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A Study of Antibiotic Sensitivity and the Effect of Corn Silk Extract on the Bacteria that Isolated from Urine of hemodialysis Patients in Kirkuk city, Iraq

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Abstract

Total (157) urine samples were collected from renal failure patients undergoing hemodialysis were evaluated in Center of Dialysis unit\ Kirkuk General Hospital in Kirkuk city, including 40 samples from General Population as control group samples. Gram-negative bacteria were isolated in 37(53.62%) of 69 cases with *Escherichia coli* 27(39.13%), *Klebsiella pneumoniae* and *Proteus mirabilis* 3(4.35 %), *Pseudomonas aeruginosa* 2(2.90 %), 1(1.45%) for both of *Citrobacter freundii* and *Citrobacter koseri*. while Gram positive 32(46.38%) in 69 were isolated, *Staphylococcus saprophuticus* 17(24.63%), *Staphylococcus aureus* 10(14.49%), *Enterococcus faecalis* 3(4.35%) and *Staphylococcus epidermidis* 2(2.90 %). The present study was aimed to identify antimicrobial activity of corn silk extract by different solvents (ethanol and chloroform) extracts (100, 50, 25, 12.5, 6.25 mg/ml each). All the extracts showed variable degree of inhibitory effect by using Agar well-diffusion method against tested bacteria. Ethanol extract showed significant antimicrobial activity against in both Gram positive bacteria and Gram negative bacteria the highest inhibition zone (15-30 mm) was observed in 100 mg/ml of ethanolic extract. In antibiotic sensitivity patterns of the isolated bacteria, both Gram positive and negative bacteria were found to be sensitive 100% towards *imipenem* and showed resistance to another antibiotics used.

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Urinary tract infection,
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Introduction

The urinary system play main role to retain the normal concentrations of water and electrolytes in body fluids, regulates the pH and size of body fluids, and assists control red blood cells (RBCs) production and blood pressure. The urinary system involves of a pair of kidneys, which is reddish-brown color, bean-shaped organ with a slick surface. An adult kidney is about twelve centimeters long, six centimeters wide, three

centimeters thick, and about 150 g weigh which remove waste products from blood and urine, and help regulate confirmed metabolic processes; a pair of ureters tubular shaped, which transport urine from the kidneys to a saclike urinary bladder, that stores a urine; and urethra tubular shaped, which conveys urine to the outside of the body (tanner, 2009 & Shier *et al.*, 2015).

Urinary tract infection (UTI), is the most common infectious diseases and is often complicated by recurrent

and chronic incidents (staerk *et al.*, 2015). *Escherichia coli* is the primary pathogen causing UTIs; it colonizes intestine of the human few hours after birth. The ability of *Escherichia coli* to colonize different anatomical sites is due in part to genome plasticity and remodeling by acquisition or genetic material losses from which it acquired resistance or virulence factors or its ability to adhere to cells. Therefore, horizontal transfer is an important factor in the evolution and adaptation of *Escherichia coli* to different places (López-Banda *et al.*, 2014). *Pseudomonas aeruginosa* is a type of bacteria that colonize kidneys and bladder, which causes infection of the urinary tract and wounds, where it has virulence factors including ability to adhere and the production of exotoxins and resistance to many antibiotics that enable it to cause disease, including urinary tract infection. *Klebsiella pneumoniae* was initially identified as a causative agent for pneumonia, but later was discovered to be cause of different diseases, including urinary tract infection. Because of the synthesis of large amounts of capsular polysaccharide, it produces large mucoid colonies of *Klebsiella* (Al-Agha *et al.*, 2017), and has the potential to cause disease in people with immune suppression. Its virulence factors including capsule, colonizes the human intestines.

Proteus mirabilis has the ability to produce urease by dissolving urea into ammonia and carbon dioxide that make the urine pH alkaline, thus encouraging the formation of kidney stones. Also *staphylococci* group causing UTIs including *Staphylococcus aureus* and *Staphylococcus saprophyticus* (Badria, 2009; López-Banda *et al.*, 2014; Khan *et al.*, 2015).

Medicinal plants have been used over the years worldwide as an alternative treatment for many diseases, Corn Silk is one of them that contains many active compounds such as volatile oils, alkaloids, flavonoids, soap, tannins, phenolic compounds, minerals and vitamins (B, C and K), as they contain antioxidants which commonly used as a traditional medicine in many countries.

It has been used against many pathogens and to treat urinary tract infections, as well as for treatment of high blood pressure and as a diuretic effect, and as previous researches suggested that it can be used to enhance immunity and treatment of bladder pain in people with urinary tract infection. And to kill the germs causing it through the change of urine pH and not allowing the germs to adhere to the cells (Sahib *et al.*, 2012; Morshed and Islam 2015; Žilić *et al.*, 2016; Haslina and Eva, 2017).

The aim of the study

The study aimed to isolate the causative bacteria from urine of hemodialysis patients and to determine the antibacterial activity of corn silk extracts on them.

Materials and Methods

The total 157 urine samples were collected from renal failure patients in the Dialysis unit in General Kirkuk Hospital in Kirkuk city, between February 2017 and May 2017 in different age ranges (between 11-90 years), including 40 samples of general populations used as a control group samples by using sterilized disposable screw cups and samples were delivered to the laboratory and cultured on the MacConkey Agar and Blood Agar then placed in the incubator for 24 hours at 37 ° C to diagnose and identify isolates.

Identification

All urine samples were delivered to the laboratory and were processed as per standard procedure, the primary diagnosis was done by morphological characteristics of the growing colonies on MacConkey Agar and Blood Agar, cultural characteristics including production of dyes and lactose fermentation and blood hemolysis and the type of hemolysis, as well as size of the colonies, pigments, convexity, smell, texture (Badria, 2009). A gram staining slide prepared (Igwe, 2014), and Biochemical tests were done to isolated microorganisms by standard procedures to confirm the diagnosis of bacteria to genus and species according to (Benson, 2001; Badria, 2009; Igwe, 2014; Chowdhury and Parial, 2015; Adnan, 2015).

Antibiotic Susceptibility Test

The antibiotic susceptibility pattern of all the isolates was performed on Mueller–Hinton agar from (Himedia, India) with the antibiotic discs from (bioanalysis, Turkish) were used (Erythromycin, Cefotaxime, Augmentin (Amoxicillin/Clavulanic acid), Nitrofurantoin, Ciprofloxacin, Azithromycin, Amikacin, Cefixime, Tetracycline, Carbenicillin, Amoxicillin, Ceftazidime, Ceftriaxone, Ampicillin, Methicillin, Bacitracin, Cephalothin, Levofloxacin, Imipenem and Nalidixic acid) according to Kirby-Bauer disc diffusion method (Benson, 2001; Kibret and Abera, 2014). Sensitivity was read after incubation aerobically at 37°C for 16-18 hours. After incubation, the plates were examined for growth inhibition zones around the

discs and diameter of zoneinhibition was measured in millimeters (mm) by a ruler, the results were compared with Clinical and laboratory Standards Institute (CLSI, 2011).

The collection and preparation of Corn Silk extraction

Fresh corn (*Zea mays*) were obtained from the local markets in Kirkuk city, and corn silk was separated from the rest of the corn and washed with distilled water and dried, by using filter papers and incubated at 40°C for 5 days, then powdered. Then powdered material was dissolved in organic solvents namely ethanol and chloroform, and using of Agar well-diffusion method (Nessa *et al.*, 2012; Sahib *et al.*, 2012; AL-kufaishi and Al-Mashhedy, 2012; Hilmi *et al.*, 2014; Morshed and Islam, 2015; Haslina and Eva, 2017).

Results and Discussion

157 urine samples were collected during 4 months from patients with renal failure in the unit of the artificial kidney in the Kirkuk General Hospital the age ranged (11-90 years), including 40 Of healthy peoples as a control group in the city of Kirkuk. In case of the (40) urine samples of control group 19(47.5%) was males and 21(52.5%) was females, and only 9(22.5%) showed positive culture growth whereas 31(77.5%) were found to be culture negative. But, in the total 117 samples of renal failure patients the number of males 64(54%) with kidney failure was higher than the females 53(45%), a similar study carried out by (Richa *et al.*, 2016). Furthermore, 69 (49%) samples grew $\geq 10^5$ cfu/ml showed

culture positivity, whereas 48(41%) were found to be culture negative. The study didnot find the similarity with the recent study conducted in (Richa *et al.*, 2016) reporting a 39(26%) culture positivity rate and 111(74%) culture negativity rate. Farther, in (Manhal *et al.*, 2012) which was done in Baghdad showed growth positivity 15(37%). Besides, our study found bacteriuria was highest in the age group of 50-70 years. Whereas in (Richa *et al.*, 2016) bacteriuria was highest in the age group 61-70 years. Which, indicates that chronic kidney failure disease is common among the elderly people with increased rate of infections including UTIs.

In the present study, among 69 (49%) growth cases, *Escherichia coli* 27(39.13%) was found to be one of the most common causes of UTI in patients with renal failure. Followed by 17(24.63%) *Staphylococcus saprophuticus*, 10(14.49%) *Staphylococcus aureus*, and 3(4.35 %) for *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* 2(2.90 %) for *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, 1(1.45%) for both of *Citrobacter freundii* and *Citrobacter koseri*. But in case of control samples among 9 positive cultures *Escherichiacoli* 4(44.45%), *Staphylococcus aureus* 3(33.33 %) and *Klebsiella pneumoniae* 2(22.22%). Also, our results near to (Ali, 2007; Badria, 2009; Richa *et al.*, 2016).

Altogether ten different bacterial isolates were found in this study and the infection rates reached higher in females than males in all isolates as shown in figure 2 and 3, the same was in (Badria, 2009) which also indicated that the number of renal patients with UTI is higher in females than males.

Fig.1 Bacterial Species Isolated from urine of renal failure patients

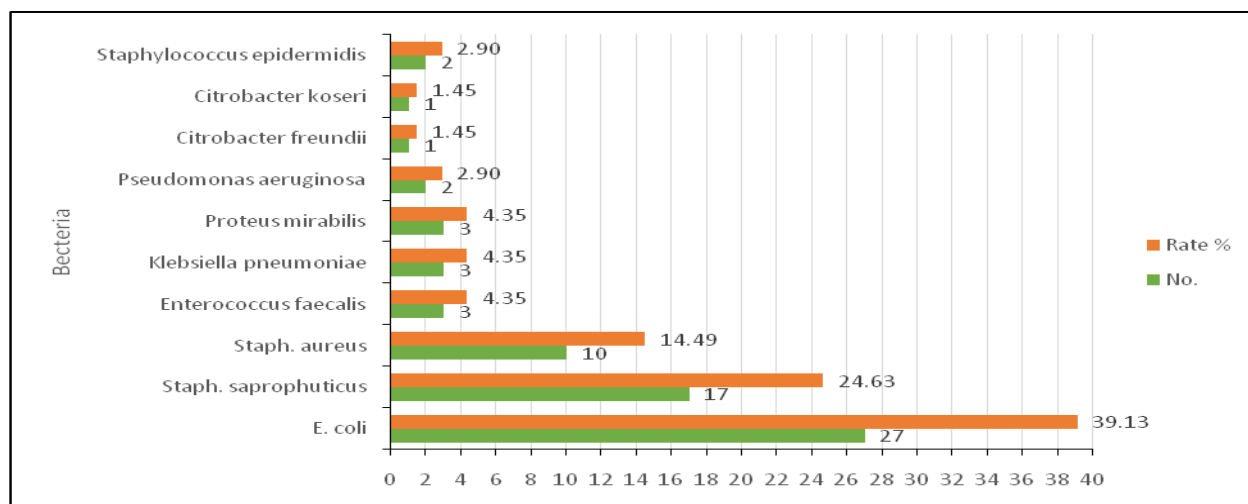


Fig.2 Rate of isolates in male and female of renal failure patients

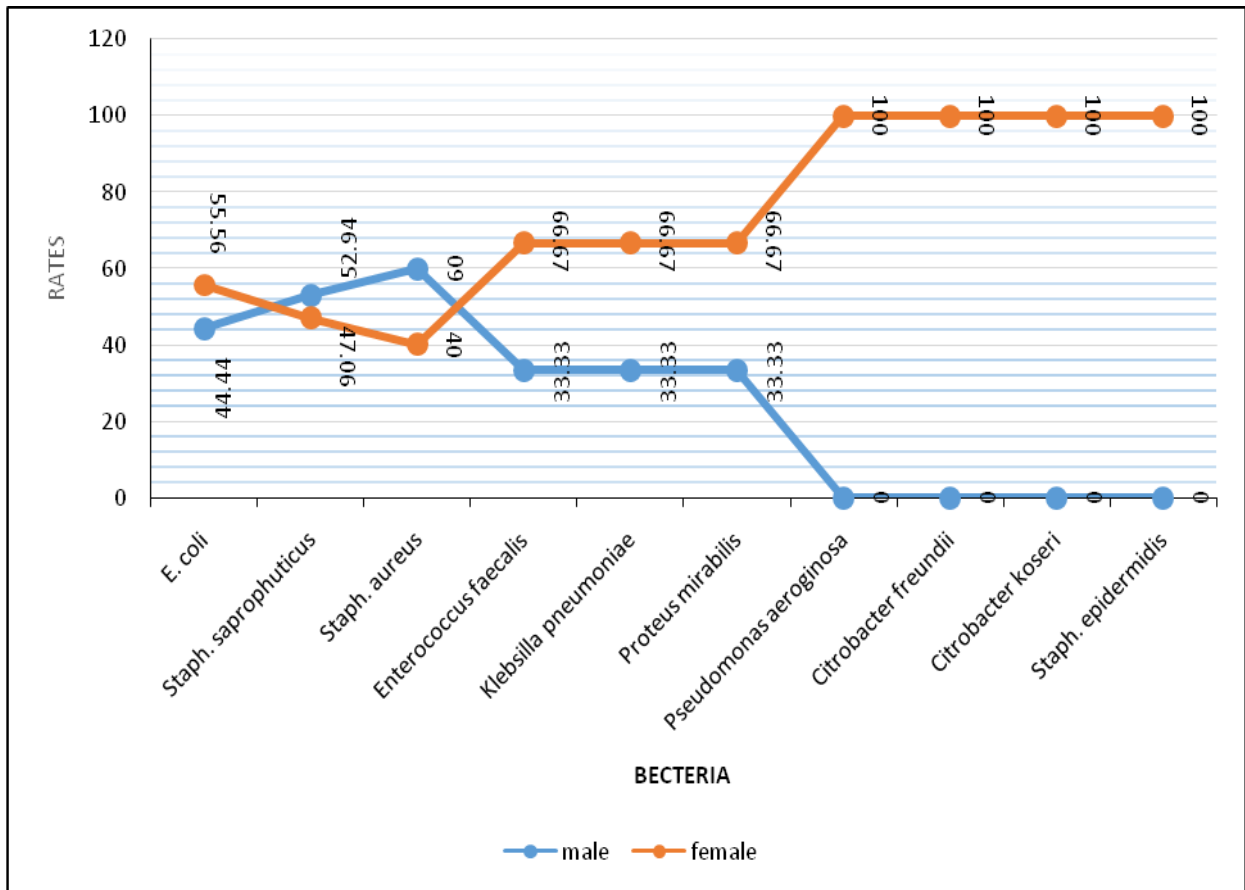


Fig.3 Rate of isolates in male and female of control group

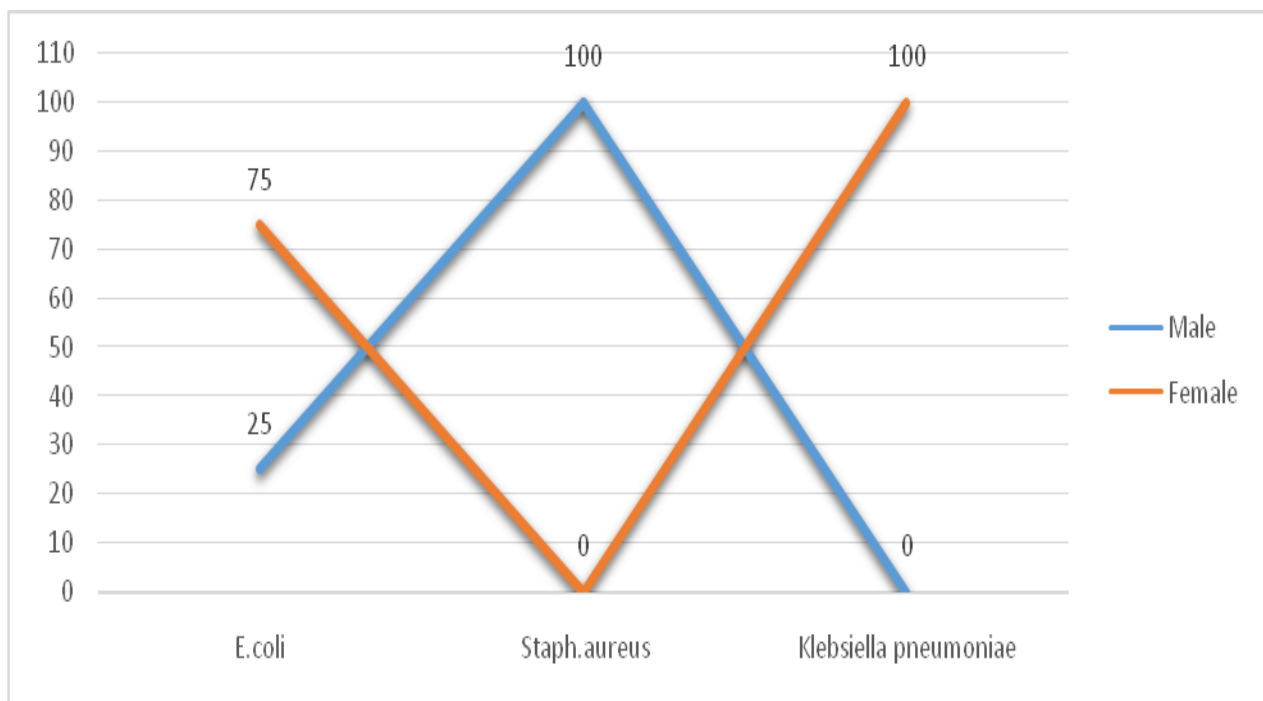


Fig.4 Bacterial Resistance and sensitivity to Antibiotics

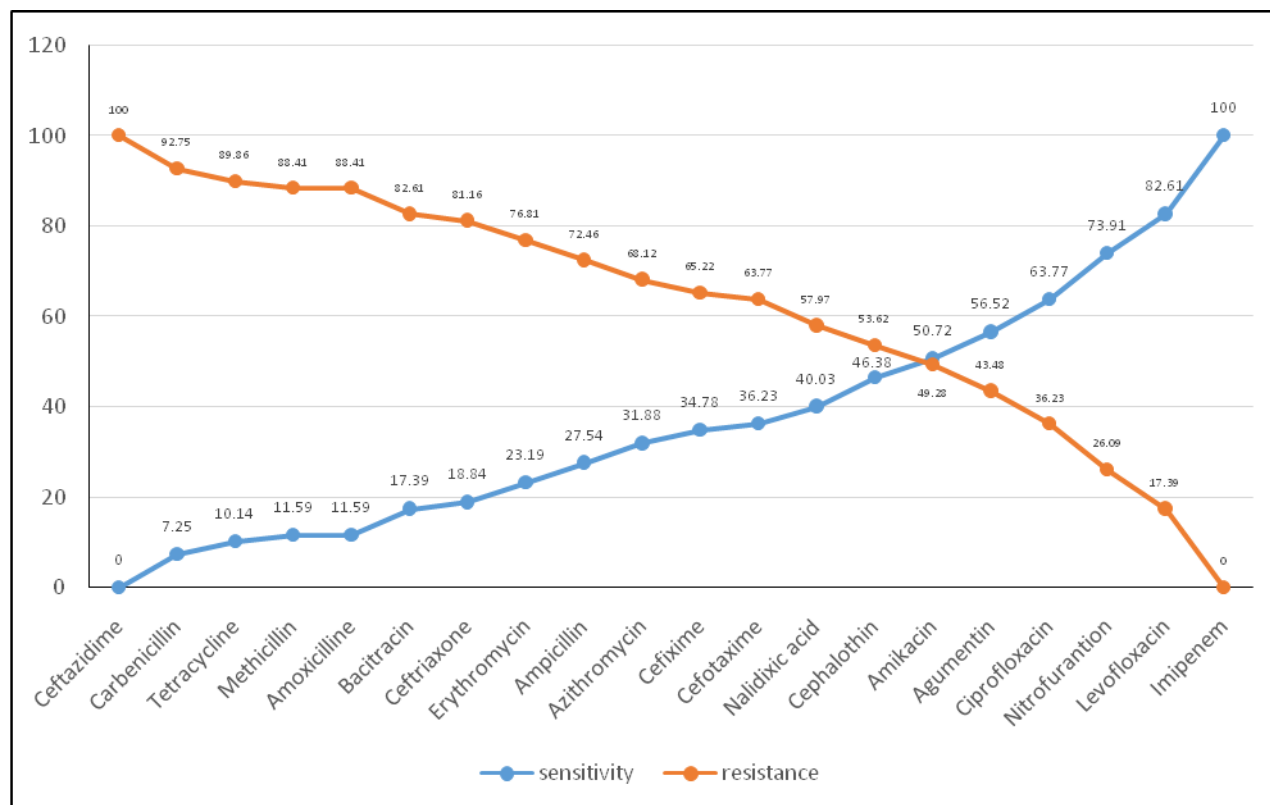


Table.1 Results of the inhibition zone of Ethanolic extract of corn silk

#	Isolations	N0.of isolates	Zone of Inhibition (mm)				
			100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
1	<i>Escherichiacoli</i>	1	20	18	R	R	R
2	<i>Escherichiacoli</i>	2	30	20	R	R	R
3	<i>Proteus mirabilis</i>	1	19	R	R	R	R
4	<i>Klebsiella pneumoniae</i>	1	30	25	R	R	R
5	<i>Pseudomonas aeruginosa</i>	1	30	15	R	R	R
6	<i>Pseudomonas aeruginosa</i>	2	25	R	R	R	R
7	<i>Enterococcus faecalis</i>	1	20	R	R	R	R
8	<i>Enterococcus faecalis</i>	2	20	R	R	R	R
9	<i>Citrobacterfreundii</i>	1	22	14	R	R	R
10	<i>Staphylococcus saprophyticus</i>	1	22	20	R	R	R
11	<i>Staphylococcus saprophyticus</i>	2	22	15	R	R	R
12	<i>Staphylococcus aureus</i>	1	15	15	R	R	R
13	<i>Staphylococcus aureus</i>	2	15	R	R	R	R

Table.2 Results of the inhibition zone of chloroformic extract of corn silk

#	Isolations	No.of isolates	Zone of Inhibition (mm)				
			100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
1	<i>Escherichiacoli</i>	1	17	15	R	R	R
2	<i>Escherichiacoli</i>	2	20	15	R	R	R
3	<i>Proteus mirabilis</i>	1	15	R	R	R	R
4	<i>Klebsiella pneumoniae</i>	1	20	14	R	R	R
5	<i>Pseudomonas aeruginosa</i>	1	15	R	R	R	R
6	<i>Pseudomonas aeruginosa</i>	2	15	R	R	R	R
7	<i>Enterococcus faecalis</i>	1	R	R	R	R	R
8	<i>Enterococcus faecalis</i>	2	R	R	R	R	R
9	<i>Citrobacterfreundii</i>	1	14	R	R	R	R
10	<i>Staphylococcus saprophyticus</i>	1	22	R	R	R	R
11	<i>Staphylococcus saprophyticus</i>	2	20	R	R	R	R
12	<i>Staphylococcus aureus</i>	1	14	R	R	R	R
13	<i>Staphylococcus aureus</i>	2	14	R	R	R	R

In this study Gram negative bacteria was more responsible for causing bacteriuria, which was 37(53.62%) than gram positive ones 32(46.38%).

We found that UTI was higher in dialysis patients compering to the control group for many reasons such as, altered immunity, uremia, low urinary flow rate, and urinary concentration defect, all of which favor growth and multiplication of bacteria(Badria,2009).In this study as shown in Figure 4, both Gram positive bacteria and Gram negative bacterial isolates showed sensitivity towards Imipeneme (100%),Such was also reported by (Chowdhury and Parial, 2015; Devmurari *et al.*, 2015), sensitivity of isolates to Levofloxacin (82.61%).

Nitrofurantion (73.91%) Such was also reported by (Devmurari *et al.*, 2015) suggest that Nitrofurantoin should be recommended for UTI. Ciprofloxacin (63.77%), Amoxicillin/Clavulanic (Augmentin) (56.52%), (Devmurari *et al.*, 2015) also pointed that Amoxicillin/Clavulanic acid should not be used unless indicated by individual patient culture Sensitivities. But all isolates show resistant for ceftazidime which (Sorlozano *et al.*, 2014) a 7-year study also pointed the evolution of resistant by the isolates to ceftazidime, also resistant to Carbenicillin (92.75%), amoxicillin (88.41%), Nalidixic acid (57.97%), Ciprofloxacin (36.23%), Erythromycin (76.81%), Ampicillin (72.46%), a similar was reported by (Ali, 2007). Resistant to tetracycline (89.86%) were similar to (Badria, 2009). In

the use of corn silks extracts we used 13 Gram negative and gram positive bacterial isolates which showed in table.1 and 2, the Ethanol extract showed highest inhibition zones (15-30 mm) in the concentration (100 mg/ml) in all isolates, but in (50mg/ml) 5 isolates were resistant(*Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staph. aureus*, and 2 isolates of *Enterococcus faecalis*) inhibition zones were between (15-25 mm), asimilar was reported by (Feng *et al.*, 2012 & Morshed, 2015) that ethanol showed highest inhibition zones, The inhibitory activity of the growth of bacterial isolates is must be due to The contents of the corn silk, which including phenols and flavonoids, that contain the aromatic ring, with one hydroxyl group Or more, which is polarized it and solubility in polar solvents more than water and the extraction of sample (corn silk) phenolic components depends on the compatibility of components with solvent systems based on principles of solvents dissolve the similarities, furthermore, alcohol is an excellent solvent for most medicinal herbs (Haslina and Eva, 2017).

But in chloroform based corn silk extract the inhibition zones were between (14-22 mm) and 2 isolates of *Enterococcus faecalis* were found to be resistant in the (100 mg/ml), since, in (50mg/ml) only 3 isolates were found to be sensitive (*Klebsiella pneumoniae*, and 2 isolates of *E.coli*) as well as, in the other Concentrations which were used in this study all isolates were found to be resistant. (Sahib *et al.*, 2012) had discovered that corn silk reduce the symptoms in patient with UTI.

These results highly agree with the uses of imipenem, and levofloxacin and Nitrofurantoin as a Therapy to the UTIs. Also, the results agree with the uses of cornsilk as antibiotic, which Ethanolic extracts of corn silk showed the broad spectrum of antibacterial activity on the tested isolates. It exhibited high inhibitory potency against *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Staphylococcus saprophyticus*. This study endorses further studies on medical plants.

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