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RESEARCH ARTICLE

ANTIMICROBIAL EFFECTS ALCOHOLIC EXTRACT OF YARROW (ACHILLE AMILLEFOLIUM) AGENTS STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI THE MOST COMMON SOURCE OF URINARY TRACT INFECTION.

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Abstract

Effect plant extract to eliminate resistance genes in E. coli andS.aureuswere isolated from urinary tract infections. All the samples in this research were taken from the pregnant woman with the age of (25-45) in Sulaimani city during May (2015-2016). Out of 200 different urine samples, 50 positive cultures were isolated and diagnosis. In (35) samples, E. coli were the predominant microorganisms and were responsible for (17.5%) of the urinary tract infections and the other 15 samples (7.5%) were S.aureus. All isolates were obtained depending on cultural, morphological identification, in addition to, API20Esystem. Theresistances of the isolateswere examined against eleven widely used antimicrobials, which were Cefotaxime(Cef),Ampicillin(Amp),Nitrofurantoin(Nit), Penicillin(Pn), Trimethoprim(Tri),Rifampicin (Rif), Tetracycline(Tet), Gentamycin(Gm),Streptomycin (Str), Ciprofloxacillin (Cip) andAmikacin(Ak). The positive cultures were classified according to their resistance to the above antimicrobials. They were grouped into (8) E. coli antibiograms and (5) *S. aureus* antibiogram. All the isolates were resistant to (Pi). Three isolated for E. coli (E3, E18, and E35) and two isolated for S.aureus (S39 and S45), resisted to all the tested antibiotics while the other samples varied in their resistance.

The ethanolic extracts inMinimum Inhibitory Concentration [MIC] for Achilleamillefolium was (3000) µg/ml. Sub-MIC of plant extract was used as eliminated antibiotic resistant genes of isolates and the results were SMIC 2500 µg/ml of ethanolic extract of Achilleamillefolium eliminated the genes that are responsible for E. coli (E3, E18, E35) isolates (Ak, Str, Rf, Gm, Tri, Tet, Cip), while reduced the percentage of resistance for these antibiotics Amp, Cef, and Pi which ranged between (100%- -10%), SMIC3500 µg/ml of ethanolic extract of Achilleamillefolium affected on (S39) genes responding on Str, Rf, Amp, Ak, Gem, Nit, Tri, Tet, Cef and Cip reduced the percentage (%100, while not affected by resistantgene in (Pi), although the genes in (S45) Responding on Ak. Str, RF, Gem, Nit, Tri, and Tet while not affected on resistant gene in (P, Cef, Cip, and Amp).

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Introduction:-

Escherichia coli (E. coli) andStaphylococcus aureus (S. aureus) are the most common cause of serous human and animal infections. They are the most common causes of soft tissues and skin infections, bone,surgical woundand joint infections. S. aureus is themost common cause of hospital-acquired bacteremia and respiratory tract infections [1]. E. coli is the most common cause of urinary tract infections(UTIs) [2], enteric infections [3], and the systemic infections including,osteomyelitis,bacteremia, nosocomial pneumonia, and infectious arthritisin humans. E. coli is also the leading cause of neonatal meningitis [4]. There is a wide range of antimicrobials affecting and limiting the growth of E. coli. Some examples of the antimicrobial agents include trimethoprim-sulfamethoxazole, fluoroquinolones,lactams,Aminoglycosides, and, that are normally used to treat community and hospital E. coliinfections [5]. However, isolates which are antimicrobial resistant for example, those producing extended-spectrumlactamases and those that are fluoroquinolone-resistant have raised significantly in certain areas that are nosocomial and community-acquired during the 2000's.

E. coli is currently resistant to the several major antimicrobial classes [5]. Penicillin, Cloxacillin, and dicloxacillinare still the antibiotics chosen for the management of serious methicillin-susceptible S. aureus (MSSA) infections, but in patients with penicillin hypersensitivity, first-generation cephalosporin,cephalothin, cefazolin, lincomycin, cephalexin, clindamycin, and erythromycin have important therapeutic roles in less serious MSSA infections. For Seriouse MRSA infections the patient should be treated with parenteral vancomycin or, if the patient is vancomycin allergic they should be treated withteicoplanin [6].

A major health concern is Antibiotic-resistant Staphylococci, since the bacteria can be easily spread in the environment. Currently we can see that Infections caused by methicillin-resistant S. aureus (MRSA) have increased around the globe during the past twenty years [7, 8]. Multiple drug-resistant S. aureus has been frequently retrieved from foodstuffs [1], water and biofilm formation [11] nasal mucosa of humans [12], clinical cases [13] and livestock [14]. Some reports onS. aureusisolate with partial or complete resistance to vancomycin portend a chemotherapeutic in which effective bactericidal antibiotics against this organism may no longer be easily available [9,10].

The World Health Organization concluded that 80% of the world population used plant extracts or their active decisions were used as medicine conventional therapies [15]. Currently Over 50% of all modern clinical drugs are derivatives that arenatural in origin [16]. Iraqi People have traditionally used a number of plants species for treatment of infectious disease and various infection [17]. Furthermore, this research was conducted in order to study and isolate E. coli and S. aureus in urinary tract infection and to test antibiotic-resistant treatment of those two strains with Achilleamillefolium (A. millefolium). A. millefolium grows wildly all around Europe, Asia, North Africa and North America and it is widespread and frequently used in Italian traditional medicine [18]. Its benefits have been known since ancient times and its spread out over many cultures from Europe to Asia: A. millefolium is recommended for the treatment of many different ailments in Greece, in the region of Thessaloniki [19]. In West Azerbaijan, Iran, they use the Infusion of dried flowers is considered, because it is considered that it is suitable for the treatment of dyspepsia, hemorrhoids, gastritis anddysmenorrhea.Another example is leaves, and flowers are used for the treatment of gastric problems and fever in the Parvati valley, West Himalaya, India.

Materials And Methods:-

200samples werecollected from urine of patients were suffering from UTI in Sulaimani City-Iraq during April (2015-2016). The patients were all pregnant woman between the age of 25-40 years. All isolated were diagnosis depending on Morphological,cultural, and biochemical analysis according to (20) in addition to using the API20E system to analyze the bacteria.

1. PlantExtraction: The alcohol extract of A. millefolium was prepared according to the instructions [21].By using usingSoxhlet extraction techniquesfifty gram of dried powder was produced. The plant extract was concentrated to dryness in the vacuum oven at 50°C. a small (1.0g)portion of the sample was sterilized and diluted in 100 mL of sterile distilled water.
2. Determination of MIC: Thedilution method of bacteria isolate inoculum Standardize using standard curve prepared previously as recommended by [22] was used to test The antimicrobial activity of ethanol extracts of A. millefolium. By further diluting the bacterial suspensions we obtained the$1 \text{ X } 10^5$ CUF inoculum. Using spectrophotometric and by the account of viable cells on nutrient agar[29] [23].

3. Determination of antibiotic susceptibility:By using dilution method in agar plate [23] The antimicrobial resistance phenotypes of all isolated bacteria were determined.The antibiotics purchasedfromsigmacompany(Germany) were:Cefotaxime(Cef), Ampicillin (Amp), Gentamycin(Gm),Trimethoprim(Tri), Penicillin (Pn),Tetracycline (Tet), Streptomycin(Str), Nitrofurantoin (Nit),Ciprofloxacillin (Cip,Amikacin (Ak), and Rifampicin (Rif). These antibiotics were added to the mediumat different concentrations after cooling and sterilization. Then, the medium was poured and mixed into Petri-dishes. After that, it was inoculated using streaking method.Resistance was recorded after incubation at 37ºc for 24h.

Result:-

50 positive cultures were isolatedfromthe 200 samples taken for this research. The 50 positive cultures concluded thatE. coli was a predominant organism and were responsible for 35(17.5%) of the cases and the restof (15 samples 7.5 %) wasS. aureus. All the isolated samples were examined for their resistance to eleven widely used antibiotics in medicine.The E. coli bacteria that was isolated bacteria were grouped into 8 biotypes shown in (Table 1)with their corresponding resistant pattern and the isolated S. aureus bacteria were grouped into 5 biotypes shown in (Table 2)with theircorresponding resistant pattern. Among 35 isolated of E. coli,3 samples(E3, E8, and E35) showeda resistance to all eleven antibiotics.Among 15 isolated of S. aureus,2samples(S39, and S45) showeda resistance to all eleven antibiotics. The alcohol extract of A. millefolium was used on the resistive samples of E. coli(E3, E8, and E35) and S. aureus(S39, and S45). Sub-MIC 2500 µg/ml was used withAk, Str, Rf, Gm, Tri,Tet,Cip, and Ampagainstthe resistive genesofE. coli(E3, E8, and E35) shown in (Table 3).Sub-MIC 3500 µg/ml was used withAk, Str, Rf, Gm, Nit, Tri, and Tet, P, Amp, Cip, and Cef against the resistive genes of S. Aureus (S39, and S45) shown in (Table 4).

The solution of Sub-MIC (2500 µg/ml)in combination withAk, Str, Rf, Gm, Tri,Nit,Tet, and Cip, was effective againstthe resistive genes in E. coli(E3, E8, and E35) and decreased resistance 100%,while the plant extract in combination with Amp was only %10 effective againstthe resistive genesshown in (Table 3).

The solution of Sub-MIC (3500 µg/ml)in combination with the antibioticsAk, Str, Rf, Gm, Nit, Tri, and Tet decreased genes resistance in S. aureus(S39, and S45) 100%, while no effects on the resistive genes for P, Amp, Cip, and Cefin(S45)although not reduced genes resistant in(S39,S45)for Cef which was shown in (Table 4).

Table 1:-Antibiogram E. Coli isolated from UTI.

Samples	Ak	P	Amx	Cip	Str	Cef	RF	GM	Tri	Nit	Tet	Amp
1,2,4,6,16	S	R	R	S	S	S	S	S	S	S	S	R
5,7,8,17,32	S	R	S	S	S	S	S	S	S	S	S	R
3,18,35	R	R	R	R	R	R	R	R	R	R	R	R
9,10,20,33	R	R	R	R	R	R	S	S	R	S	S	R
11,12,13,14,15	S	R	S	R	S	S	S	R	R	R	R	R
19,,21,24,25,27	S	R	R	R	R	R	R	R	R	S	S	R
28,31,34	S	R	S	R	R	R	S	S	R	R	S	R
22,23,26,29,30,	S	R	S	R	R	R	S	R	R	S	S	R

Table 2:-AntibiogramS. aureus isolated from UTI.

Samples	Ak	P	Amp	Cip	Str	Ceff	RF	GM	Tri	Nit	Tet	Am
39,45	R	R	R	R	R	R	R	R	R	R	R	R
36,37,40	S	R	R	R	S	S	S	R	R	S	S	S
38.41,44,46	S	R	R	S	S	R	S	R	S	R	S	S
42,43,47	R	R	S	S	R	R	S	R	S	S	S	R
48,49,50	S	R	R	R	S	R	S	R	R	S	R	R

Table 3:-Effect ofA.millefolium on resistant genes in E. coli.

Samples	Plant extract	Ak	P	Amp	Cip	Str	Cef	Rf	Gm	Tri	Nit	Tet
E coli 3	SMIC 2500	S	R	R	S	S	R	S	S	S	S	S
E18	SMIC 2500	S	R	R	S	S	R	S	S	S	S	S
E35	SMIC 2500	S	R	S	S	S	R	S	S	S	S	S

Table 4:-Effect ofA.millefolium on resistant genes in S. aureus.

Samples	**Plant extract**	**Ak**	**P**	**Amp**	**Cip**	**Str**	**Cef**	**Rf**	**Gm**	**Tri**	**Nit**	**Tet**
S39	SMIC 3500	S	S	S	S	S	R	S	S	S	S	**S**
S45	SMIC 3500	S	R	R	R	S	R	S	S	S	S	**S**

Discussion:-

The evolution and spreading of antimicrobial resistance bacteria is generally due to,consumption,mistreatment, self-medication by patients,excessive prescription in which, it is anticipated that 70-80% of prescriptions for antimicrobials are probably advised intentionally by the health professionals like doctors, nurses and pharmacists. In spite of the fact that most acute viraldiarrhealand nasopharyngitisevent are not viral in origin, yet, antibiotic are used at random to treat them. Reasons for over definition are often in absence of certainty,pharmaceutical company pressure or patient pressure to get treatment. Furthermore; poverty and lacking access to antibiotics constitute are a major factor in the development of antibiotic-resistance bacteria in addition to improper diagnosis and wrong treatments which,in many instances the laboratory diagnostic compels the physician to define antibiotics empirically, thus, increasing the likelihood of the patient receiving a wrong antibiotic. The accessibility of antibiotics over-the reverse and sales promotion schemes by the pharmaceutical companies also leads to pressure on the doctors to promote wholesales to the patients,thus, increasing the likelihood of over usage and increasing the probability of emerging antimicrobial resistantbacteria. Another major issue is changing drugs which is contributing to the development of resistance because different drugs contain either the wrong ingredient or lesser amount of the active ingredient.

The treatment of infectious diseases with antimicrobial agents carrying onto present problems in the modern day.Medical studies showed a significant increase in the occurrence of side effects and the resistance that pathogenic microorganism against several antibiotics [28]. However, in the modern day attention has been paid to plant and their biologically active compounds which are isolated and used in herbal medicine such asflavonoids, alkaloids, tannins, Phenolic andglycosides Compounds [29]. The activating of the plant on resistant genes because Plants, is a source of medicinal compounds have continued to play a major role in the maintenance of human health since ancient times.So that, plants with potential antimicrobial activity has to be tested against the right microbial model to confirm its activity [29]. Thus, the aim of this research was to studytwo Pathogenic bacteria (E. coli and S. aureus) from the urine of pregnant women who were suffering from Urinary Tract Infections (UTI) and study the effects of A.millefoliumextract on their antibiotic-resistant gene.In this research, 200 samples were taken from a pregnant woman at age 25 to 40 years old at Sulaimani City.From those 200 samples, 50 samples were positive cultures.The most common bacteria isolated in these patients was E. coli (35 samples, 17.5%)and the rest (15 samples 7.5 %) was

S. aureus.This finding is similar to other reports, which indicated that UTIisolated in patients is caused by the commonestpathogenE. colithat a gram-negative bacterium [25, 21]. This was because of the fact that strains of E. coli are causing the urinary tract and possess a variety of virulence characteristics which facilitate their intestinal carriage and persistence in the vagina and then ascension and invasion of the normal urinary tract [26].

All the isolates were tested for antibiotic-resistant as it is shown in (Table 1) and (Table 2). It was concluded that three strains of E. coli (E3, E18, and E35) were totally resistant to all used antibiotics, and two strains of S. aureus (S39, and S45) were also totally resistant to the antibiotics. The alcoholic extract of A.millefolium was used to reduced antibiotic-resistant, which was shown in the (Table 3) and (Table 4).Antibacterial effect of A. millefolium was evaluated in vitro against the three pathogenic bacteria species of E. coli (E3, E18, and E35) were more responsive to the A. millefolium than S.aureusat the applied concentrations (SMIC 2500µg/ml)and reduced resistant genes 100%in combination withAk, Str, Rf, Gm, Tri, Nit,Tet and Cip, but did not reduce any genes resistant forP and Cefshown in T3. Also, The Ethanolplant Extract, Which Was Used For Reducing Resistant Genes In S. aureus At The Applied Concentrations (Smic 3500µg/ml) affectedthe resistant genes in S39and S45, which was shown in (Table 4). SMIC Plant extracts converted genes resistant forAk,Str, Rf,Gm,Nit,Tri and Tet in S39 and S45 to sensitive but there was no effect on genes resistant to Pi, Amp and Cipin (S45)andit did not reduce any genes resistant for Cef. The result of this study agreed with the recent investigation, which has also pointed out to the notable effect of extracts of aerial parts of A. millefolium as it showed a board spectrum antimicrobial activities against S. aureus andE. coli[24, 3]. It could be concluded that the alcoholic extract of A. millefolium has considered be able to reduce the resistive genes of both S. aureus or E. coli.

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