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The Effect of Nettle Leaves and Corn Silk Extracts on The Isolated Bacteria From Children UTI

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Key words:
Effect , Nettle Leaves , Corn Silk , Isolated Bacteria.

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ABSTRACT

The antibacterial effect of ethanol and aqueous extracts of *Zea mays L* and *Urtica dioica L*, was investigated on bacteria were obtaind from human Urinary Tract Infection (UTI) from Hawlery Ferkary Hospital in Erbil City-Iraq. ,by using the well diffusion technique .A total of 120 isolates bacterial isolates were from 180 UTI patient, isolates were identified ***as E.col***i, ***Staph. albus***, ***Staph .capitis***, ***Staph epidermidis*** , ***Staph. aureus***, ***Pseudo luteola, Pseudo.aeruginosa***, ***P. mirabilis***, ***Morganella.Morganii***, ***K. Pneumonia***, ***K. oxytoca, Micrococcus*** and ***Citrobacter freundii*** following different morphological , physiological and biochemical test. Antibiotic sensitivity of17 commerical antibiotic discs AK, AMC, AMP, AT, ATM, AZM, CAZ, CD, CEP, CFM , CIP, COT, CRO, GM, IPM, MEM and NA was screened by disc diffusion assay , It was observed that the extracts of both plant had an inhibitory effect on the bacteria under study , except ***Pseudo luteola*** to extracts of *Zea mays L* and ***Pseudo luteola, K. Pneumonia*** and ***K. oxytoca*** to extracts of *urtica dioica L*. The inhibitory concentration of *Zea mays L*. extracts was 75% and 100% and *urtica dioica L* extracts was 50%, 75% and 100%. The antibacterial activity of extracts of *U.dioica* showed the best action as inhibitor against test bacteria than the extracts of *zea mays*. The result showed that there is an antibacterial activity of alcoholic extract of *U.dioica* higher sensitivity when compared with standard antibiotics.

تأثير مستخلص نبات حرير الذرة والقريص على البكتيريا المعزولة من اصابات القناة البولية للأطفال

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الخلاصــة

اظهر المستخلص المائي والكحولي لنبات كفشة ذرة ونبات الحريق (القريص) فعالية مثبطة للبكتريا المعزولة من القناة البولية المصابة لاطفال راقدين في مستشفى رابرين في اربيل (العراق) .عزلت الانواع البكتيرية وشخصت بعد زرعها. فكانت 120 عزلة بكتيريه من 180 مريض مصاب بالتهاب القناة البولية منها *E.coli* ، *Staph.albus*، *Staph.capitis* ، *Staph epidermidis* ، *Staph.aureus* ، *Pseudo.luteola, Pseudo.aeruginosa* ، *P.mirabilis*، *Morganella Morganii*، *K. Pneumonia Micrococcus*، و *Citrobacter frenudii*، بعد اجراء الاختبارات الكيمياوية والمظهرية المختلفة، وتم تشخيص حساسية الانواع البكتيرية بطريقة انتشار الاقراص لسبعة عشر مضاد حيوي منها AK، AMC، AMP، AT، ATM، AZM، CAZ ، CD، CEP، CFM، CIP، COT، CRO، GM، IPM ، MEM و N وكذلك شخص التأثير التثبيطي للانواع البكتيريه لكلا النباتين قيد الدراسة ماعدا *Pesudo luteola* تجاه مستخلص كفشة الذرة وكل من *K. oxytoca*،*Pneumonia K.* تجاه مستخلص الحريق، وتراوحت التراكيز المثبطة البكتيرية للمستخلص حريرالذره 75% و100% اما نبات القريص كانت 50% ,75% و100% . اظهر نبات القريص تاثيرا مثبطا للبكتريا افضل من نبات حرير الذره وكذلك كان للمستخلص الكحولي لنبات القريص تاثيرا كبيرا تجاه الانواع البكتيريه قيد الدراسة لدى مقارنته بالمضادات الحياتية المثالية.

الكلمات المفتاحية :
مستخلص ، حرير الذرة ، القريص ، البكتريا المعزولة ، اصابات القناة البولية ، الاطفال .

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Introduction :

More than 1500 herbal preparations are sold as dietary supplements or ethnic traditional medicines (Tambekar, & Dahikar 2011). There are several reports on antimicrobial activity of crude extracts prepared from plants that inhibit various bacterial pathogens, because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, phenolic compounds, which are part of essential oils, Tannin, terpenoids, alkaloids, and flavonoids (Akrayi & Tawfeeq 2012) .The antimicrobial activity of different plant species in various geographical regions in search for new antibiotics (Akrayi & Abdulrahman 2013). In recent years, human pathogens have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Undesirable side effects of certain antibiotics and the emergence of previously uncommon infections led the scientists to look for new antimicrobial substance from various sources, especially from medicinal plants. (Rahman, *etal*., 2010 & Johnson,*etal*.,2010).Urinary tract infection (UTI) is one of the most common causes of hospitalization and referral to outpatient settings in children. It is estimated that at least 3% of girls and 1% of boys experience one episode of UTI before the 11th years of age .About 30-50% of these patients will have another episode within three months to two years,(Adwan, *etal*., 2009). Early treatment of UTI with an effective antibiotic is essential for prevention from long-term consequences. Delay in treatment increases the risk of scar formation in kidneys, (Sharifian, *etal*. ,2006). Common nettle (*Urtica dioica L*), an herbaceous perennial flowering plant, is a member of the *Urticaceae* family. Traditional herbal medicine in the Balkan countries uses stinging nettle leaves in the form of an herbal infusion as a remedy for the treatment of diarrhea, vaginal discharge, internal/external bleeding . Being rich in chlorophyll, nettle leaves are used for the treatment of anemia as well as general well-being, and more recently as natural food colorant (Tucakov,*etal*.,1997)*Zea mays* L. is fine soft thread 10-20 cm long, commonly cultivated in warm climates. It is medicinally used as a mild stimulant, diuretic and demulcent, useful in acute and chronic cystitis and in the bladder irritation of uric acid and phosphatic gravel; has also been employed in Gonorrhoea (Grieve, 1971). In Chinese medicine, *Zea mays L*. are used for oedema of various origins and for hepato-biliary disease (Al-Kareemi , 2012) . The medicinal properties of *Zea mays* L. supported by several authors as it exhibited antioxidant activity (Maksimovic *etal*.,2005) anti-diabetic activity (Guo,*etal*.,) antibiotic activity towards corn earworm (Waiss, *etal*.,1979) resistance to insect attacks (Guevara,*etal*.,)and antitumour activity (Habtemariam,1998). Phytochemical studies on *Zea mays* L. revealed that it contained a number of flavonoids, chlorogenic acid, p-coumaric, ferulic acid, saponins, phytosterols, volatile oil, fixed oil, resin, sugars, allantoin, tannin and minerals (Elliger ,1980& Fazilatun *etal*.,2001).

Materials and Methods :

Bacteria tested:

The bacteria under study (***E.coli***, ***Staph. albus***, ***Staph .capitis***, ***Staph epidermidis, Staph. aureus***, ***Pseudo luteola, Pseudo.aeruginosa***, ***P. mirabilis***, ***Morganella Morganii***, ***K. Pneumonia***, ***K. oxytoca, Micrococcus*** and ***Citrobacter freundii***) were obtained from human Urinary Tract Infection (UTI) from Hawlery Ferkary Hospital in Erbil City–Iraq. The isolates were inoculated on agar to obtain a single colony, which were subcultured on the same medium to check for the purity of the isolated bacteria. Purified isolates were identified using morphological, cultural, and some bio- chemical tests, as a more accurate method for identification (Baron, *etal*., 2007).

Plant Extraction:

Collection and preparation of plant samples both plants Nettle leaves (*Urtica dioical* L) and Corn silk (*Zea mays* L) with tap water using soap powder, and then were washed with distilled water. They were then air dried, powdered, and stored in polyethylene bags in refrigerator at 4°C for further processes (Parekh *etal*.,2005).

Extract preparation:

The ethanol and aqueous extracts of both plants were prepared by maceration method according to the procedure discussed in Reference 11 with slight modification. A total of 10 gm of the plant powder was steeped in 100 ml of each solvent (ethanol and sterilized distilled water) for 3 days, and then filtered through eight-layered muslin cloth. They were further filtered using filter paper (Whatman No.1) and centrifuged at 3000×g for 10 minutes. The supernatants were collected separately and stored in sterile bottles at 4°C. (Parekh *etal*., 2005).

Well diffusion technique:

Screening of antibacterial activity was performed by well diffusion technique (13). The Nutrient agar (NA) plates were seeded with 0.1 ml of the inoculums of each tested organism. The inoculums were spread evenly over the plates with a loop. A standard cork borer of 8-mm diameter was used to cut uniform wells on the surface of the NA, and 100 µl of each concentration (100, 75, 50, 25.12.5 %) of plant extracts or juices was introduced in the well. The plates were incubated for 24 hours at 37°C, and the zones of inhibition was measured to the nearest millimeter (mm).(Olaleye, 2007).

Antibiotic susceptibility test:

Antibiotics susceptibility was carried out on all isolate using Kirby Bauer disc diffusion method. Results were interred operated by measuring the zone of inhibition in mm (Vandepitte ,etal. ,2003).

Result and Discussion:

One handred and eighty (180) Swabs were collected from patients (1-15) years old with obtained UrinaryTract Infection (UTI) from Hawlery Ferkary Hospital in Erbil City- Iraq.120 isolates of bacteria were obtained from these samples and were diagnosed , of which E. coli comprised the highest percentage 56 (46.66%) , Staphylococcus aureus was19 (15.8%) and 0.83-5% for other type of bacteriae as Staph. albus, Staph. capitis, Staph epidermidis, Psuedo luteal Pseudo.aeruginosa, P.mirabilis, Morganella Morganii, K. Pneumonia, K. oxytoca, Micrococcus and Citrobacter freundii(Fig 1). Twenty three (19.6) collected from patients (>1) years old ,18 (7.5-15%) from (1-5) years old ,2-8 (1.66-6.66%) from (6-19).(fig2).Eighty(6.66) female and 40 (33.3%)male(Table1) .

In the present investigation (Table 2) the inhibitory effect of crude aqueous and ethanol extracts of silk of Zea mays against bacterial isolated from UTI, results showed that aqueous extract possess strong antibacterial activity against Staph .capitis, Staph.aureus and Speudo.aeruginosa while moderate against Staph. albus , Morganella.Morganii , E.coli , Staph. epidermidis , Micrococcus , K. Pneumonia and Citrobacter freundii and weak against Proteus. Spp, P. Vulgaries, Klebsiella .Spp, P. mirabilis, K. oxytoca , and Pseudo. Spp . Ethanolic extracts was strong antibacterial activity against, Staph .capitis, Staph. albus, Morganella Morganii and Staph.aureus while moderate against Pseudo. Spp, Citrobacter frenudii, Proteus Spp , E.coli, P. mirabilis , P. Vulgaries, and weak against K. oxytoca at concentration of 100% showed a zone of inhibition in the concentration till reach to height of 16 mm to aqueous extracts and 20 mm to ethanol extracts . All bacterial species included in the test showed susceptibility toward aqueous and ethanol extracts of Silk of Zea mays under study except Pesudo luteola . It was also observed that the ethanolic extracts acted as better antibacterial agents than the aqueous extracts , ethanolic and aqueous extracts had less or no antibacterial activity at concentration of 25% and 12.5%.

The inhibitory effect of crude aqueous extract of Urtica dioica possess strong antibacterial activity against Citrobacter frenudii , Proteus. Spp , P. Vulgaries , Staph. epidermidis , Staph .spp , Staph.aureus , Staph .spp ,Pseudo.aeruginosa and P. Vulgaries and weak against Micrococcus E.coli , Morganella.Morganii and Klebsiella .Spp ,while no effect moderate against any bacteria, Ethanol extracts showed strong antibacterial activity against Proteus. Spp , P. mirabilis , E.coli , Citrobacter freundii , Pseudo. Spp, Staph. Albus , Staph. epidermidis, Staph.aureus, Pseudo.aeruginosa and P. Vulgaries while moderate against , Morganella.Morganii , Micrococcus and Klebsiella .Spp . All bacterial species included in the test showed sensitivity toward aqueous

and ethanolic extracts of Urtica dioica under study except K. oxytoca and K. Pneumonia. It was also observed that the ethanolic extracts were better antibacterial agents than the aqueous extracts, aqueous extracts showed less or no antibacterial activity at concentration of 50, 25 and 12.5 % while at concentration of 100% showed a zone of inhibition which reach to height of 20 mm to aqueous extracts and 38 mm to ethanolic extracts . The antibacterial activity of Urtica dioica L. were better than that of silk of *Zea mays* . It is clear that extracts of Urtica dioica L., especially the ethanolic extracts, had maximum antibacterial activity (Table 2,3) and showed the best action as inhibitor against test bacteria than the extracts of zea mays .This is observed in other studies as well (AL–wasfi, etal.,2012) in which the alcohol extract of U.dioica inhibit the growth of gram positive and negative bacteria and confirm that alcohol extract has more activity than the water extracts , this may be attributed to the difference in the activity of the active compounds when extracted with different solvents (Cowan, 1999) but (Kukrića, etal.,2012) observed the U.dioica extract exhibited best antibacterial activity against the B.subtillis and E.coli with the lowest inhibitory activity against speudo.aeruginosa , water extract had no effect on the growth of pseudo.aeruginosa .the total phenolic content in ethanol extract of U.dioica is high and flavonoid and flavonols is low . It is noted from the present result that the extracts of Zea mays had minimum antibacterial activity , which is don't identical with results obtaind from other researchers (Nessa, etal.,2012). Extract and flavonids of Zea mays showed higher sensitivity against a number of bacteria than gentamysin, However negative results do not indicate that the bioactive constituents are absent or that the plant is inactive. Active compounds may be present in insufficient quantities in the crude extracts; therefore, the dose levels employed would not be sufficient enough to exhibit the inhibitory activity. The lack of inhibitory activity can thus only be proven by using large doses. Alternatively, even if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects on the positive effects of the bioactive agents, thus zeroing the antibacterial activity of the principle. It is also possible that the extracts may be active against other bacterial species that were not tested (Parekh & Chanda, 2008).Antibiotic provide the main basis for the therapy of microbial infection , since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious disease , But worldwide emergence of resistant bacteria has become a major therapeutic problem at the recent time , In addition multidrug resistant strains are also increasingly being isolated from community acquired infections(Fuad, etal.,2012) . All isolate was multi drug resistant against antibiotic. All bacteria of E. coli, resistance to all antibiotic and sensitive to Meropenem , Nitrofuration , Gentamicin , Aztreomycin, and Co-trimoxazole , Morganella morganii resistance to all antibiotic and sensitive to Ampicillin, Aztreomycin, Azithromycin, Ciprofloxacin, Gentamicin, Impinim , Meropenem and Nitrofuration. Micrococcus resistance to all antibiotic and sensitive to Amoxicillin/clavulanic acid , Ampicillin, Aztreomycin ,Cephathiane, Cefixime .Ciprofloxacin , Impinem, Nalidixic acid , Nitrofuration Citrobacter frenudii resistance to all antibiotic and sensitive to AT, Aztreomycin ,Azithromycin, Ceftazidime , Ciprofloxacin , Impinem, Meropenem Nalidixic acid , Nitrofuration. All bacteria Staphylococcus sp. were resistance to Amoxicillin/clavulanic acid , Ampicillin , Aztreomycin , Meropenem , Azithromycin , Ceftazidime , Ceftridacin , Cyclodextrin , AT , Ciprofloxacin , Co-trimoxazole, Cefixime , Cephathiane and sensitive to Amikacin , Impinem, Gentamicin , Nalidixic acid, Nitrofuration. Proteus sp. showed resistance to all antibiotic only Aztreomycin, Ciprofloxacin, Meropenem. Pseudomonas sp. showed sensitive only to Amikacin AT, Ciprofloxacin, Gentamicin Meropenem and Impinem. Klebsiell sp. were resistance against all antibiotic while sensitive against Aztreomycin ,Gentamicin , Meropenem, Nalidixic acid , Nitrofuration and Impinem. In another study showed all isolates were resistant to one or two of cephalosporin, penicillin and β-lactam groups. Resistance of bacteria means that these bacteria have antibiotic resistance genes. Increasing of infections based on antibiotic resistant microorganisms has to be using new and natural antimicrobials. Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development (Adwan,etal.,2009).

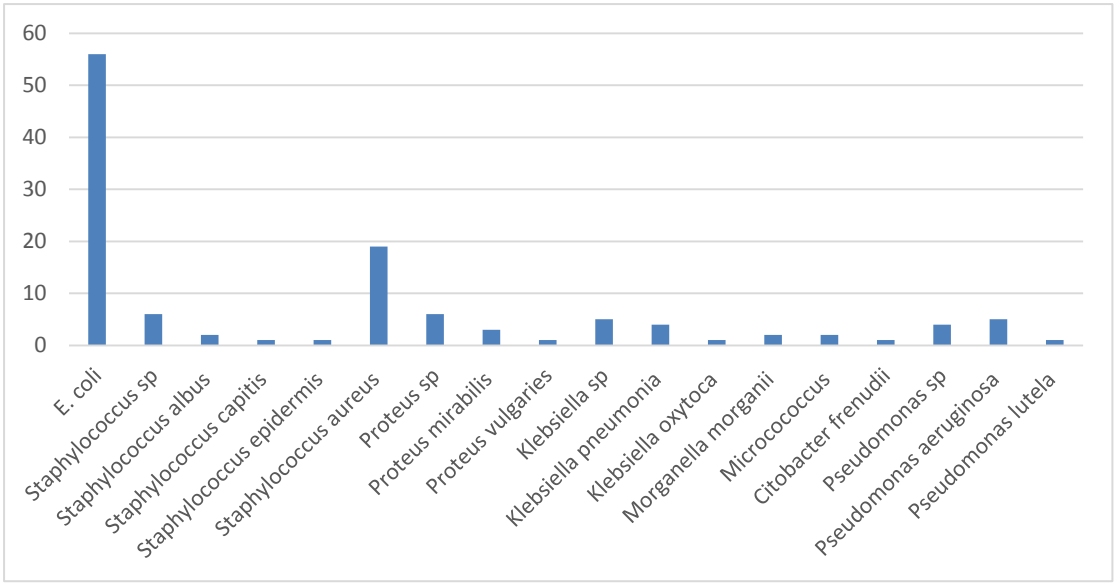

Fig 1: Number of Bacteria isolates from UTI

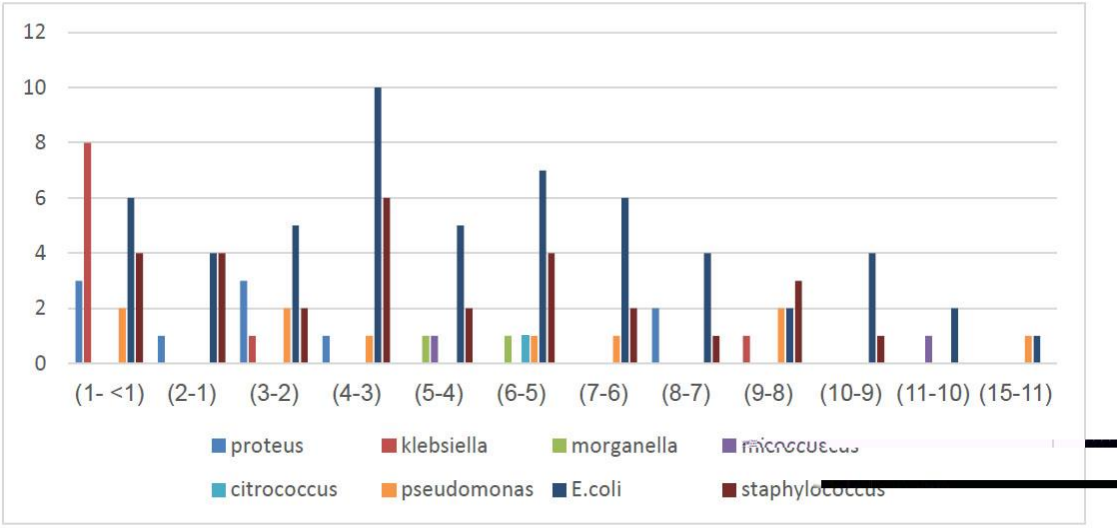

Fig 2: Percentage bacteria isolate of different age

Table 1: Percentage bacteria isolate of different male and femal

Gender	*E.coli*	*Staph.*	*Pseud..*	*Proteus*	*Klebsiella.*	*Morganella*	*Micrococcus*	*Citro.*	Total	%
Female	41	19	3	6	7	2	1	1	80	66.6
Male	15	10	7	4	3	0	1	0	40	33.3
total	56	29	10	10	10	2	2	1	120	99.9

Table 2: Showed the inhibition zone produced by using the extracts of (*Zea mays* L)

Species	Concentration of aqueous extract %					Concentration of alcoholic extract %					IPM
	100	75	50	25	12.5	100	75	50	25	12.5	
E.coli	5	5	2	-	-	30	25	22	7	4	S
Staph .spp	12	10	8	-	-	20	18	12	6	-	S
Staph. Albus	11	11	7	4	-	23	20	12	7	-	S
Staph .capitis	12	10	7	-	-	16	13	9	3	-	S
Staph epidermis	14	13	8	4	-	21	19	10	5	2	S
Staph. Aureus	13	12	9	-	-	19	14	14	8	4	S
Pseudo. Spp	3	-	-	-	-	25	20	20	5	-	S
Pesudo luteola	-	-	-	-	-	8	5	-	-	-	S
Pseudo.aeruginosa	12	5	-	-	-	19	19	12	5	-	S
Proteus. Spp	20	19	12	8	4	38	35	30	18	8	S
P. mirabilis	20	17	11	6	3	32	27	24	14	5	S
P. Vulgaries	12	10	7	-	-	18	15	10	5	-	S
Morganella. Morganii	4	-	-	-	-	10	7	4	-	-	S
Micrococcus	8	6	4	-	-	10	7	4	-	-	S
Klebsiella .Spp	2	2	-	-	-	8	2	-	-	-	S
K. Pneumonia	-	-	-	-	-	-	-	-	-	-	S
K. oxytoca	-	-	-	-	-	-	-	-	-	-	S
Citrobacter freundii	22	20	12	7	2	26	22	15	6	3	S

−No inhibition zone appeared

Table 3: Showed the inhibition zone produced by using the extracts of (*Urtica*

Species	Concentration of aqueous extract %					Concentration of alcoholic extract %					IPM
	100	75	50	25	12.5	100	75	50	25	12.5	
E.col	8	7	6	2	-	9	7	4	2	-	S
Staph .spp	3	3	-	-	-	20	12	8	-	-	S
Staph. Albus	10	7	4	-	-	13	10	9	6	-	S
Staph .capitis	16	11	7	5	-	17	15	10	4	-	S
Staph epidermidis	8	6	-	-	-	8	7	4	-	-	S
Staph. Aureus	12	10	4	-	-	12	11	8	5	2	S
Pseudo. Spp	2	5	-	-	-	10	6	5	-	-	S
Pesudo.luteola	-	-	-	-	-	-	-	-	-	-	S
Pseudo.aeruginosa	12	8	4	-	-	13	11	7	-	-	S
Proteus. Spp	7	5	-	-	-	10	9	5	-	-	S
P. mirabilis	5	3	2	-	-	9	5	4	-	-	S
P. Vulgaries	7	6	5	-	-	9	4	-	-	-	S
Morganella.Morganii	10	9	4	-	-	13	12	8	5	-	S
Micrococcus	8	8	7	-	-	9	7	4	2	-	S
Klebsiella .Spp	6	-	-	-	-	9	7	2	-	-	S
K. Pneumonia	8	6	-	-	-	8	7	4	-	-	S
K. oxytoca	4	-	-	-	-	7	5	-	-	-	S
Citrobacter freundii	8	6	-	-	-	10	8	5	2	-	S

No inhibition zone appear

Table 4: Resistance of bacteria under study to antibiotic

Isolate bacteria	Ak	AMC	AMP	AT	ATM	AZM	CAZ	CD	CEP	CFM	CIP	COT	CRO	GM	IPM	MEM	NA
E. coli	33.9	66	93	40	13	43.7	52	98	59	63	60	21	65	20	2	13	68
Citobacter frenudii	100	100	100	0	0	0	0	100	100	0	100	100	0	100	0	0	0
Micrococcoccus	50	0	0	100	0	100	100	100	0	0	0	100	100	100	0	50	0
Morganella morganii	50	50	0	50	0	0	100	100	50	50	0	50	50	0	0	0	100
Staphylococcus sp	20	66.6	75	0	50	66.6	60	33.3	17	40	80	100	100	100	0	20	50
Staphylococcus albus	0	50	100	0	100	100	100	50	0	100	100	0	0	0	0	0	50
Staphylococcus capitis	100	100	100	0	0	100	100	100	100	0	0	0	100	0	0	0	50
Staphylococcus epidermidis	0	100	100	100	100	100	100	0	100	100	30	100	100	0	0	100	40
Staphylococcus aureus	33.3	33.3	64	100	87	79	95	22.2	25	85	85	10	50	43	7	33	67
Proteus sp	0	60	50	50	0	80	60	75	75	100	0	70	100	100	80	20	70
Proteus mirabilis	70	70	100	100	0	100	33	100	100	0	0	70	50	30	0	0	50
Proteus vulgaries	0	0	100	0	100	0	100	100	100	100	0	0	100	100	0	0	100
Pseudomonas sp	0	100	100	0	100	50	75	100	50	75	0	100	75	0	0	25	100
Pseudomonas aeruginosa	50	100	100	0	0	25	50	100	100	100	0	33	100	0	0	25	70
Pseudomonas luteola	0	100	100	0	100	100	100	100	100	100	0	100	100	0	0	0	0
Klebsiella sp	25	100	100	100	0	60	100	100	100	80	0	75	100	50	0	20	50
Klebsiella pneumonia	50	75	100	100	0	70	70	100	70	70	0	50	70	0	0	30	0
Klebsiella oxytoca	100	100	100	100	0	0	100	100	100	100	50	100	100	0	0	0	0

AK: Amikacin , AMC: Amoxicillin / Clavulanic acid , AMP: Ampicillin , AT , ATM: Aztreomycin , AZM: Azithromycin , CAZ: Ceftazidine , CD: cyclodextrin , CEP :Cephathiane , CFM: Cefixime , CIP: Ciprofloxacin , COT: Co-trimoxazole , CRO: Ceftridacim , GM: Gentanmicin, IPM: Impinem, MEM: Meropenem, NA: Nalidixic acid , NIT: Nitrofuration.

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