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Urinary Tract Infection with *Esherichia coli* and Antibacterial Activity of Some Plants Extracts

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Abstract: Urinary tract infections are the most common pathogenic infections, these infections lead to many difficult problems, especially increasing resistance to antibiotic drugs. Generally humans in many countries, especially developing countries expose to urinary tract infection in different ages and sex. The aim of this study was to evaluate the antibacterial activities of natural extracts against pathogenic *E. coli* causing urinary tract infections. A total 130 urine samples was collected from patients examined for urinary tract infection in King Abdulaziz University Hospital, Urology Department, Saudi Arabia. Results indicated a positive bacterial growth on cled, macConkey's agar and nutrient agar. *E. coli* was the most predominant pathogen causing urinary tract infection with ratio 43.9%. The incidence of bacteriuria was higher among patients ages 41-52 years old (35.3%) and decrease to 15.5% with age of 29-40 years old and less incidence was detected among 77-87 years old (1.7%). Also, the infection was more prevalent among females (56.9%) compared to males (43.1%). Ciprofloxacin, levofloxacin and norfloxacin were the best antibiotics used against *E. coli* isolates. Clove, ginger (dry, fresh), peppermint, spearmint and thyme alcoholic extracts were the most effective plant extracts against selected isolates of *E.coli.* It was concluded that combination between clove alcoholic extract and antibiotics as gentamicin, levofloxacin, amikacin and norfloxacin increase the synergistic effect of antibiotics against selected *E. coli* isolates.

Key words: Urinary Tract · Infection · *E. coli* · Plants Extracts

INTRODUCTION

Urinary tract infection (UTI) is the second most common type of infection in the body. There are an estimated 150 million urinary tract infections per year world wide.Urinary tract infection is a bacterial infection that affects any part of urinary tract [1].

In most cases bacteria infection if not treated travel to the urethra and multiply causing kidney infection.Urinary tract bacterial infection are common in women because they have a shorter urethra than men [2, 3].

The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae. Members of these families include *E. coli*, Klebsiell, Enterobacter and Proteus. Also Gram positive Stapylococcus sp. plays a role in the infection [4]. *E. coli* is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infections [5].

The frequency of *E. coli* in urine samples varies in different studies from 32% [6], 40% [7-9]. A recent study in France gave a higher value 75% [10]. During 1999, a total of 13774 non hospital urine samples were analyzed and a total of 2798 strains were isolated. About half of these were *E. coli* with Proteus mirabilis, Enterococcus spp. And K. Pneumonia occurring relatively small numbers. Non glucose fermenting Gram negative bacilli and other enterbacteria made up 27% of isolates, Gram positive cocci 19% [11].

The antimicrobial agent with the highest levels of activity against Gram negative bacilli was amikacin which was restricted to hospital use while cefuroxime, ciprofloxacin, fosfomycin, gentamicine and nitroufurantoin showed acceptable levels of activity. Nitrofurantoin was active against all strains of *Staph. aureus* but there were a lower effect on *E. coli* when used amoxicillin with cavulanate, cotrimoxazole and quinolon [11].

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The strains of *E. coli* isolated from hospitalized patients were more resistance to amoxicillin. Amoxicillin – clavulanate, trimethoprim, ciprofloxacin, cephalexin and gentamicin compared to those from outpatients. The resistance rate of *E.coli* to amoxicillin and trimethoprim among outpatients isolates was still high than resistance among the gram negative isolates of Enterobacteriaceae.

Antimicrobial activity of some medical plants against *Bacillus subtilis*, *E. coli*, *Staphylococcus aureus, Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumonia* were screened and showed that, among these bacteria, *E. coli, Proteus vulgaris* and *Staphylococcus aureus* were highly inhibited [12, 13].

Oils of caryacrol, oregano, geraniol, eugonol, cinnamon leaf, citral, clove bud, lemon grass, cinnamon bark and lemon showed antibacterial activity against *Escherichia coli* O157:H7 and salomnella enteric. The antimicrobial sensitivity of volatile oil against some gram–negative bacteria (*E. coli* ATCC 35218, *Klebsilla pneumonia*, *Salmonella paratyphi*, Citrobacter spp. and *Enterobacter cloaca*, a Gram –positive bacterium (*Staphylococcus aureus* ATCC25923) and fungus (*Candida albicans*) showed a broad spectrum of activity [14].

This study aimed to diagnosis *E. coli* as the most important cause of UTI and investigates the most effective antibiotic against pathogenic *E. coli*. Also, to detect the antibacterial activities of some natural plant extracts and investigate the effect of combination between antibiotics and plant extract on *E. coli* isolates.

MATERIALS AND METHODS

Collection of Urine Samples: Urine samples for isolation of *E. coli* were collected during June to October 2008 from 51 subjects (5 to 88 years) clinically suspected to suffer from urinary tract infection according to Chessbrough [15].

Isolation of *E. coli*: Urine samples were transfered immediately to Microbiology Department at King Abdulaziz University Hospital. The samples were examined microscopically by Gram's stain. Samples showed Gram negative results were inoculated on plates of nutrient agar, clede agar, macConkey's and blood agar then incubated at 37°c for 24 hour [16]. The colony showed fermenting of lactose on macConkey agar and cled agar media were purified and identified according to their morphology as circular, rose - pink to red colonies on macConkey agar medium and yellow colonies on cled agar. The isolates were identified by biochemical reactions e.g. catalase enzyme, potassium hydroxide test, Indole and methyl red test, voges proskaur reaction, urease and citrate, H2S and oxidase test [17].

Detection of Susceptibility to Antibacterial Agents:

- Concentrations of(1000, 500, 250, 125, 62.5, 31.65, 15.32 and 7.8 mg / ml) were prepared from each antibiotics used (ciprofloxacin, levofloxacin, norfloxacin, nalidixic acid, gentamicin and amikacin).
- Each antibiotic concentration was loaded on sterile filter paper disc (5mm diameter) and applied on the surface of Muller -Hinton agar plates inoculated with *E. coli* isolates and incubated at 37°C for 24 h. the lowest concentration which inhibit growth is known as minimum inhibitory concentration (MIC) [18].

Effect of Different Plants Extract on Growth of *E. coli*: Different extracts of clove (Flower), ginger (dry, fresh Rhizome thyme Leal marjoram (Leaf). oliban, Product of Orange peel (Fruit), peppermint (Leaf), kroon peel (Fruit), cinnamon (Bark) and rosemary (Leaf) plant were extracted with water (cold, boiling) andalcohol according to the methods of Huang *et al*. [19] and Saeed andTariq [20].

The Method Used for Plant Extract Was as Follows:

- The plant part was dried in air, then was grinded 50 gram from each selected plant part.
- Five gram of each plant were dissolved in known amount of distilled water and left 24 hours at room temperature with occasional shaking. They were filtered to obtain clear infusion.
- The aqueous decoction was prepared by boiling 5 gram of each plant in known amount of distilled water in flask for 20 minutes. The flask was removed from heat and allowed to cool. The content was filtered to obtain clear decoction [21].

The Method Used for Alcoholic Extract as Following: The alcoholic extract was prepared by similar way (5 gram of plant with known amount of ethanol and left 24 hours at room temperature with occasionale shaking and filtered to obtain clear extract.

Screening of antimicrobial activity was performed by standard disc diffusion method [21]. Sterilized disc of filter paper (6mm dimeter) were soaked in 1ml infusion (cold) decoction (boiling) and alcoholic, for 1-2 minutes then used for screening.

Effect of Combination Between Natural Plant Extracts and MICs of Antibiotics: Highest effective alcoholic plant extracts of clove,ginger(fresh),peppermint,thyme, rosemary and cold water extract of thyme were tested after addition the antibiotics amikacin, Jevofloxacin, ciprofloxacin, norfloxacin and gentamicin with MIC concentration and measuring diameter of inhibition zones (mm).

RESULTS

Isolation of *E. coli:* *E. coli* was the most predominant species were isolated from cases suffering from urinary tract infection patients. The isolated *E. coli* was identified by microscopic examination and biochemical reactions. The most frequent isolated species from UTIs patients was *E. coli* (43.9% 51 out 116 positive urine samples).

Effect of Antibiotics on *E. coli*: The most effective antibiotics against *E. coli* isolates were ciprofloxacin, levofloxacin and norfloxacin (percent of susceptibility 96%) followed by nalidixic acid 92%, gentamicin 84% and amikacin 76.5% where all *E. coli* isolates were resistant to sulbactam/ampicillin(percent of resistance 100%). As shown in table l the most effective antibiotics were ciprofloxacin and levofloxacin which recorded inhibition zone of 34 mm in diameter with *E. coli* no.102 followed by nalidixic acid winch yield inhibition zone of 30 mm in diameter with *E. coli* no.17 while all selected isolates of *E. coli* were resistant to sulbactam/ampicillin.

Minimum Inhibitory Concentration (MIC) of Selected Antibiotics: The results in table 2 indicated that the highest MIC was obtained when exposed *E. coll* n. 54 to ciprofloxacin, gentamicin and norfloxacin antibiotics drugs which recorded 500 |ug /ml and *E. coli* n. 102 to ciprofloxacin. Lower MIC recorded 7.8 ug /ml for *E. coli* n. 19 with levofloxacin antibiotics drugs.

Effect of Different Plant Extracts on Growth of *E. coli*: The results in tables 3- 5 showed that alcoholic extract of different plant gave inhibitory effect against most isolates compared with cold and boiling water extract which were not active against most isolates except cold water extract of lemon peel and boiling extract of clove.

Minimum Inhibitors Concentration of Different Plant Extract Against *E. coli* Isolates: The MICs for *E. coli* were determined. MICs defined as the lowest dilution of a plant extract required to inhibit the growth of *E.coli.* Higher MIC was obtained when exposed *E. coli* n. 17 and54 to alcoholic extract of clove which recorded 4 gm/L. Lower MIC recorded 0.05 gm/L for *E. coli* n. 19 with alcoholic extract of peppermint as in table 6.

Effect of Combination Between MIC of Plant Extracts and Different Antibiotics on Selected *E. coli* Isolates: Combination between alcoholic extract of clove and some antibiotics as gentamicin, levofloxacin, amikacin, ciprofloxacin and norfloxacin was increase the antimicrobial agent effectivity of antibiotics against

Table l: Susceptibility and inhibition zone (mm) of different antibiotics drugs used against clinical *E. coli* isolates

	Susceptibility and inhibition zone (mm)													
	NOR (10 µg)		SAM (20 µg)		CIP (5 µg)		NA (30 µg)		LEV (5 µg)		AK (30µg)		CN (10 µg) streptpmyci	
Number of *E. coli* isolates	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST
17	27	S	0	R	23	S	30	S	25	S	16	I	0	R
19	0	R	0	R	0	R	0	R	0	R	20	S	0	R
54	25	S	0	R	25	S	23	S	26	S	16	I	0	R
102	27	S	0	R	34	S	21	S	34	S	24	S	21	S

NOR = Norfloxacin SAM = Sulbactam/Ampicillin Cip = Ciprofloxacin
NA = Nalidixic Acid LEV= Levofloxacin AK = Amikacin CN=Gentamicin
(IZ=Inhibition zone; ST=Susceptibility test; R =resistance; I = Intermediate; S = Sensitive)

Table 2: Minimum inhibitory concentration (MIC) used antibiotics

	MIC (µg/ ml)				
E. coli isolates n.	Amikacin	Ciprofloxacin	Gentamicin	Levofloxacin	Norfloxacin
17	125	15.63	250	125	62.5
19	62.5	125	31.25	7.8	62.5
54	250	500	500	125	500
102	125	500	31.25	62.5	31.25

Table 3: Effect of cold water plant extracts against isolates of *E. coli*

	Diameter of inhibition zone (mm) of different cold water plant extract											
E. coli isolates n.	Cinnnamon IZ	Dry ginger IZ	Fresh ginger IZ	Spearmint IZ	Peppermint IZ	Maejoram IZ	Thyme IZ	Clove IZ	Lemon peel IZ	Orange peel IZ	Rosemary IZ	Oliban IZ
17	ND	ND	ND	ND	ND	ND	ND	ND	9	ND	ND	ND
19	ND	ND	ND	ND	ND	ND	ND	ND	12	ND	ND	ND
54	ND	ND	ND	ND	ND	ND	ND	ND	20	ND	ND	ND
102	ND	ND	ND	ND	ND	ND	ND	ND	16	ND	ND	ND

IZ = inhibition zone ND= Not detected (0.0)

Table 4: Effect of boiled water plant extract against isolates *E. coli*.

	Diameter of inhibition zone (mm) of different boiled water plant extract											
E. coli isolates n.	Cinnnamon IZ	Dry ginger IZ	Fresh ginger IZ	Spearmint IZ	Peppermint IZ	Maejoram IZ	Thyme IZ	Clove IZ	Lemon peel IZ	Orange peel IZ	Rosemary IZ	Oliban IZ
	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ
17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	ND	ND	ND	ND	ND	ND	ND	11	ND	ND	ND	ND
54	ND	ND	ND	ND	ND	ND	ND	10	ND	ND	ND	ND
102	ND	ND	ND	ND	ND	ND	ND	10	ND	ND	ND	ND

IZ= inhibition zone ND=Not detected (0.0)

Table 5: Diameter of inhibition zone (mm) of different alcoholic plant extracts against isolates of *E. coli*

	Diameter of inhibition zone (mm) of different alcoholic plant extracts											
E. coli isolates n.	Cinnnamon IZ	Dry ginger IZ	Fresh ginger IZ	Spearmint IZ	Peppermint IZ	Maejoram IZ	Thyme IZ	Clove IZ	Lemon peel IZ	Orange peel IZ	Rosemary IZ	Oliban IZ 17
	9	12	11	13	14	ND	15	19	13	11	10	ND
19	12	11	12	9	15	ND	ND	20	12	10	11	ND
54	11	10	15	12	19	7	10	18	15	14	ND	10
102	11	12	13	11	13	ND	9	16	15	14	ND	ND

IZ= inhibition zone ND= Not detected (O.O)

Table 6: MICs of different natural plant extract against isolates of *E. coli*

	MIC of]			Dlant extracts (gm/L)		
E. coli isolates number	Clove	Ginger (fresh)	Rosemary	Peppermint	Thyme (alcoholic)	Thyme(cold water)
17	4	0.5	0.3	0.2	0.5	0.2
19	0.5	0.5	0.3	0.05	0.25	0.2
54	4	0.5	0.6	0.4	0.5	0.2
102	2	0.5	0.3	0.4	0.5	0.2

Table 7: Combination effect between MICs of clove extract and different antibiotics against *E. coli* isolates

	Diameter of inhibition zone (mm)				
E. coli isolates n.	Gentamicin+clove	Levofloxacin+clove	Norfloxacin+clove	Amikacin +clove	Ciprofloxacin+clove
17	25	31	25	26	30
19	18	12	14	21	20
54	18	20	16	21	13
102	24	19	21	20	14

E. coli isolates. The diameter of inhibition zone of gentamicin alone in *E. coli* n. 19 was 15 mm while when combined gentamicin with clove increase and recorded to 18 mm. Also, amikacin recorded 17 mm in diameter with *E. coli* n. 102 while with combination with clove recorded 20 mm in diameter.

DISCUSSION

Urinary tract infection (UTI) is one of the most important causes of morbidity in the general population and is the second most common cause of hospital visits [22]. Urinary tract infection is an old problem that

continues to present new challenges due to change in the etiology of UTl and in the antimicrobial susceptibility of urinary pathogens over the years. Factors such as the changing in patient population and extensive use and abuse of antimicrobial agents could contribute to changes in the microbial profile of urinary tract isolates [23]. *E. coli* is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infection (UTI) [5].

In the present study, the incidence of bacteriuria was higher in patient's ages 41-52 years old (35.3%) and this percentage decrease by 15.5% with age 29-40 years old and less incidence among age of 77-87 years (1.7%).

The present study declared high prevalence of infection among females (56.9%) than males (43.1%). The prevalence of bacteriuria increase with age from 9.5 to 35.5% and the sex ratio of infections become nearly equal over the age of 77 years (1.7%).This results agree with Geo *et al*. [24], who found that the prevalence of bacteriuria increase with age and sex ratio of infection become nearly equal over the age of seventy years. On the other hands, these results disagree with Gupta *et al*.[25], who found that the incidence of UTI was higher in males than females. Also, after 50 years old the incidence of UTI was almost as high in men as in women. In the present study, *E. coli*.was the most prevalent isolates (43.9%), *Staphylococcus aureus* (21.6%), *Candida albicans* (16.4%), *Klebsiella pneumonia* (14.7%) and *Pseudomonase aeruginosa* (3.4%). This result agreed with Al-Hadad, [26] who found that *E.coli* was the most frequently isolated organism (41.5%). Also, with Iroha *et al*.[5], who–reported that *E. coli* is one of the most common bacteria capable of causing infection in humans particularly urinary tract infection.

Fifty one *E. coli* isolates were identified by microscopic and biochemical reactions.The method used to detect antibiotic susceptibility was disc diffusion methods [27].

In the present study, antibiotic susceptibility tests were performed to *E. coli* isolates associated with urinary tract infections, the results showed that the antibiotics norflxacin, ciprofloxacin, levofloxacin were the most effective against *E. coli* isolates.The percentage of sensitive organisms to them were (96.1%), followed by nalidic acid (92.2%), gentamycin (84.3%), amikacin(76.5%) and ofloxacin (56.9%). On the other hand, salbactam-ampicillin showed no effect on the isolated *E. coli* followed by amoxicillin –clavulanic acid and cefotaxime. These results agree with those reported by Kader *et al*. [28] who found that *E. coli* showed high rates of resistance to amoxicillin, amoxicillin-clavulanate [28], also agree with Rosa *et al*. [11] who reported that the antimicrobial agents with the highest levels of activity against Gram negative bacilli was amikacin which was restricted to hospital use, while ciprofloxacin and nitrofuranoin showed acceptable levels of activity [11].

The results indicated that *E. coli* isolate were resistance to amoxicillin and clavulanic acid and sensitive to fluoroquinolones. Resistance of *E. coli* was observed against ampicillin and TMP/SMX (55and 51%). The antibiotics resistance rates for *E. coli* were as follows nitrofourantoin (6%), nalidixic acid (14%) and first generation of cephalosporin (13%), third generation of cephalosporin (5%), aminoglycosides (2%), norfloxacin (9%) and ciprofloxacin (4%) [29].

The obtained data showed that, *E. coli* showed minimum inhibitory concentration to amikacin (MIC >125 mg/l, ciprofloxacin, gentamicin, levofloxacin and norfloxacin(MIC >250 mg/l).The MICs for all quinolone against *E. coli* isolates reaching 1.000 micro g/ml [30]. This result is in agreement with our study as fluroquinolonea (ciprofloxacin, levofloxacin and genamicin) show high minimum concentration (MIC>250 mg/I) except for ofloxacin which show resistace rate of (41%) to *E. coli*.

In the present study, the alcoholic extracts of clove, ginger (dry and fresh), peppermint, spearmint and thyme were the most effective than the aqueous extracts against *E. coli* isolates. Clove extracts had potent antimicrobial activity against E.col. These results are in agreement with that obtained by Ayoola *et al*. [31].

In the present study, many of multistrains of *E. coli* showed that MIC of clove ranged from (6.25-50%), ginger (fresh) was 50%.MIC of peppermint ranged from (6.25-50%), MIC of rosemary ranged from (50-25%), MIC of thyme (cold extract) was 50%. *E. coli* isolates showed that MIC values for ethanolic extract from clove ranged from 0.5 to 5.5 mg / l.

In this study to verify the synergism between 13 antimicrobial drugs and 8 plant extracts, guaco (*Mikania glomerata*), guava (*Psidium guajava*), clove (*Syzgium aromaticum*), garlic (*Allium sativum*), lemongrass (*Cymbopogon citrates*), ginger (*Zingiber officinale*), carqueja (Baccharis trimera and mint (*Mentha piperita*) were tested against *Staphylococcus aureus* strains. Antibiotics used were pencillin (PEN;10 IU), oxacillin (OXA;1 µg),vancomycin (VAN; 30 µg), ampicillin (AMP; 10 µg),cephalothin(CFL;30ug)cefoxitin(CFO;30 µg), chloramphenicol (CLO;30 µg), gentamycin (GEN; 10 µg), netilmicin (NET; 30 µg), tetracycline (TET; 30 µg), erythromycin (ERI; 15 µg), cotrimoxazole (SUT; 25 µg) and ofloxacin(OFX; 5 µg).

Tetracycline showed synergism with all the extracts, followed by chloramphenicol and netilmicim. The synergistic capacity was promising for the extracts of some plants such as *S. aromaticum, C.citates,P. guajava*. which presented synergism with 11, 11 and 9 drugs, respectively; while garlic and ginger showed synergism with only 3 and 2 drugs, *respectively*.

The synergism recorded to plant extract with weak action on *Staphylococcus aureus* growth, such as lemongrass which showed a synergism profile similar to that of clove extract which considered the most efficient *Staph. Aureus* growth inhibitor in this study, thus the researchers should investigate the synergistic capacity of plant extracts or other natural products; independent of the antimicrobial activity they have [32]. The results illustrated that there is synergism between the combination of alcoholic extract of clove and antibiotics (*Amikacin, gentamicin*,levofloxacin and norfloxacin). Plant could be considered as a source of compounds that increase the sensitivity of bacterial cells to antibiotics [33].

In conclusion, urinary tract infections in human are considered the most serious health Problems facing the world. *E. coli* is the most frequent isolated species. The results of this study demonstrated that alcoholic extracts of (clove, ginger (fresh and dry), peppermint, spearmint and thyme) were effective plants extracts against selected isolated *E. coli.*

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