43 (88.37%) for Amoxicillin/clavulanic (20/10g), 39/43 (90.70%) for piperacillin (100g), 40/43 (93.02%) for Cefoxitin (30g), 32/43 (74.42%) for cefotaxime (30 (5).

Results showed that E. coli was most resistant to (AmoxicillinClavulanate (20/10 g), Cefoxitin (30 g), and Cefotaxime (30 g), and most sensitive to (Ciprofloxacin (5 g), Norfloxacin (10 g), and Levofloxacin (5 g))

(3). *Escherichia coli* were shown to be sensitive to meropenem and amikacin (Patil & Patil, n.d.), but resistant to cephalosporins, piperacillins, and quinolones. All *E. coli*

35/135 (31.8%) isolates were resistant to cefotaxime (100%), aztreonam (100%), ciprofloxacin (100%), gentamicin (40%) and tetracycline (40%), as reported by Hana et al..

Table (6): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *E. coli*.

Antibiotic µg/disk	<i>Escherichia coli</i> n=43	
	R (%)	S (%)
Amoxicillin/Clavulanate (20/10 µg)	38 (88.37%)	5 (11.63%)
Piperacillin (100µg)	39 (90.70%)	4 (9.30%)
Cefoxitin (30µg)	40 (93.02%)	7 (16.28%)
Cefotaxime (30µg)	32 (74.42%)	11 (25.58%)
Ceftazidime (30µg)	30 (69.77%)	13 (30.23%)
Cefepime (30µg)	29 (67.44%)	14 (32.56%)
Aztreonam (30µg)	37 (86.05%)	6 (13.95%)
Meropenem (10µg)	28 (65.12%)	15 (34.88%)
Imipenem (10 µg)	24 (55.81%)	19 (44.19%)
Doxycycline (30µg)	31 (72.09%)	12 (27.91%)
Amikacin (30µg)	34 (79.07%)	9 (20.93%)
Gentamicin (10µg)	35 (81.40%)	8 (18.60%)
Ciprofloxacin (5µg)	9 (20.93%)	34 (79.07%)
Norfloxacin (10µg)	14 (32.56%)	29 (67.44%)
Levofloxacin (5µg)	12 (27.91%)	31 (72.09%)

And the results showed that the levels of resistance of *Klebsiella pneumoniae* isolates these isolated from catheterized patients for all age groups and both sexes to the antibiotics are 38/43 (88.37%) for Amoxicillin/clavulanic (20/10g), 39/43 (90.70%) for piperacillin (100g), 40/43 (93.02%) for Cefoxitin (30g),

32/43 (74.42%) for cefotaxime (30g), 30/ (6). In their study, Hanan and colleagues found that *Klebsiella pneumoniae* isolates were resistant to aztreonam, amikacin, gentamicin, cefotaxime, tetracycline, and ciprofloxacin with respective percentages of 100%, 60%, 80%, 100, and 60%.

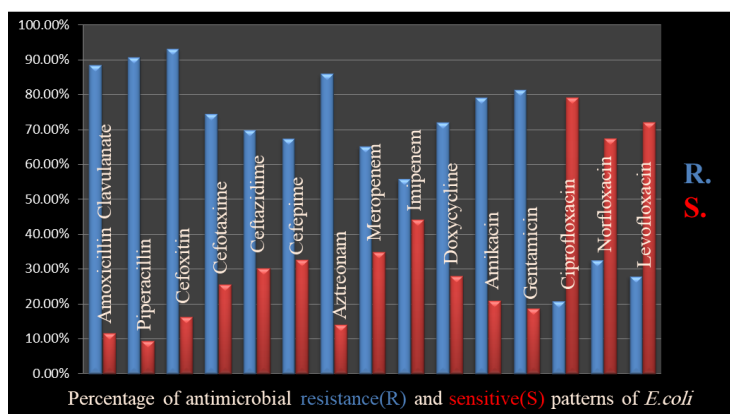


Figure (6): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *E. coli*.

Table (7): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *Klebsiella pneumoniae*.

Antibiotic µg/disk	<i>Klebsiella pneumoniae</i> n=21	
	R (%)	S (%)
Amoxicillin/Clavulanate (20/10 µg)	15 (71.43%)	6 (28.57%)
Piperacillin (100µg)	18 (85.71%)	3 (14.29%)
Cefoxitin (30µg)	12 (57.14%)	9 (42.86%)
Cefotaxime (30µg)	9 (42.86%)	12 (57.14%)
Ceftazidime (30µg)	10 (47.62%)	11 (52.38%)
Cefepime (30µg)	10 (47.62%)	11 (52.38%)
Aztreonam (30µg)	12 (57.14%)	9 (42.86%)
Meropenem (10µg)	11 (52.38%)	10 (47.62%)
Imipenem (10 µg)	2 (9.52%)	19 (90.48%)
Doxycycline (30µg)	12 (57.14%)	9 (42.86%)
Amikacin (30µg)	12 (57.14%)	9 (42.86%)
Gentamicin (10µg)	14 (66.67%)	7 (33.33%)
Ciprofloxacin (5µg)	1 (4.76%)	20 (95.24%)
Norfloxacin (10µg)	4 (19.05%)	17 (80.95%)
Levofloxacin (5µg)	3 (14.29%)	18 (85.71%)

So the results showed the highest resistance of *Klebsiella pneumoniae* for (Amoxicillin\Clavulanate (20/10 µg), piperacillin (100µg), and gentamicin (10µg)) antibiotics and highest sensitivity of *Klebsiella pneumoniae* for (imipenem (10µg), Ciprofloxacin (5µg), and Levofloxacin (5µg)) antibiotics as shown in

Table (6) and Figure (4). (Patil & Patil, n.d.) mentioned *K. pneumoniae* were best sensitive to Meropenem, Imipenem, Aminoglycosides, Ceftazidime with moderate resistance to quinolones, ceftriaxone, Cefepime, Amoxicillin\Clavulanate.

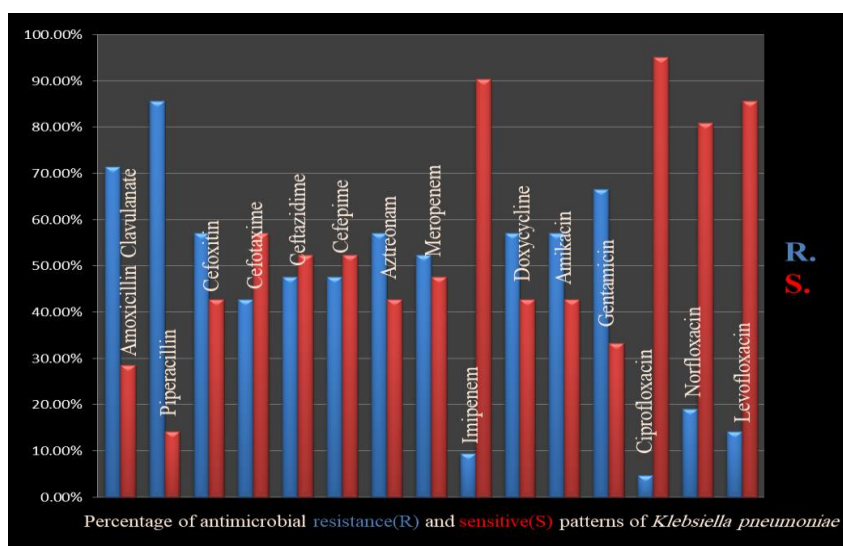


Figure (7): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *Klebsiella pneumoniae*.

And the results showed the resistance levels of *Staphylococcus aureus* isolates these isolated from catheterized patients for all age groups and both sexes to the antibiotics are

15/17 (88.24%) for Amoxicillin/clavulanic (20/10µg), 17/17 (100%) for piperacillin (100µg), 13/17 (76.47%) for Cefoxitin (30µg), 14/17 (82.35%) for cefotaxime (30µg), 12/17

(70.59%) for ceftazidime (30µg), 13/17 (76.47%) for cefepime (30µg), 15/17 (88.24%) for Aztreonam (30µg), 8/17 (47.06%) for Meropenem (10µg), 6/17 (35.29%) for imipenem (10µg), 13/17 (76.47%) for Doxycycline (30µg), 13/17 (76.47%) for Amikacin (30µg), 14/17 (82.35%) for gentamicin (10µg), 8/17 (47.06%) for Ciprofloxacin (5µg), 6/17 (35.29%) for norfloxacin (10µg), and 9/17 (52.49%) for levofloxacin (5µg) as shown in Table (7).

So the results have shown the highest resistance of *Staphylococcus aureus* for (Amoxicillin\Clavulanate (20/10 µg), piperacillin (100µg), and Aztreonam (30µg)) antibiotics and highest sensitivity of

Staphylococcus aureus for (imipenem (10µg), Ciprofloxacin (5µg), and norfloxacin (10µg)) antibiotics as showed in Table (8) and Figure (8).

And the results showed the resistance levels of *Pseudomonas aeruginosa* isolates these isolated from catheterized patients for all age groups and both sexes to the antibiotics are 13/15 (86.67%) for Amoxicillin/clavulanic (20/10µg), 14/15 (93.33%) for piperacillin (100µg), 12/15 (80%) for Cefoxitin (30µg), 11/15 (73.33%) for cefotaxime (30µg), 12/15 (80%) for ceftazidime (30µg), 10/15 (66.67%) for cefepime (30µg), 11/15 (73.33%) for Aztreonam (30µg), 9/15 (60%) for Meropenem (10µg), 10/15 (66.67%) for imipenem (10µg), 13/15 (86.67%)

Table (8): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *Staphylococcus aureus*.

Antibiotic µg/disk	<i>Staphylococcus aureus</i> n=17	
	R (%)	S (%)
Amoxicillin/Clavulanate (20/10 µg)	15 (88.24%)	2 (11.76%)
Piperacillin (100µg)	17 (100%)	0 (0.00%)
Cefoxitin (30µg)	13 (76.47%)	4 (23.53%)
Cefotaxime (30µg)	14 (82.35%)	3 (17.65%)
Ceftazidime (30µg)	12 (70.59%)	5 (29.41%)
Cefepime (30µg)	13 (76.47%)	4 (23.53%)
Aztreonam (30µg)	15 (88.24%)	2 (11.76%)
Meropenem (10µg)	8 (47.06%)	9 (52.94%)
Imipenem (10 µg)	6 (35.29%)	11 (64.71%)
Doxycycline (30µg)	13 (76.47%)	4 (23.53%)
Amikacin (30µg)	13 (76.47%)	4 (23.53%)
Gentamicin (10µg)	14 (82.35%)	3 (17.65%)
Ciprofloxacin (5µg)	8 (47.06%)	9 (52.94%)
Norfloxacin (10µg)	6 (35.29%)	11 (64.71%)
Levofloxacin (5µg)	9 (52.94%)	8 (47.06%)

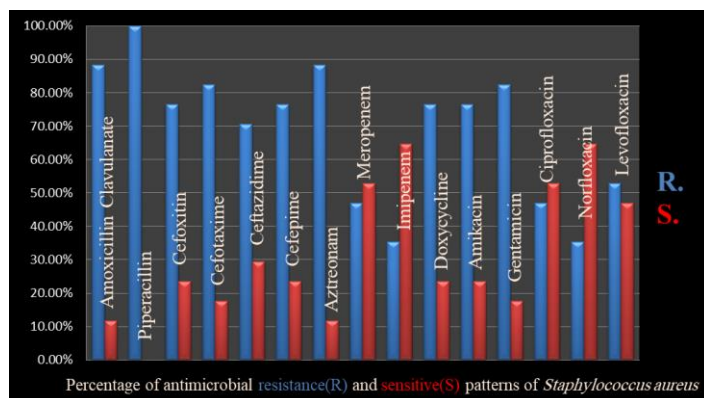


Figure (8): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *Staphylococcus aureus*.

for Doxycycline (30µg), 15/15 (100%) for Amikacin (30µg), 15/15 (100%) for gentamicin (10µg), 6/15 (40%) for Ciprofloxacin (5µg), 8/15 (53.33%) for norfloxacin (10µg), and 6/15 (40%) for levofloxacin (5µg) as shown in Table (8). (Patil & Patil, n.d.) mentioned *Pseudomonas aeruginosa* isolates were resistant to

aztreonam, amikacin, gentamicin, cefotaxime, tetracycline, and ciprofloxacin with intermediate resistance to quinolones, ceftriaxone, and Amox-Clav, according to Hanan and et. al findings. 's *Pseudomonas aeruginosa* 100%, 40%, 100%, 100%, 60%, and 40%, respectively.

Table (9): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *Pseudomonas aeruginosa*.

Antibiotic µg/disk	<i>Pseudomonas aeruginosa</i> n=15	
	R (%)	S (%)
Amoxicillin Clavulanate (20/10 µg)	13 (86.67%)	2 (13.33%)
Piperacillin (100µg)	14 (93.33%)	1 (6.67%)
Cefoxitin (30 µg)	12 (80.00%)	3 (20.00%)
Cefotaxime (30µg)	11 (73.33%)	4 (26.67%)
Ceftazidime (30µg)	12 (80.00%)	3 (20.00%)
Cefepime (30µg)	10 (66.67%)	5 (33.33%)
Aztreonam (30µg)	11 (73.33%)	4 (26.67%)
Meropenem (10µg)	9 (60.00%)	6 (40.00%)
Imipenem (10 µg)	10 (66.67%)	5 (33.33%)
Doxycycline (30µg)	13 (86.67%)	2 (13.33%)
Amikacin (30µg)	15 (100.00%)	0 (0.00%)
Gentamicin (10µg)	15 (100.00%)	0 (0.00%)
Ciprofloxacin (5µg)	6 (40.00%)	9 (60.00%)
Norfloxacin (10µg)	8 (53.33%)	7 (46.67%)
Levofloxacin (5µg)	6 (40.00%)	9 (60.00%)

According to the findings, *Pseudomonas aeruginosa* exhibited the highest level of resistance to the antibiotics piperacillin (100 g), amikacin (30 g), and gentamicin (10 g),

and the highest level of sensitivity to the antibiotics ciprofloxacin (5 g), norfloxacin (10 g), and levofloxacin (5 g), as shown in Table (10) and Figure (9).

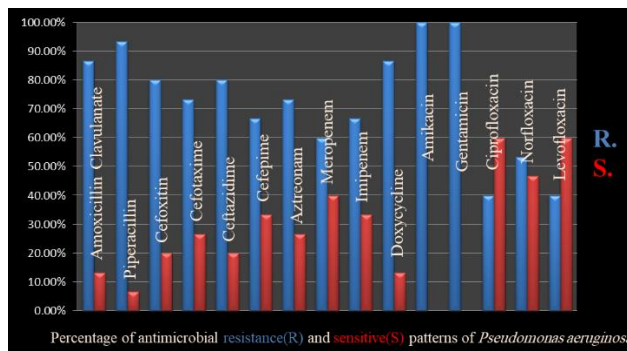


Figure (9): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *Pseudomonas aeruginosa*.

And the findings demonstrated that the resistance levels of *Proteus mirabilis* isolates that were isolated from catheterized patients for all age groups and both sexes to the antibiotics are as follows: 4/10 (40%) for Amoxicillin/clavulanic (20/10g), 9/10 (90%) for piperacillin (100g), 8/10 (80%) for Cefoxitin (30g), 3/10 (30%) for cefotaxime (30g), 6/10 (60%) for ce (9). According to Hanan and colleagues' findings, *Proteus mirabilis* isolates exhibited a high level of resistance to aztreonam, amikacin, gentamicin, cefotaxime, tetracycline, and ciprofloxacin, with respective percentages of 100%, 60%, 80%, 100%, 100%, and 60%.

According to the findings, *Proteus mirabilis* exhibited the highest level of resistance to the

antibiotics piperacillin (100 g), cefoxitin (30 g), and ceftazidime (30 g), while also demonstrating the highest level of sensitivity to the antibiotics ciprofloxacin (5 g), norfloxacin (10 g), and levofloxacin (5 g), as shown in Table (10) and Figure 10.

And the findings demonstrated that the resistance levels of *Proteus vulgaris* isolates that were isolated from catheterized patients for all age groups and both sexes to the antibiotics are as follows: 4/10 (40%) for Amoxicillin/clavulanic (20/10g), 9/10 (90%) for piperacillin (100g), 8/10 (80%) for Cefoxitin (30g), 3/10 (30%) for cefotaxime (30g), 6/10 (60%) for cef.

Table (10): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *Proteus mirabilis*.

Antibiotic µg/disk	<i>Proteus mirabilis</i> n=10	
	R (%)	S (%)
Amoxicillin Clavulanate (20/10 µg)	4 (40.00%)	6 (60.00%)
Piperacillin (100µg)	9 (90.00%)	1 (10.00%)
Cefoxitin (30 µg)	8 (80.00%)	2 (20.00%)
Cefotaxime (30µg)	3 (30.00%)	7 (70.00%)
Ceftazidime (30µg)	6 (60.00%)	4 (40.00%)
Cefepime (30µg)	3 (30.00%)	7 (70.00%)
Aztreonam (30µg)	2 (20.00%)	8 (80.00%)
Meropenem (10µg)	1 (10.00%)	9 (90.00%)
Imipenem (10 µg)	3 (30.00%)	7 (70.00%)
Doxycycline (30µg)	2 (20.00%)	8 (80.00%)
Amikacin (30µg)	4 (40.00%)	6 (60.00%)
Gentamicin (10µg)	2 (20.00%)	8 (80.00%)
Ciprofloxacin (5µg)	0 (0.00%)	10 (100.00%)
Norfloxacin (10µg)	0 (0.00%)	10 (100.00%)
Levofloxacin (5µg)	0 (0.00%)	10 (100.00%)

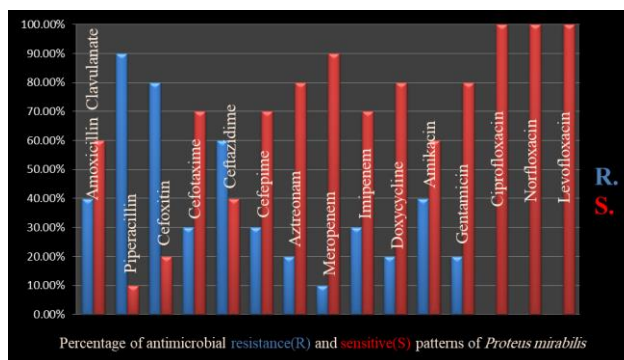


Figure (10): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *Proteus mirabilis*.

Table (11): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *Proteus vulgaris*.

Antibiotic µg/disk	<i>Proteus vulgaris</i> n=3	
	R (%)	S (%)
Amoxicillin Clavulanate (20/10 µg)	3 (100.00%)	0 (0.00%)
Piperacillin (100µg)	2 (66.67%)	1 (33.33%)
Cefoxitin (30 µg)	1 (33.33%)	2 (66.67%)
Cefotaxime (30µg)	1 (33.33%)	2 (66.67%)
Ceftazidime (30µg)	1 (33.33%)	2 (66.67%)
Cefepime (30µg)	1 (33.33%)	2 (66.67%)
Aztreonam (30µg)	1 (33.33%)	2 (66.67%)
Meropenem (10µg)	1 (33.33%)	2 (66.67%)
Imipenem (10 µg)	1 (33.33%)	2 (66.67%)
Doxycycline (30µg)	2 (66.67%)	1 (33.33%)
Amikacin (30µg)	3 (100.00%)	0 (0.00%)
Gentamicin (10µg)	3 (100.00%)	0 (0.00%)
Ciprofloxacin (5µg)	0 (0.00%)	3 (100.00%)
Norfloxacin (10µg)	0 (0.00%)	3 (100.00%)
Levofloxacin (5µg)	0 (0.00%)	3 (100.00%)

According to the findings, *Proteus vulgaris* exhibited the highest level of resistance to (Amoxicillin/clavulanic (20/10 g), Amikacin (30 g), and gentamicin (10 g), while also

exhibiting the highest level of sensitivity to (Ciprofloxacin (5 g), norfloxacin (10 g), and levofloxacin (5 g), as shown in Table (11) and Figure (11).

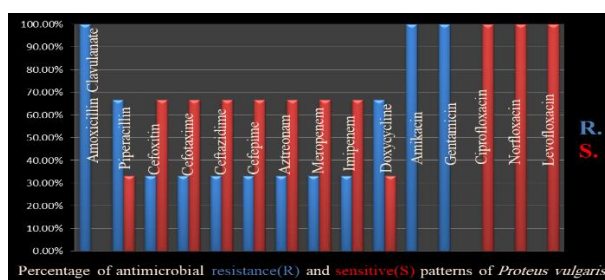


Figure (11): Percentage of antimicrobial resistance(R) and sensitive(S) patterns of *Proteus vulgaris*.

5. CONCLUSIONS AND RECOMMENDATIONS

Antibacterial activity may be found in both the alcoholic and aqueous extracts of the fruit of the Cucurbita pepo plant. This activity is directed against multi-resistant pathogenic bacteria that infect the urinary tract of humans. Because cucurbita pepo is readily available in the area where it is grown and can be purchased for very low costs, it is likely that its usage, among other applications, as an adjuvant to antibiotics will increase, leading to more successful treatment of bacterial illnesses. These findings may imply that the total antimicrobial activities of fruit extract are the consequence of the individual activities of many different components present in the fruit, each of which has an impact that is distinct from the others. It is essential to do research on the efficacy of these components found in the fruit of Cucurbita pepo using modern research techniques in order to evaluate the genuine effect that each component has on multi-resistant microorganisms..

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