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Study of Rosemary Essential Oil Antibacterial Effect on bacteria Isolated from Urinary Tract Infections in some Hospitals of Baghdad

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ABSTRACT

Sixty urine samples were collected from women with urinary tract infection in different ages. The aims of this study were determined the dominancy of pathogens isolated from urine of women with UTI and evaluating the antibacterial activity of *Rosmarinus officinalis* L. essential oil against these pathogenic isolates. Identification of bacteria was done on Chromogenic orientation agar while disc diffusion method was used for determination the sensitivity of bacterial isolates to antibiotics and Agar well diffusion method was used for evaluation the antibacterial effect of Rosemary essential oil on these isolates. The results showed that 50% of women infected with *Escherichia coli*, it was dominants in ages above 15 years old while *Staphylococcus aureus* seems to be dominants in ages under 10 years old. Meropenem (10mcg) showed highest effect on *E.coli* and *Klebsiella* while Cefixime (5mcg) showed highest effect on *S. aureus* as well as neither of these antibiotics had no effect on *Candida albicans*. On the other hand, Rosemary essential oil showed effect to bacterial isolates between5000-10000ppm and to *Candida* at 5000ppm.

Keywords: UTI, Chromogenic orientation agar, *R. officinalis*.

1. INTRODUCTION

Millions of people affected by urinary tract infections (UTI) and considered as a serious health problems [1]. 8.3 millions visit to the hospital each year so it represent the second most common type of infections in the body [2] UTI more frequent in women than men [3,4]. Bacterial UTIs can involve the bladder, prostate, urethra, or kidneys. Symptoms may include dysuria, urgency, lower abdominal pain and flank pain. Kidney infection may be accompanied with symptoms and even sepsis may occur with. UTI are about 50-fold more common in women than men. Cystitis or pyelonephritis, are dominant types of UTIs in women in adult aged 20 to 50 years [5].

1.1 Type of urinary tract infection

Cystitis or pyelonephritis (kidney infection) considered as uncomplicated UTI and is usually associated with lower urinary tract (the bladder and urethra) that occurs in women in premenopausal adult stage with no structural or functional abnormality of urinary tract and who are not pregnant [6,7]. Complicated UTI can involve either sex at any age, where the patient has urinary tract abnormality (structural or functional) and urinary flow obstruction, and patients had the catheter or surgery of the urinary tract evolving as the pyelonephritis or cystitis, this type being more sever and recurrent [8]. Postmenopausal and sexually active premenopausal women had higher morbidity than others by recurrent urinary tract infection. Adult women with first episode infected with recurrence in 25–30% of UTI, and another infection can expect in about 25-50% of these women within a year of previous one. Recurrence occurs in most women who had an uncomplicated UTI [9].

1.2 Most common causative agent of UTI

Escherichia coli represent the commonest causative agent in 80% of UTI, it has specific attachment factors that attach on transitional epithelium of bladder [10]. Younger women mostly infected with *Staphylococcus saprophyticus* (10%), with higher incidence in the summer [11]. 5% of infection occur by Gram negative such as *Klebsiella, proteus* and *enterobacter* [12]. *Candida albicans*, virus and sexually transmitted bacteria e.g. *Chlamydia* formed approximately 3%. *Staphylococcus aureus*, group B streptococci an ***Enterococcus*** represent 2% of other Gram positive bacteria [13].

1.3 Rosemary

Scientific Name(s): *Rosmarinus officinalis* L. Family: Lamiaceae (mints) Common Name(s): Rosemary, (*Rosmarinus officinalis*). Traditionally, it is used medicinally to improve memory, stimulate hair growth, support the circulatory and nervous systems and relieve muscle pain and spasm. Long term daily intake of rosemary prevents thrombosis in humans (14)**.** It is considered as safe when taken in recommended doses. Vomiting, spasms, coma and, in some cases, pulmonary edema (fluid in the lungs) considered as serious side effects when rosemary leaves used in large quantities of, because of their volatile oil content. Rosemary should not be taken as a supplement by women because higher doses of rosemary may cause miscarriage, pregnant and nursing but it is safe to eat as a spice in food**.** People with high blood pressure, ulcers, Crohn's disease, or ulcerative colitis should not take rosemary**.** Rosemary oil can be toxic if ingested and should never be taken orally [15]**.**

In the last few years, due to the misuse of antibiotics and an increasing incidence of immunodeficiency-related diseases, the development of microbial drug resistance has become more pressing problem. Recently, phytochemicals with antimicrobial potential have been extensively explored to identify components for possible medical applications.

Essential oils or some of their components are used in perfumes and make-up products, in sanitary products, in dentistry, in agriculture, as food preservatives and additives, and as natural remedies [17]. In recent years, due to the new interest in natural products, plant essential oils have come more into the focus of phytomedicine [16, 18]. It is important to develop a better understanding in basic research applications, especially, of the anti-microbial and anti-oxidant activities of essential oils as well as their potential anti-cancer activity [19, 20].

The aims of this study were determined the dominancy of pathogens isolated from urine of women with UTI and evaluating the antibacterial activity of *R. officinalis* L. essential oil against these pathogenic isolates.

2. MATERIALS AND METHODS

2.1 Samples collection

Sixty specimens were collected from patients (women) suffering from urinary tract infection without on any type of antibiotics. The specimens were collected from the educational laboratories of Madienat al-teb and Al qanat Laboratories.

2.2 Preservation of bacterial isolates

Bacteria that obtained on chromogenic agar orientation were preserved by taking number of colonies and inoculated on nutrient agar slants then incubated at 37 °C for 24 hours.

2.3 Activation of bacterial isolates

Isolates that preserved in slants were activated by inoculating it in nutrient broth before using in sensitivity test.

2.4 Sensitivity test

Bacteria that isolated from UTI cases were examined against 10 different antibiotics (discs) on Muller Hinton agar. Bacteria spread on the plate (0.1 ml of inoculums from culture 24 hr age) then by sterile forceps, discs of antibiotics were placed on the inoculated media and incubated for 24 hr at 37 °C. Diameter of inhibition zones were calculated.

2.5 Rosemary essential oil extraction

Taken 250gm of air-dried, cut, aerial parts of rosemary and blended it in the blender to make it as powder then the powder is placed in the round-bottom flask of Clevenger-type apparatus, added 500-600 ml pure water and boiled for 4 hours. The essential oil hydro-distilled and after condensation water drained and essential oil collected.

2.6 Effect of Rosemary essential oil as antimicrobial agent

Four different concentrations were prepared from oil (10000ppm, 5000ppm, 2500ppm, 1250ppm) by using DMSO as diluents. Five wells on Muller hinton agar plate covered with bacterial culture previously were done by sterile cork borer. Four wells filled with different concentrations of oil by adding 25µl from each diluents, the fifth well (in the center) was filled with DMSO only as control. Plates were incubated for 24 hr at 37°C, then the diameter of inhibition zones were calculated.

3. RESULTS AND DISCUSSION

3.1 Manual definition of the culture

The chromogenic orientation agar is used to differentiate the gram-negative from other gram-positive bacteria causing the disease, especially for UTI infection. The cases classified according to age of patients are given in table 1.

Table 1: Samples classification according to patients age.

Age	No. of samples	Percentage
Under 10 years old	9	15%
Between 15-50 years old	36	60%
Above 50 years old	15	25%
Total number of samples	60	

Cultivation of urine samples found 30 cases were infected by *E.coli* out of 60 cases (50%), color and shape of colony is different from what appears in the rest of the agar media, it seems small, dark purple color colonies, sometimes purple or pink diagonal for a little redness.

Since the culture has differential nature of bacteria, it is possible that these bacteria show up with one or two other forms of bacteria for example with *Proteus* and *Enterococcus* or *Staph.aureus*. The second most bacteria appeared is the *Enterococcus* which appeared in 22 cases out of 60 (36.6%), Celestial show -colored or light blue diagonal of greenness and its colonies are small. Third prominent bacteria emerged is *Staphylococcus aureus* for 16 cases (26.6%) where it was identified by its transparent color. The fourth is *Proteus* appeared in 11 cases (18%). Colony color yellow to brown. *Klebsiella* appeared in 4 samples (6.6%). It has dark blue color and appeared with other species like *E.coli* and *Enterococcus*. The less one appeared on agar among the others is *Candida*, which is one of the common types of yeasts that infect the urinary system. *Candida* colony has white color; it appeared in two specimens only (3.3%) (table 2).

Table 2: Isolates that obtained from 60 urine samples and there percentage.

Bacterial species	No. of isolates	Percentage %
E. coli	30	50%
Enterococcus	22	36.6%
Staph. Aureus	16	26.6%
Proteus	11	18%
Klebsiella	4	6.6%
Candida albicans	2	3.3%
E. coli +Enterococcus	6	10%
Proteus+Enterococcus	6	10%
Total samples number = 60		

The more bacteria causing the children UTI who are under the age of ten are *Staphylococcus* which appeared in 5 samples out of 9 samples (55.5%) then *E. coli* and *Enterococcus* where each one of them appeared when two out of 9 and therefore the ratio will be (22.2%) (Table 3).

Table 3: Isolates that obtained from children UTI (under 10 years old) and there percentage.

Bacterial species	No. of isolates	Percentage %
E. coli	2	22.2%
Enterococcus	2	22.2%
Staph. Aureus	5	55.5%
Samples number = 9		

When collecting samples that appeared for the range between (15-50) years found that ***E. coli*** appeared in 21 samples out of 36 and the proportion of high- more than half the sense caused 58.3 % followed thereafter *Enterococcus* appeared either Staph. Categorized in 9 samples followed by *Klebsiella* and Candida and *Proteus* in few numbers (table 4).

Table 4: Isolates that obtained from adult(15-50 years old) and there percentage.

Bacterial species	No. of isolates	Percentage %
E. coli	21	58.3%
Enterococcus	14	38.8%
Staph. Aureus	9	25%
Proteus	7	19.4%
Klebsiella	1	2.7%
Candida albicans	1	2.7%
Samples number = 36		

But women aged 50 and older were hit by ***E. coli*** and ***Enterococcus*** which appeared in 6-7 samples out of 15 samples followed by ***Proteus*** appeared in 4 samples and then **Staph**, ***Klebsiella*** and ***Candida*** (table 5).

Table 5: Isolates that obtained from adult (above 50 years old) and there percentage.

Bacterial species	No. of isolates	Percentage %
E. coli	7	46.7%
Enterococcus	6	40%
Staph. aureus	2	13.3%
Proteus	4	26.7%
Klebsiella	3	20%
Candida albicans	1	6.7%
Samples number = 15		

3.2 Antibiotics sensitivity result

Ten types of antibiotics were used to detect the sensitivity of some microbes isolated from UTI cases such as *E.coli* , *Klebsiella* , *Staphylococcus aureus* and *Candida*.

All antibiotics have no effect on *Candida*, tetracycline (10mcg), bacitracin (10µ) and Cephalothin (30mcg) have no effect on all microbes mentioned above. Cefixime (5mcg) worked *on Staphylococcus aureus* only in the same time it showed the highest effect on it while Meropenem (10 mcg) showed highest effect on *E.coli* and *Klebsiella* as showed in table 6.

Table 6: Inhibition zone (cm) of different antibiotics on isolates from UTI.

Antibiotics	*E.coli*	*Klebsiella*	*Staphylo*	*Candida*
Azithromycin (15mcg)	1.2	2	NO	NO
Tetracycline (10mcg)	NO	NO	NO	NO
Bacitracin (10µ)	NO	NO	NO	NO
Cefixime (5mcg)	NO	NO	3.2	NO
Meropenem (10mcg)	2.65	3	2.7	NO
Ampicillin (10mcg)	1.5	2.1	2.4	NO
Amoxicillin (25mcg)	0.3	1.6	0.6	NO
Cephalothin (30mcg)	No	No	NO	NO
Clarithromycin (15mcg)	1.7	2.1	1.4	NO
Gentamicin (10mcg)	2.4	1.9	1.9	NO

3.3 Antimicrobial effect of Rosemary essential oil

The same bacteria that used to detect the sensitivity to antibiotics were used to detect the antimicrobial effect of Rosemary essential oil, 4 concentrations were used (10000ppm, 5000ppm, 2500ppm and 1250 ppm). No effect seen on all bacterial types in 2500ppm and 1250 ppm, in contrast, effect appeared on all bacterial types in 10000ppm, the effect on *Klebsiella* and *Candida* seems more than on the others. In 5000ppm, no effect observed on *E.coli* and *Klebsiella* while highest effect appeared on *Candida* (table 7).

Table 7: Antimicrobial effect of Rosemary essential oil (inhibition zone in cm)

	E.coli	*Klebsiella*	*Staphylo*	*Candida*
10000ppm	1.2	2	1.3	2
5000 ppm	NO	NO	1.8	2.2
2500 ppm	NO	NO	NO	NO
1250	NO	NO	NO	NO

Rosemary essential oil was bactericidal on Gram-positive and Gram-negative bacteria at 0.1 and 0.3 % respectively. Fennel essential oil concentration at 0.1 and 0.3% had bacteriostatic effect on Gram-positive bacteria and Gram-negative bacteria respectively. The highest bactericidal concentration (1.0 %) of sage essential oil was observed on *Y. enterocolitica* and lowest (0.05 %) *on L. monocytogenes* [21].

From the total number of samples, 60% of them were found from patients with their ages between 15-50 years and this may be as a result to the alteration of conditions monthly during cycle that lead to minimize the immunity during this period. *E.coli* represents 50% of bacterial isolates as a result to the short distance between anal opening and urinary tract opening.

Antibiotics, in general, show low effect on bacterial isolates while there is no effect on *Candida* was observed, these results may be as a result to the wrong use of antibiotics in Iraq, it's usual in Iraq to get any antibiotics without clinician decision this lead to develop strains with high resistance to different antibiotics. Rosemary essential oil showed good effect on *Candida.*

Resistance in Gram-negative bacteria has been increasing, particularly over the last 6 years. This is mainly due to the spread of strains producing extended-spectrum b-lactamases (ESBLs). Many of the isolates producing these enzymes are also resistant to trimethoprim, quinolones and aminoglycosides, CTX-M-producing *Escherichia coli* often occurs in the community and as *E. coli* is one of the commonest organisms causing urinary tract infections (UTIs) [22]. Data from the Australian Group on Antimicrobial Resistance (AGAR) show that resistance to fluoroquinolones may be increasing. Isolates taken from patients with UTIs in the community in 2008 and 2012 showed an increase in non-susceptibility to ciprofloxacin; 4.2% vs. 6.9 %. *E coli*, defined by the AGAR as 'acquired resistance to more than three antibiotics', is of significant concern. AGAR surveys indicate that multidrug-resistant *E coli* increased from 4.5% of isolates in 2008 to 7.6% of isolates in 2012 [23]. Amoxycillin and clavulanate may be an alternative in uncomplicated UTIs caused by multidrug-resistant isolates if susceptibility is confirmed by laboratory testing. Also consider nitrofurantoin, which currently retains activity against most multidrug-resistant strains [24]. UTIs caused by multidrug-resistant *E coli* have also been observed in patients without obvious risk factors. This may be due to an increase in the number of otherwise healthy individuals colonised with multidrug-resistant *E coli* [25]. Severe problems (eg, urosepsis, kidney scarring, end-stage renal disease) are more likely to occur with complicated infections [26]. So it is important to order a urine culture to ensure appropriate antibiotic treatment in patients with a complicated UTI [27].

Significant changes in antimicrobial susceptibility were evident. A greater proportion of isolates were resistant to ampicillin, amoxicillin-clavulanic acid, cefuroxime, ceftazidime and clotrimoxazole in 2003 when compared to 1999. With respect to *E coli*, there were significant increases in prevalence of resistance to cefuroxime and amoxicillin-clavulanic acid. The overall resistance rate for norfloxacin remained relatively low and was unchanged for *E coli* [28]. *E coli* strains were suppressed by both treatments. Staphylococcus spp. and enterococci colony counts increased following PIV treatment in the periurethral flora but remained neither stable with NOR [29]. 73,675 individuals were identified with UTI, of whom 54,796 (74.4%) received trimethoprim-sulfamethoxazole (TMP-SMX), 4184 (5.7%) received ciprofloxacin, 3142 (4.3%) received levofloxacin, 5984 (8.1%) received ofloxacin, and 5569 (7.6%) received norfloxacin. Compared with TMP-SMX, the composite treatment failure was significantly lowered for norfloxacin in propensity score (PS) matching analyses (OR, 0.73; 95% CI, 0.54-0.99). Both norfloxacin (PS-matched OR, 0.68; 95% CI, 0.47-0.98) and ofloxacin (PS-matched OR, 0.70; 95% CI, 0.49-0.99) had significantly lowered composite treatment failure rate when compared with ciprofloxacin [30]. Significant increases were seen in the frequency of ofloxacin (8.9 to 16.7%) and ciprofloxacin resistance (6.2 to 10.1%) ($p < 0.001$). It was found that an increased use of one defined daily dose (DDD)/1000 patient-days for ofloxacin, ciprofloxacin and norfloxacin induced average increases of 0.81%, 0.65% and 0.53% in *E. coli* ofloxacin resistance ($p < 0.01$), with average delays of 4, 4 and 6 months, respectively [31].

Sienkiewicz and coworkers studied the antibacterial activity of the *Rosmarinus officinalis* essential oil on standard strain *Escherichia coli* ATCC 25922 as well as 60 other clinical strains of *Escherichia coli*. Strains isolated from patients with infections of the abdominal cavity, respiratory tract, skin, urinary tract and from hospital equipment. *Rosmarinus officinalis* essential oil are active against all of the clinical strains from *Escherichia coli* including extended-spectrum β-lactamase positive bacteria, [32] Luqman and coworkers found that rosemary essential oil more active against the gram-positive pathogenic bacteria compared to gram-negative bacteria. (except *E. faecalis* and drug-resistant mutants of *E. coli*) [33]. Santoyo *et al* work on effect of Rosemary essential oil antimicrobial activity against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), a yeast (*Candida albicans*)and fungus(*Aspergillus niger*) and found that S. aureus be the most sensitive bacteria, whereas the least susceptible was *A. niger* [34]. The MIC (minimal inhibitory concentration) and the MBC (minimal bactericidal concentration) of Rosemary essential oil was investigated and revealed that *Aeromonas sobria* and *Candida* strains were the most susceptible micro-organisms [35]. Zaouali from Tunisia and coworkers studied the variation in antimicrobial and antioxidant activity in the essential oil composition of *Rosmarinus officinalis* var. *typicus* and var. *troglodytorum* endemic to Tunisia, and growing wild in different bioclimates. oils from var. *troglodytorum* showed higher bactericidal effect than those from var. *typicus* [36].

5. REFERENCES

1. Okonko, I. O., Ijandipe, L. A., Ilusanya, A. O., Donbraye-Emmanuel, O. B., Ejembi, J., Udeze A. O.,Egun O. C., Fowotade A. and Nkang A. O.(2010) Detection of Urinary Tract Infection (UTI) among pregnant women in Oluyoro Catholic Hospital, Ibadan, South-Western Nigeria Malaysian Journal of Microbiology, Vol 6(1), pp. 16-24.
2. Ojo O. and Anibijuwon I.(2010) Urinary tract infection among female students residing in the campus of the University of Ado Ekiti, Nigeria O .African Journal of Microbiology Research Vol. 4 (12), pp. 1195-1198.
3. Vazquez, J. C. and Villar, J. (2000). Treatment for symptomatic urinary tract infections during pregnancy (Cochrane Review). In: Cochrane Library. Issue 3. John Wiley and Sons Ltd., Chichester, UK.

4. Tambekar DH, Dhanorkar DV, Gulhane SR, Khandelwal VK, Dudhane MN (2006). Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. Afr. J. Biotechnol., 5(17): 1562-1565.
5. Wood M. W, Breitschwerdt E. B., and . Gookin J. L(2010) Autocrine Effects of Interleukin-6 Mediate Acute-Phase Proinflammatory and Tissue-Reparative Transcriptional Responses of Canine Bladder Mucosa▽ Infect Immun. Feb; 79(2): 708–715.
6. Hatzichristou D, Rosen RC, Broderick G, Clayton, A, Cuzin B, Derogatis L, Litwin M, Meuleman E,O'Leary M, Quirk F, Sadofsky R and Seftel A (2004) Clinical Evaluation and Management Strategy for Sexual Dysfunction in Men and Women. J Sex Med 1:1, 49 – 57.
7. Gupta K1, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, Moran GJ, Nicolle LE, Raz R, Schaeffer AJ and Soper DE(2011) International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A Clin Infect Dis. 1;52(5):e103-20.
8. Thomas M. Hooton, M.D.(2012).Uncomplicated Urinary Tract Infection the new engl j med 366;11
9. Falagas M. E., Betsi G. I., Tokas T. and Athanasiou S. (2006) Probiotics for Prevention of Recurrent Urinary Tract Infections in Women A Review of the Evidence from Microbiological and Clinical Studies Drugs; 66 (9): 1253-1261
10. Karen E, Dorthe S, Bettina L, Suen F, Stig H, Tor M, Rolf L and Niels F (2006). Pulse-field gel electrophoresis typing of *Escherichia coli* strains from samples collected before and after pivmecillinam or placebo treatment of uncomplicated community acquired urinary tract infection in women. J. Clin. Microbiol. 44: 1776-1781.
11. Michael W, Johan W, Suen F, Carina K, Tor M (2007). Molecular epidemiology of Staphylococcus saprophyticus isolated from women with uncomplicated community-acquired urinary tract infection. J. Clin. Microbiol. 45: 1561-1564.
12. Benjamin WD, Brian KP, Gary VD (2009). *Lactobacillus deldrueckii* as the cause of urinary tract infection. J. Clin. Microbiol. 47: 275-277.
13. Ecke T. H.(2013) When do we need a prophylactic treatment for asymptomatic bacteria? Cent European J Urol. 2013; 66(3): 328.
14. Angioni A, Barra A, Cereti E, Barile D, Coïsson JD, Arlorio M, Dessi S, Coroneo V and Cabras P(2004)chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. J Agric Food Chem. Jun 2;52(11):3530-5.
15. Debersac P1, Heydel JM, Amiot MJ, Goudonnet H, Artur Y, Suschetet M and Siess MH (2001)Induction of cytochrome P450 and/or detoxification enzymes by various extracts of rosemary: description of specific patterns. Food Chem Toxicol. Sep;39(9):907-18.
16. Zu, Y.G.; Yu, H.M.; Liang, L.; Fu, Y.J.; Efferth, T.; Liu, X.; Wu, N. (2010). Activities of Ten EssentialOils towards *Propionibacterium acnes* and PC-3, A-549 and MCF-7 Cancer Cells. *Molecules*,*15*, 3200–3210.
17. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. *(*2008). Biological effects of essential oils–A review. *Food Chem. Toxicol.*, *46*, 446–475.
18. Sylvestre, M.; Pichette, A.; Longtin, A.; Nagau, F.; Legault, J. (2006). Essential oil analysis andanticancer activity of leaf essential oil of Croton flavens L. from Guadeloupe. *J. Ethnopharm.*, *103*, 99–102.
19. Mimica-Dukic, N.; Bozin, B.; Sokovic, M.; Simin, N.(2004). Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. *J. Agric. Food Chem.*, *52*, 2485–2489.
20. Sylvestre, M.; Legault, J.; Dufour, D.; Pichette, A. (2005). Chemical composition and anticancer activity of leaf essential oil of *Myrica gale L. Phytomedicine*, *12*, 299–304.
21. Erkmen O. and Özcan M. (2004) Antimicrobial effects of essential oils on growth of bacteria, yeasts and molds .Journal of Essential Oil Bearing Plants Volume 7, Issue 3 pages 279-287.
22. Pallett A. and Hand K. (2010). Complicated urinary tract infections: practical solutions for the treatment of multiresistant Gram-negative bacteria J Antimicrob Chemother 65 . 3: 25–33
23. The Australian Group on Antimicrobial Resistance. Gram-negative survey. (2012) antimicrobial susceptibility report. (accessed 22 August 2014).
24. Therapeutic guidelines. antibiotic version 15, (2014). Melbourne: Therapeutic Guidelines Ltd, 2014.
25. Oteo J, Perez-Vazquez M, Campos J. (2010). Extended-spectrum [beta]-lactamase producing Escherichia coli: changing epidemiology and clinical impact. Curr Opin Infect Dis 23:320–6.
26. Melekos MD, Naber KG (2000). Complicated urinary tract infections. Int J Antimicrob Agents;15:247–56.
27. Jarvis T, Chan L and Gottlieb T (2014). Assessment and management of lower urinary tract infection in adults. Aust Prescr 37:7–9.
28. Orrett FA and Davis GK. (2006). A comparison of antimicrobial susceptibility profile of urinary pathogens for the years, 1999 and 2003. West Indian Med J. ;55(2):95-9
29. Norinder BS; Norrby R; Palmgren AC; Hollenberg S;Eriksson U and Nord CE. (2006).Microflora changes with norfloxacin and pivmecillinam in women with recurrent urinary tract infection. Antimicrob Agents Chemother. 50(4):1528-30.
30. Lee MT, Lee SH, Chang SS et al. (2014).Comparative effectiveness of different oral antibiotics regimens for treatment of urinary tract infection in outpatients: an analysis of national representative claims database. Medicine (Baltimore).;93(28):e304.
31. Mahamat A, Lavigne JP, Fabbro-Peray P et al. (2005). Evolution of fluoroquinolone resistance among Escherichia coli urinary tract isolates from a French university hospital: application of the dynamic regression model. Clin Microbiol Infect.11(4):301-6.
32. Sienkiewicz M1. Łysakowska M, Pastuszka M, Bienias W. and Kowalczyk E.(2013) The potential of use basil and rosemary essential oils as effective antibacterial agents. Molecules 5;18(8):9334-51.
33. Luqman S Dwivedi GR, Darokar MP, Kalra A and Khanuja SP. (2007) Potential of rosemary oil to be used in drug-resistant infections. Altern Ther Health Med. 13(5):54-9.
34. Santoyo S, Cavero S, Jaime L, Ibañez E, Señoráns FJ and Reglero G. (2005)Chemical composition and antimicrobial activity of *Rosmarinus officinalis L.* essential oil obtained via supercritical fluid extraction. J Food Prot. ;68(4):790-5.
35. Pintore G, Marchetti M, Chessa M, Sechi B, Scanu N, Mangano G and Tirillini B(2009). *Rosmarinus officinalis* L.: chemical modifications of the essential oil and evaluation of antioxidant and antimicrobial activity. Nat Prod Commun.;4(12):1685-90
36. Zaouali Y , Bouzaine T and Boussaid M. (2010) Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities.Food Chem Toxicol. 48(11):3144-52.

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