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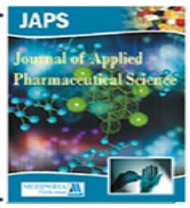

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Susceptibility of Bacteria Infecting Urinary Tract to Some Antibiotics and Essential Oils

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ABSTRACT

This study investigates the most common urinary tract infection bacteria and their sensitivity to antibiotics and some essential oils from Egyptian plants. The Urinary tract bacteria were sampled from patients expressing any symptoms of urinary tract infection except those taking antibiotics. The bacteria were isolated, cultured, and identified. 64 bacterial isolates were identified as *E.coli* (28 isolates), *Klebsiella pneumonia* (9 isolates) *Pseudomonas aeruginosa* (6 isolates), *Proteus mirabilis* (6 isolates), *Staph.aureus* (5 isolates) , *Enterococcus faecalis* (4 isolates), *Morganella morganii* (4 isolates) and *Pseudomonas fluorescens* (2 isolates). The isolates showed different degrees of sensitivity to different antibiotics . Among the essential oils of five medical plants known for their application in folk medicine in Egypt , oil of Dill *(Anethum graveolens*) showed the highest effect , affecting more than 50 % of both gram +ve and gram –ve bacteria ,followed by Parsley *(Petroselinum hortense)* and Celery *(Apium graveolens)* affecting 48% & 41% of the isolates respectively . The oil of Thyme *(Thymus valgare)* was effective against Gram –ve bacteria only. The lowest effects were recorded to the oil of Chamomile *(Marticaria recutita)* affecting only 5% of the tested isolates.

Keywords: urinary tract infection, antibiotics, bacteria, essential oils.

INTRODUCTION

The urinary tract is the body's filtering system for removing waste liquid, or urine; it comprises the kidneys, ureters, bladder and urethra (Ramadan, 2003). A urinary tract infection is caused by bacteria that enter the urinary tract; women are more likely than men to get UTI because of their urinary tract's design, men have a larger urethra, so it is more difficult for bacteria to enter the urinary tract. Nearly half of all women will have a UTI at some point in their lives (Marild *et al*., 1998; Craig, 2001; Foxman, 2003; Parlak *et al.*, 2007). Urinary tract infection are categorized into either lower tract infection, located in the bladder and/or urethra (cystitis and urethritis) and upper tract infection, located in ureters, collecting system, and parenchyma (pyelonephritis). It is necessary to understand the difference between both types to make an accurate diagnosis**.** ***Cystitis*** is defined as an inflammatory condition of the urinary bladder, whereas ***pyelonephritis*** is defined as a diffuse pyogenic infection of the pelvis and parenchyma of kidney.

Signs and symptoms of cystitis include dysuria, frequency, urgency, malodorous urine, enuresis, hematuria and suprapubic pain. On the other hand, the signs and symptoms of pyelonephritis include; fever over 38.5°C, chills along with cost vertebral angle or flank pain and tenderness with pyuria and positive urine culture (Dulczak & Kirk, 2005). Most of UTI are caused by gram-negative bacteria like *Escherichia coli, Proteus mirabilis, Proteus vulgaris Klebsiella* sp, *Pseudomonas aeruginosa, Acinetobacter, Serrati, and Morganella morganii*. Also UTI are caused by Gram positive bacteria include *Enterococcus, Staphylococcus* especially coagulase-negative staphylococci, and *Streptococcus agalacticae* (Tangho & Mcaninch, 2004). At least 80% of the uncomplicated cystitis and pyelonephritis are due to *E.coli*. Whereas *Proteus mirabilis* and *Klebsiella pneumoniae* infection accounts 10% and 6% respectively. Adherence properties of some organisms prevent the normal washout of these organisms by bladder emptying and mucosal host defense mechanisms (Ashkenazi *et al.*, 1991; Tangho & Mcaninch, 2004). Treatment of UTI with the appropriate antibiotic can minimize mortality, morbidity and any renal damage from acute UTI. Choosing the appropriate antimicrobial agents sounds difficult, but advances in the understanding of the pathogenesis of UTI, the development of new diagnostic tests, and the introduction of new antimicrobial agents have allowed physicians to appropriately tailor specific treatment for each patient (Schlager, 2001). Down the ages essential oils and other extracts of plants have evoked interest as source of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe *et al.*, 2004).The present work aims at the isolation and identification of bacteria infecting urinary tract and testing the susceptibility of the isolates to some antibiotics and essential oils from some plants of medicinal uses in Egypt.

MATERIALS AND METHODS

Sample collection

A total of 100 clinical samples were collected equally from Al-Seguiny Hospital, Alexandria and El-Miry Hospital, Kafr Eldwar (50% females and 50% males). Samples were obtained from patients in different ages, expressing any symptoms of urinary tract infections excluding those treated with antibiotics prior to sampling. Patients were sampled by clean catch midstream urine. All samples were coded and processed at the time of collection according to the standard method. Samples were then transferred the lab of microbiology for further investigation using a portable cooler to avoid any bacterial multiplication (Robert *et al.*, 1991**).**

Urinalysis, Microscopy

Urinalysis was performed on all collected specimens using. Dipstick screening technique for leukocyte esterase and nitrite using multisticks of Medi-Test Combi 10 ® SGL, a routine direct microscopy of a centrifuged sample was performed for the examination of white blood cells and bacteria examination was performed, The presence of more than 5 WBC/HPF indicated pyuria (Hindler & Munro, 2007).

Microbiological investigation

All samples positive for one or both leukocyte and nitrite and positive for one or both WBCs, and bacteria were inoculated on chromogenic media and incubated at 37°C for 24 hours. After the incubation period, the plates were examined for growing bacterial colonies. The isolated colonies were purified and sub cultured for characterization. Streaking technique was used; an isolated colony can then be used as a source of inoculums for a pure culture. The agar media were used are, MacConkey's (Oxid ®) Agar selective for: gram-negative bacteria, the growth of gram-positive bacteria was inhibited by the crystal violet dye and bile salts in the media (Schlager, 2001).

Hemolysis with Blood Agar this medium contains 5% sheep's blood differential for hemolysis, based on the ability to break down hemoglobin or red blood cells, 3 groups of microorganisms can be described alpha-hemolysis a green to light-brown halo is seen around the colonies, Beta-hemolysis a clearing is seen around the colonies, and Gamma-hemolysis no hemolysis is observed (Dulczak, & Kirk, 2005).

Isolates maintenance

Isolates were maintained on nutrient agar plates and slants (Magdigan & Martinko, 2005). Subculture were made Bimonthly transfer, and kept in the refrigerator for further investigation

Characterization of bacterial isolates

Colony characteristics the isolates colonies were examined for appearance, smell, and pigmentation (Cowan & Steel, 1965; Black, 1996).

Gram stain and morphology characters

Gram staining is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical, primarily the presence of high levels of peptidoglycan, and physical properties of their cell walls. A Gram positive results in a purple/blue color while a Gram negative results in a pink/red color. Smears of isolated bacterial were stained with Gram technique and examined microscopically for gram stain response, cell shape, and aggregation (Bergey *et al*., 1994).

Biochemical characterization test

The bacterial isolates were tested for their biochemical characteristics using Oxidase test; to detect the presence of the intracellular cytochrome oxidase enzyme which allows the organism to use oxygen as part of respiration. Organisms that are oxidase positive called oxidative fermentative, will cause the reagent to turn purple within 10-20 seconds. 24 h colonies were used for this test. This test was performed as follows: a drop of the oxidase reagent (tetramethyl-p-phenylene diamine hydrochloride)

was placed on a disk of filter paper. A small amount of the bacterial colony was rubbed on the filter paper using a toothpick. Blue-black development was observed within 10-20 seconds for positive results. Any changes after 20 seconds were disregarded. A positive test indicates a non-fermenting bacteria and *vice versa* (MacFaddin, 2000).

Catalase test was carried out by placing a drop of hydrogen peroxide on the bacterial culture. A positive test is indicated by froth formation. A positive test indicates production of catalase enzyme to facilitate cellular detoxification (Taylor & Achanzar, 1972).

Indole test was carried out by culturing pure bacterial strain in sterile peptone broth for 24-48 h before dipping a strip wetted with Kovac's reagent. A positive result is shown by the presence of a red or violet color indicating the presence of tryptophanase system, which splits indole from tryptophan (MacFaddin, 2000).

Antibiotic sensitivity tests

All purified isolated strains were inoculated on Muller-Hinton Media (Oxid ®) and then incubated at 37˚C for 24 hours in an incubator. Antimicrobial susceptibility of isolates was tested by the disk diffusion method using antibiotic disc (Oxid®) with the minimum inhibitory concentration (MIC) (Alan *et al*., 2006). Agents tested were Amoxicillin/ Clavulanate, Pipracillin/ Tazobactam, Cefotaxim, Imipenem, Amikacin, Norfloxacin, Trimethoprim/ Salfamethoxazole (oxoid®). These antibiotics were chosen as they are the antibiotics of choice in the treatment of UTI (Wood & Washington, 1995).

Cluster analysis and isolates identification

For cluster analysis, positive and negative readings were coded as 1/0, respectively. The data obtained in this study subjected to statistical analysis and a matrix Euclidean distance among the isolates was formed for the construction of dendogram. The statistical calculations were done by the System of Statitics SYSTAT 12 software. Cluster analysis was used to investigate similarities in bacterial profiles of the samples (Sharma, 1996).

Identification of isolates

All bacterial isolates were identified using highly automated VITEK®2 System that depends on supplemental tests and provides same-day right identification (Bosshard *et al*., 2006).

Three identification cards GN ID (Gram negative identification), GP ID (Gram positive identification cards) and YST ID (yeast and yeast like organisms) were used (Wallet *et al*., 2005).

Effect of natural oils on isolated strains

The crude natural oils of five Egyptian plants namely, Celery *(Apium graveolens)*, Chamomile *(Matricaria recutita)* , Dill *(Anethum graveolenes)*, Parsley *(Petroselinum hortense)* and Thyme *(Thymus vulgare)* were tested for their antibacterial effects. The tests were performed by the disk diffusion method, using sterilized filter paper saturated with reach natural oil separately. Discs were placed on Muller- Hinton agar plates inoculated with the bacterial isolates. Plates were then incubated at 37˚C for 24 hours. The diameter of growth inhibition zones were measured across the disk and data were recorded to the nearest millimeter (Milhau *et al*., 1997).

RESULTS

Sampling design and handling

Hundred patients were selected from Kafr El-Dawar (a public hospital lab situated in a marginal area) and from Alexandria (a public hospital lab situated in one of the most populated commercial area) according to clinical investigations and symptoms. Gender represented 50% of each. Half of the patients were above 40 years old, and the other half were under 40 years old. Only 55 samples showed positive results 63.6% females and 36.4% males. Among them 66% were above 40 years old and 44% were under 40 years old table (1) fig (1).

Table. 1: Distribution of UTI in the sampling areas.

	Number of infected samples / Kafr Eldwar patients	Number of infected samples / Alexandria patients	% of infected samples
Females	22 / 25	13 / 25	63.6%
Males	14 / 25	6 / 25	36.4%
>40 years old	20/ 25	13 / 25	66%
<40 years old	16 / 25	6 / 25	44%

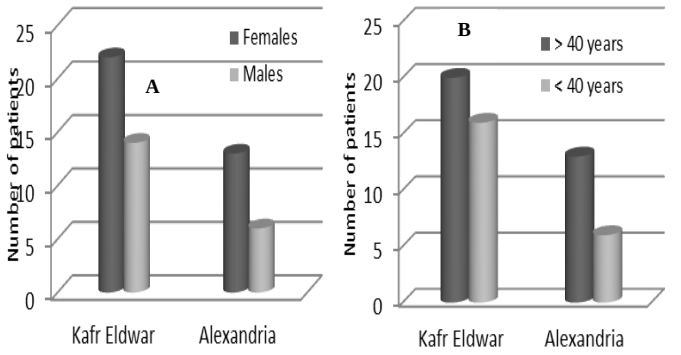

Fig 1: Distribution of UTI in the sampling areas (a): gender, (b): age.

URINALYSIS RESULTS

Investigation of leucocytes, nitrites, WBC and pyuria

Leucocytes and nitrites were investigated using the dipstick technique. This technique is a routine lab test. Phase contrast light microscopy was used to test the samples for pyuria and bacterurea.

Results in figure (2) show 26 cases (24%) of samples were positive for leukocytes, 14 cases (13%) of samples were positive for nitrite, 15 cases (14%) of samples were positive for both leukocyte and nitrite of the dipstick test, and 17 cases (15%) of samples were positive for pyuria, and 38 cases (34%) of samples were positive for bacteriuria of microscopy test.

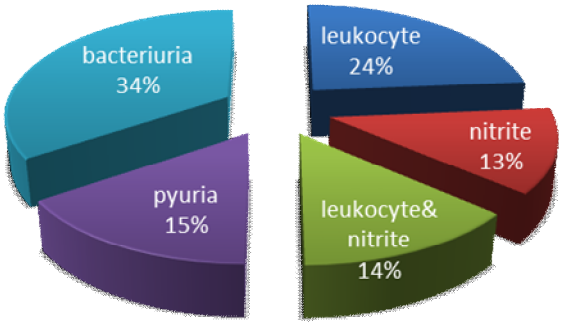

Fig. 2: Urine analysis (%).

UTI causative organisms

Samples of positive leucoscytosis and bacteriuria were selected for further microbiological investigations. One ml of bacterial suspension was plated on MacConkey and Blood agar plates. Samples were characterized for single or multiple infections according to the morphological description of the plates. Developed colonies were coded and purified using the streaking plate method technique. 64 bacterial strains and 4 fungal strains were obtained from this process. Bacterial strains were examined phenotypically for Gram reaction, cell shape and culture description. According to Gram reaction and cell shapes, isolates were grouped into 3 categories: Gram- positive cocci, Gram-negative cocco bacilli, and Gram- negative bacilli.

Phenotypic characterization of the isolates

Gram- positive cocci

Nine strains were included in this group. All of the nine strains were not able to grow on MacConkey agar. They were oxidase negative and non-indole producers. 60% were of entire, large, yellow colonies. Cells were forming irregular grape-like group. They were all of β-hemolytic type on blood agar medium. The other 40% were of entire small white colonies. They were all of γ-hemolytic type on blood agar medium..

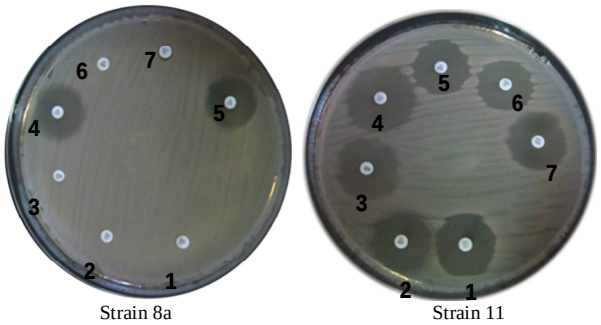

Fig. 3: Antibiotic sensitivity test of strains 8a and 11 representing the Gram-positive cocci, where 1: Amoxicellin, 2: Pipracillin; 3: Cefotaxin 4: Imipenem, 5: Amikacin, 6: Norfloxacin, and 7: Trimethoprin.

Gram- negative coccobacili

It comprises of twenty-eight strains. All of them were of entire medium white colonies. Cells were very short rods. All were able to grow on MacConkey agar giving pink colonies. 100% of the strains were oxidase negative, catalase positive, and indole positive.

They all showed non-haemolytic reaction on blood agar medium. Although all strains seemed to be identical, they showed differences in antibiotic sensitivity pattern.

Gram- negative bacilli

This group comprises of twenty-seven strains, all growing on MacConkey agar. 33% of them were of entire medium white colonies. Cells were long straight rods. They were of γ-haemolytic type on blood agar medium. 22% of the strains of them were of irregular small green colonies. Cells were short rods and of β-haemolytic type on blood agar medium. Other 22% of the strains were of entire large yellow colonies. Their cell shape was long straight rods of gamma γ-hemolytic type on blood agar medium. 15% of them were of entire small white colonies. Cells were of long straight rods and of beta (β) hemolytic type on blood agar medium. The last 8% strains were of entire small green colonies of alpha α-hemolytic. Members of this group showed differences in antibiotic sensitivity.

For studying the antibiotic sensitivity pattern of members of this group, strains 5, 42, 36a and 50b were selected (Fig. 4). Strain 5 was sensitive to all tested antibiotics except Norfloxacin and trimethoprin. Strain 42 was sensitive to all tested antibiotics except Pipracillin, Norfloxacin and Trimethoprin. Strain 36a was sensitive to all tested antibiotics except Imipenem and Amikacin while strain 50b was sensitive to all tested antibiotics except Amoxicillin, Pipracillin and Trimethoprin.

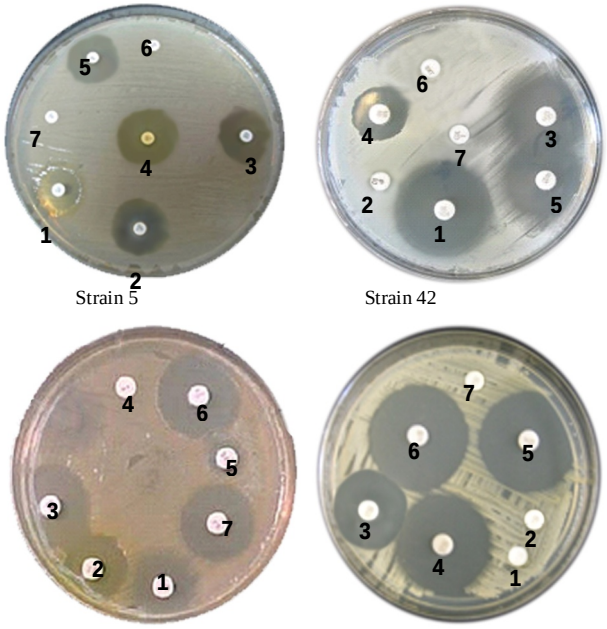

Fig. 4: Antibiotic sensitivity test of representative strains of the Gram- negative bacilli where 1: Amoxicillin, 2: Pipracillin, 3: C*efotaxin*, 4: Imipenem, 5: Amikacin, 6: N*orfloxacin*, and 7: Trimethoprin..

Other strains

Four other strains were found in some female patient samples. Their colonies were large shiny and cells were large, compared to bacteria, with very clear nucleus. These strains were very similar to yeasts.

Numerical analysis and Identification of the UTI isolates

Characteristic data were transformed into 0/1 codes to be analyzed using SYSTAT program. Cluster analysis was chosen to classify members within each group into clusters based on similarity matrix. From each cluster, a representative strain was selected for identification using VITEK® system.

Gram- positive cocci

The numerical analysis of this group resulted in 2 major clusters. Strains 1b, 11, 18, 34b, and 41 were grouped in one cluster at a similarity distance of 98.8. While, strains 8a, 16, 32 and 36b were grouped in another cluster with similarity distance of 98.8. Strains 11 and 8a were chosen to represent these clusters, respectively, for identification.

Results of the VITEK® identification of the representative strains showed that strain 8a was found to belong to *Enterococcus faecalis*, and strain 11 was found to belong to *Staphylococcus aureus*.

Gram- negative coccobacilli

Results showed two main clusters. Strains 2, 4, 7, 9, 14, 19, 20, 21, 26, 25, 28, 33, 35, 38, 40, 43, 46 and 48 were grouped in one cluster at a similarity distance of 98%, while strains 6a, 8b, 22b, 23a, 24a, 27b, 30b, 34a, 45a, 50a were grouped in another cluster with similarity distance of 97%. The whole group was homogenous and very similar in all phenotypic characters except in the sensitivity pattern to antibiotics. 50% of all strains were chosen for VITEK® system identification, and they were all found to belong to *E. coli*.

Gram- negative bacilli

The results showed that members of this group were classified into two clusters: A was separated at 75% and B was separated at 83%. Cluster B was sub-divided into two sub-clusters (C at 74% and D at 73%).

Cluster A was separated at a distance of 75% and comprised 4 strains (50b, 45b, 30a and 31a). Strains 50b and 31a were chosen as representative to this cluster for identification and both found to belong to *Morganella morganii*.

Cluster C was separated at a distance of 76% and contained 8 strains, subdivided into 2 sub-clusters (c1 and c2) at a distance of 72%. Four strains of sub-cluster c1 were chosen for identification and all found to belong to *Pseudomonas aeruginosa*, while the stain chosen from sub-cluster c2 was found to belong to *Pseudomonas fluorescens*. These results lead to the conclusion that all members of cluster C were classified among genus Pseudomonas.

Cluster D was separated from Cluster B at a distance of 74%. Members of this cluster were also sub-clustered to d1 at 72% and d2 at 71%. 50% of the members included of each sub-cluster were submitted for VITEK ® system for identification. Members of sub-cluster d1 were found to belong to *Klebsiella pneumoniae* while members of sub-cluster d2 were found to belong to *Proteus mirabilis*.

Distribution of uropathogens in collected samples

The results indicate that the most predominant uropathogen, in the study area, was *Escherichia coli* as it was obtained in 43.7% of the isolates followed by *Klebsiella Pneumonia* (14.1%). Both *Pseudomonas aeruginosa* and *Proteus mirabilis* were represented in 9.4% of the isolates while *Staphylococcus aureus* was recorded in 7.8% of the samples. *Enterococcus faecalis* and *Morganella morganii* were represented in 6.2% of the isolates however, only 3.2% was recorded for *Pseudomonas fluorescens* (Table 2).

Table. 2:: Frequency of the uropathogenic isolates .

Microorganism	Frequency	Percentage
Escherichia coli	28	43.7%
Klebsiella pneumoniae	9	14.1%
Pseudomonas aeruginosa	6	9.4%
Proteus mirabilis	6	9.4%
Staphylococcus aureus	5	7.8%
Enterococcus faecalis	4	6.2%
Morganella morganii	4	6.2%
Pseudomonas fluorescens	2	3.2%

Effect of natural oils on bacterial isolates

Results in figure (5a) show that Dill oil was the most effective oil that reduced 61% of E. coli isolates. It also affected 56% of the Gram- positive cocci, and interestingly, it affected only 33% of the Gram- negative bacilli. Generally, dill affected 48% of all isolated uropathogens (Fig. 5b).

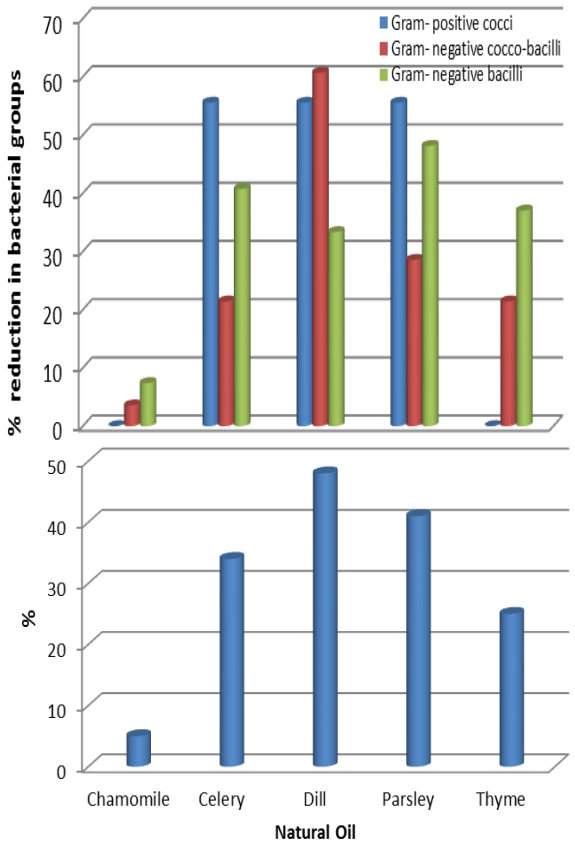

Fig. 5: the effect of natural oil on the uropathogens isolated from the study area.

Results also indicated the effect of Parsley and Celery, their stronger effects were against Gram- positive cocci (56%) for both oils, followed by Gram- negative bacilli (48% and 41%, respectively) while their effects on *E. coli* was much less (29% and 21%, respectively). Parsley and Celery followed Dill in their general effect. They affected 41% and 34% of the local uropathogens, respectively (Fig. 5a and b).

Thyme showed effects only against Gram- negative bacilli and cocco bacilli (37% and 21%, respectively). It had no effect on Gram- positive cocci. Its effect generally on all isolates did not exceed 25%.

Chamomile was the weakest oil among the tested oils. It affected only the Gram- negative while had no effect on Gram-positive cocci. Generally, it affected only 5% of all isolated uropathogens (Fig. 5a and b).

The inhibition zones with different diameters caused by different oils on plates of different isolates in (Fig.6) reflect the antibacterial potency of the used essential oils against different bacterial species infecting urinary tract.

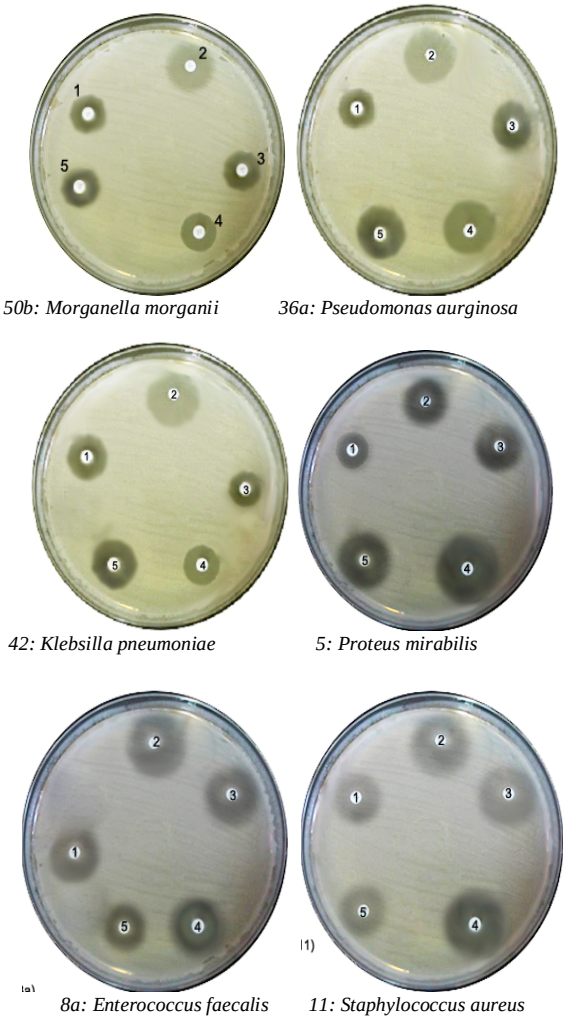

Fig. 6: Oils sensitivity test of strains 50b, 36a, 42, 5 representing the Gram-negative bacilli, 8a and 11 representing the Gram- positive cocci where, 1: Chamomile oil, 2:Celery oil, 3:Dill oil, 4:Parsely oil, and 5: Thyme oil.

DISCUSSION

Urinary tract infection (UTI) is an extremely common clinical problem. It is important because it may involve the urethra, bladder, uterus, and kidney**.** Because they are not reportable diseases, it is difficult to assess accurately the incidence of UTIs, not only in Egypt but also in many other countries. This situation is further complicated by the fact that accurate diagnosis depends on both the presence of symptoms and positive urine cultures, although in most outpatient settings this diagnosis is made without the benefit of culture (Foxman, 2003).

55% of tested patients were found to suffer from UTI, where about 11% of them had renal failure and 16% were from ICU all had mixed urinary tract infection. These results show that UTI represent a real risk in Egypt, as it is twice higher than those reported by Foxman (2003). Jonathan & Evan (2006) also stated that the relation between nosocomial infection as UT mortality in hospitals remains unclear; however, the highest rates of nosocomial infections are observed in ICUs.

The presence of both pyuria and bacteriuria from a fresh urine sample is highly indicative for UTI (Watson, 2004). The study of bacteriology of UTI in 114 patients revealed a high al failure among patients with bacteriuria was a complicated one (Kaye, 1972). Another study reported infection rates ranging from 1%–5% after a single brief catheterization to virtually 100% for patients with indwelling urethral catheters draining into an open system for longer than 4 days (Fridkin *et al.*, 1997).

According to the current study Gram- negative bacteria was responsible for 85.9% of UTIs in comparison to Gram-positive bacteria which was 14.1%.. In our study *Escherichia coli* was the most predominant uropathogen with 43.7%, followed by *Klebsiella Pneumoniae* 14.1%, *Pseudomonas auruginosa* and *Proteus mirabilis* 9.4%, *Staphylococcus aureus* 7.8%, *Morganella morganii* and *Enterococcus faecalis* 6.2%, and *Pseudomonas fluorescens* 3.2%. In a cross sectional study by the University of Florida, USA of a group of patients, urine specimens were collected in an emergency department and grew cultures with greater than 100,000 cfu/ml of single organism on Mackonky and blood agar. All patients lacking UTI symptoms were excluded. After making all exclusions, 81 patients met the inclusion criteria of this study. Of these 81 patients 89% had UTI due to *Escherichia coli*, 3.7% to *Klebsiella*, 1.2% to *Proteus*, 1.2% to *Citrobacter*, 1.2% to *Staphylococcus* 1.2% and *Enterococcus* 3.7% (Mcloughlin & Joseph 2003).

In this study, there was a significant difference between the frequency of UTI and gender. The prevalence of UTI was in female more than in males as after screening 100 (50 males and 50 females) urine samples suspected of having urinary tract infection that were collected from two areas Alexandria and Kafr Eldwar using two method of examination urinalysis and urine culture. Only 55 (55%) patients (35 of 63.6% females and 20 of 36.4% males) gave positive urinary tract infection culture test that is meaning the infection spread in females than males. In another study, it was seen that significantly higher incidence rate for girls

(34.4 episodes per 1000 person years) than for boys (4.4 episodes per 1000 person years). Another study also showed that urinary tract infections are more common in girls (Brooks & Houston, 1977).

In current microbiological study patients divided into two groups; the first group contains 50 patients above 40 years, and the second contains 50 patients below 40 years, thirty nine (39%) of first group, and only sixteen (16%) from second group had infection of urinary tract. One study showed increase of women's UTIs between the age groups 20-25 and 41-46 years. The result is not surprising since this age span corresponds to a woman's most fertile period and to parturition, especially the first delivery, which is a well-known risk factor for the development of UI, also in women, a low estrogen level increases the intravaginal pH, resulting in the lactobacillus being replaced by a pathogenic agent (Hagglund *et al*., 2004).

According to the National Institute for Health and Clinical Excellence (NIHCE) guidelines, prevention of UTI recurrence includes; relieving constipation and dysfunctional elimination syndromes in patient who have had a UTI, encouraging them to drink an adequate amount of water. A prolonged course of low-dose antibiotics is effective in reducing the frequency of UTI in those with recurrent UTI. Also cranberry (juice or capsule) may decrease the incidence of UTI in those with frequent infections (Tangho & Mcaninch, 2004).

The data of antibiotic sensitivity pattern has revealed a close relationship with their prophylactic usage. The more frequently used antibiotics like Penicillin, Erythromycin, Chloramphenicol, and Ampicillin revealed very low levels of sensitivity (<25%) to all organisms in a group as a whole. The avoidance of prophylactic usage of antibiotics may help surmount this to a certain extent (Harkness, *et al*., 1975).

In this study, the antibiotic sensitivity tests showed that *Enterococcus faecalis and Ps. Aeruginosa* were sensitive to Cefotaxin, Amoxicillin/ Clavulanate, Norfloxacin, Pipracillin/ Tazobactam, Trimethoprim/ Slfamethoxazol and resistant to Imipenem and Amikacin. *Staphylococcus aureus* was resistant to all selected antibiotics. But *Proteus mirabilis* and *K. pneumoniae* were sensitive to Cefotaxin, Amikacin, Amoxicillin/ Clavulanate, Imipenem, Pipracillin/ Tazobactam and resistant to Trimethoprim/ Slfamethoxazol and Norfloxacin. *M. morganii* was sensitive to Cefotaxin, Amoxicillin/ Clavulanate, Imipenem, Norfloxacin and resistant to Amikacin, Trimethoprim/ Slfamethoxazol, Pipracillin/ Tazobactam.

In a study, imipenem demonstrate good activity against Enterobacteriaceae, (100% for *E coli*, 99% for other Enterobacteriaceae), also piperacillin/tazobactam was the most potent antibiotic against *P. aeruginosa* (90% of susceptible strains versus 84% for carbapenems) as reported in other studies (Turner, 2008). In a nother study, the researcher reported that meropenem, imipenem and piperacillin/ tazobactam are very active against Gram-negative bacilli, including Enterobacteriaceae, and the susceptibility data obtained from this multicentre study were similar to data previously published for studies conducted in Canada and other European countries (Zhanel *et al.,* 2008).

Often the antibacterial agents in herbs, volatile essential oils are extracted from plants using steam distillation. These highly concentrated oils are often complex mixtures of chemicals possessing wide-ranging properties. Before modern medicine started emphasizing chemically synthesized drugs, herbal remedies were the cornerstone of most of the world's healing traditions and even today, are used by 80% of the world's population who cannot afford Western pharmaceutics. As concerns grow about drug side effects or bacterial resistance, many are once again turning to herbal remedies to treat diverse ailments, including UTIs (Knobloch *et al*., 1989). Some studies had shown that Celery Seed oils can help as an herbal remedy today, Celery Seed is most commonly used as a natural diuretic, as well as a treatment for Urinary Tract Infections due to it is anti-bacterial properties (Maruzzella & Sicurella, 1960). The potent anti-septic action and mild diuretic effect of the celery comes in handy in the treatment of many disorders affecting the human body (Farag, *et al*., 1989). Antibacterial activity of various constituents of leaves, flowers and mixtures of *Thymus vulgaris* extracted with distilled water and 90% ethanol was reported in a study. Alcoholic extracts are more efficient on various pathogenic bacteria and mixed extracts have a highly antibacterial activity. In general, all extracts in various concentrations with few exceptions are more efficacious on Gram positive bacteria than on Gram negative bacteria (Goodner *et al*., 2006). The antibacterial activity of *Thmus vulgaris* extracts may be due to presence of phenolic constituents (thymol and carvacrol), which make up a large percentage of the volatile oil (Janssen *et al*., 1987).In general the results agree the results recorded by many workers investigating the used plants ,Celery (Celestin *and* Heiner, 1993), Dill (Delaques *et al*, 2002), Thyme (Goodner *et al* , 2006) and Camomile (Tayel and El-Taras, 2009).

Evaluation of Vitek GNI+ and Becton Dickinson Microbiology Systems Crystal E/NF identification systems for identification of members of the family *Enterobacteriaceae* and other gram-negative, glucose-fermenting and non-glucose-fermenting bacilli. The system is aiming at rapid identification for which time-consuming supplementary tests are contraindicated and/or not often performed in a routine clinical laboratory (Miller, 1999).

One major advantage of the VITEK 2 system is its speed in reliably identifying gram-negative rods within 3 h. This is basically achieved by the more sensitive fluorescence-based technology used in the system. As a broader and more detailed database has been built by the company and allows a better discrimination between related taxa. However, even the more sensitive fluorescence-based technology used in the ID-GNB card did not significantly change the outcome of the identifications of some slowly metabolizing non-fermenting bacteria, which were categorized as various non-fermenting gram-negative bacilli (Stager *et al*., 1998). Obviously, the VITEK 2 system in conjunction with the ID-GNB card represents an improvement

regarding speed compared with the previous VITEK system. In one evaluation, 88.5% of all strains were correctly identified after 3 h, whereas in the evaluation of O'Hara et al., applying the previous GNI+ card, only 47% of all enteric strains were identified in 3 h or less (O'Hara, et al., 1997). Other advantages of the VITEK 2 system are the decreased turnaround and hands-on times since the system is nearly fully automated. The high degree of automation may also improve accuracy (Cuziat *et al.*, 1997). Factors affecting the quality of the identification are the age of the culture (8- to 24-h cultures are best) and the inoculums but not the age of the inoculum suspension (Guicherd *et al*., 2002).

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