Vol. 6 (2), July 2015, 35 - 43

Antimicorbial Efficacy of Green Tea Extract and their inhibitory action against uropathogens from Clinical Sample

Yusra Iqbal

Department Of Microbiology, Jinnah University For Women, Karachi.

ABSTRACT

Green tea extract is an all natural way to obtain fluoride and they have a primary antimicrobial influence on a number of gram positive and gram negative bacteria plus on some fungi. Tea catechins have straight impact to the lipid bilayer. These catechins in turn affect the membrane. The main catechins in green tea extract are epicatechin (EC), epigallocatechin-3-gallate (EGCg), epigallocatechin (EGC), epicatechin-3-gallate (ECG). EGCg and EGC have been proven to really have the biggest antimicrobial outcome. It also inhibits the formation of fatty acid solution. Inhibition of fatty acidity synthesis by green tea extract has been found to inhibit bacterial development of harmful metabolite also. In today's study we noticed the antimicrobial effectiveness of green tea extract on the bacterial and fungal strains that have been obtain from the urine samples of UTI patients. Of January 2016 a total of 14 samples were gathered in month. The collected urine samples were organized to isolate the bacterial agents by using systematizes etiquette of isolation and determined by cultural characteristics and biochemical tests. 27 strains of pathogenic bacteria and fungus were collectively determined were 64.4% was E.coli, *Staphylococcus aureus* 50%, Pseudomonas 28.5% and Candida was 35.7%. The antibacterial activities of green tea extract contrary to the isolates were dependent on agar well diffusion method and broth dilution solution to performed Minimum inhibitory concentrations (MIC). The MIC of green tea extract against all the 27 isolates discovered positive effect at 12.25 mg/ml where 66.66% was E.coli, S.aureus was 57.14%, Pseudomonas 50% and Candida was 40%. On the other hand only S. aureus and Candida will be the most sensitive isolates inhibited by agar well diffusion technique. Inhibition of the isolates inhibited by agar plate were dependent on zone of inhibition in mm. Urinary system infections is one of the normal infection to have an effect on human which is the second most typical cause of clinic visits. It really is more prevalent in feminine than in man.

INTRODUCTION

The enlargement of anti-microbial resistance in bacteria is a developing issue around the world. In this way, treatment alternatives are supplanted with a second or third decision of antibiotics, which are considerably more costly (Payam Behzadi, Elham Behzadi, *et al* 2015). These difficulties have been getting developing enthusiasm to discover elective antimicrobial operators from plant extract that should be produced and used to control multidrug-resistant bacteria

Determination of empiric anti-infection agents for urinary tract diseases (UTIs) has turned out to be all the more difficult as a result of the expanding rates of multidrug-resistant Enterobacteriaceae (MDRE) infection.When discussing about the UTI, the proper frame work of urinary system must be known. The urinary tract is recognized as the renal system also. It involves the kidneys, ureters, bladder, and the urethra (C. Dugdale, David 2011). The kidneys make urine by filtering wastes and further water from bloodstream (Kim Ann Zimmermann, 2015). Each kidney involves

millions of practical items called nephrons (C. Dugdale, David 2011). Its key function is to modify the attention of normal water and soluble chemicals like sodium by filtering the blood vessels, reabsorbing what's needed and excreting the others as urine (Maton, Anthea; Jean Hopkins, *et al* 1993).

The kidneys create and collaborate with a few hormones that are included in the control of frameworks outside of the urinary framework. The hormones are Calcitriol. It is the dynamic type of vitamin D in the human body. Calcitriol cooperates with parathyroid hormone (PTH) to raise the level of calcium particles in the circulation system.

Another one is Erythropoietin. Erythropoietin, otherwise called EPO, is a hormone that is created by the kidneys to invigorate the generation of red blood cells.

The next in the line is renin. Renin is not a hormone itself, but rather a catalyst that the kidneys produce to begin the renin-angiotensin system (RAS).

There are several pathogens which are involved in the UTI some of them are E. coli, Pseudomonas, and Staph. aureus and Candida.

The E.coli is one of the major causes of UTI. Escherichia (E.) coli is in charge of most uncomplicated cystitis cases in women, particularly in more youthful ladies. E. coli is by and large a safe microorganism beginning in the digestion tracts. On the off chance that it spreads to the vaginal opening, it might attack and colonize the bladder, bringing on a disease. The spread of E. coli to the vaginal opening most generally happens when ladies or young ladies wipe themselves from back to front in the wake of urinating, or after sexual action. UTI and a considerably more noteworthy number create asymptomatic bacteriuria (ABU) (Johnson. 1991). Uropathogenic E. coli (UPEC) speak to a particular subset of E. coli fit for colonizing the urinary tract and evoking the indications of cystitis and pyelonephritis.

Staphylococcus aureus is the next organism used in the recent study. *Staphylococcus aureus* is as often as possible isolated from urine samples got from long term care patients. The centrality of staphylococcal bacteriuria is indeterminate. We estimated that S. aureus is a urinary pathogen and that colonized urine could be a source of future staphylococcal disease.

Pseudomonas is the third most typical pathogen connected with hospital-acquired catheter-associated UTIs. Pseudomonas is a typical reason for urinary tract infection and for the most part is found in patients who have had urologic control or have obstructive uropathy

Funguria is normal in hospitalized patients and is for the most part amiable. Intrusive disease of the kidney is surprising and is hard to treat. Most by far of contagious diseases of the kidney and bladder result from *Candida albicans* and other Candida species. The vicinity of C.albicans in urine is known as candiduria, which may happen in both asymptomatic and symptomatic UTIs. In spite of the high rate of morbidity in UTIs brought on by C.albicans, the mortality is low.

Numerous studies uncovered that Green tea has an immediate antimicrobial impact on microorganisms and parasite. For green tea creation, newly reaped tea leaves should be prepared with minimal measure of oxidation (Tem Isikgoz Tasbakan, Raika Durusoy *et al.* 2013). Green tea is: as a cancer prevention agent, mitigating, hostile to cancer-causing in oral contaminations, Urinary Tract diseases and others. Large portions of the immediate impacts of tea catechins are results of the catechins tying to the bacterial lipid bilayer membrane which then makes harm to membrane. The most critical catechins in green tea are epicatechin (EC), epigallocatechin-3-gallate (EGCg), epigallocatechin (EGC), epicatechin-3-gallate

(ECG). EGC and EGCg have been appeared to have the best antimicrobial impacts. Is harm can then prompt an assortment of related antimicrobial impacts (Barbara W. Trautner, MD, PhD, 2010). Tea catechins additionally hinder the unsaturated fatty acid. Restraint of fatty acid synthesis by green tea has additionally been found to repress bacterial creation of dangerous metabolites (Johnson. 1991). In vivo investigations have shown that green tea extract decreases the UTI infection by repressing the uropathogens, generally E. coli, Pseudomonas, Candida and S. aureus (Tem Isikgoz Tasbakan, Raika Durusoy *et al.* 2013). Urinary tract infection (UTI) is a standout to be the most imperative reasons for morbidity in communities and it is the second most normal reason for health center center visits. It is more basic in female than in male. Klebsiella, Proteus, Enterobacter, Citrobacter, Staphylococcus and Enterococcus are likewise the pathogens included in UTI (Barbara W. Trautner, MD, PhD, 2010).

MATERIAL AND METHOD:

Collection of Samples: 14 fresh urine sample were collected in a month of January at the Dr. Essa's Laboratory & Diagnostic Center located at North Nazimabad, Block H in Karachi, Pakistan. Early morning samples were collected by using sterile, wide mouthed container with screw cap tops.

Agar Medium:
3 specific agars were used, Mannitol Salt Agar (MSA), Pseudomonas Agar Base (PS) and Sabouraud Dextrose Agar (SDA), for the isolation of *Staphylococcus.aureus*, Pseudomonas and Candida respectively. For E.coli we used Luria Broth (LB).

Culture: A loop full of well mixed urine samples were inoculated on LB broth and on each plate of MSA, PS and SDA. In next step all plates and broths were then incubated at 37°C aerobically for 24 to 48 hours. After incubation examined the plates macroscopically and microscopically. Each agar shows growth of their specific organism.

Observations: On MSA yellow colony should observe which shows the Mannitol fermentation by S.aureus. Dark green and blackish colonies were observed on PS by Pseudomonas. While creamy colored and smooth colonies were produced by Candida on SDA plate. On LB broth turbidity show the growth of E.coli.

For further confirmation of E.coli we used Eosin methylene blue agar (EMB).Take a loop full culture from LB broth and inoculate on EMB plates and incubate the plates at 37°C for 24 hours. After incubation the green metallic sheen was observe on EMB which showed the growth of E.coli.

Biochemical Testing: Citrate utilization test for the identification of Pseudomonas. If there is growth in the citrate agar, the agar is blue and if there is no growth the agar is green.

For S.aureus catalase and coagulase test were performed. The S.aureus has ability to coagulate the plasma.

Preparation of Green Tea Extract: For the preparation of green tea stock solution, take 12.5 gm. green tea and mix it into 100 ml sterile distilled water. Gently stir the extract on the low flame so the extract is completely mixed in the distilled water. After cooling passed the solution from 0.2 mm membrane filter assembly to get the 12.5% stock solution.

Antimicrobial Activity: To examine the antimicrobial activity of green tea extract against the isolates two methods were employed in our research project.

Agar Well Diffusion Method: The method used with standardization of inoculums size was agar diffusion method. For this method we used Muller Hinton Agar (MHA) which is a special agar used to check the sensitivity of

Table I: showing the percentage of isolated organisms

Organisms	Percentages
E.coli	64.28%
Pseudomonas	28.57%
S.aureus	50%
Candida	35.71%

an organism by measuring the zone around the well (measured in millimeters).

With the help or cotton swab lawn the purified colony sterile MHA plate. In next step, with help of sterile borer make a well on each plate and pour 0.2 to 0.5 ml green tea extract solution into the well and then placed were allowed to stay for few minutes for pre diffusion to take place. Then Incubate these plates at 37 C for 24 hours. After incubation the zone was observed which show the sensitivity of an organism.

Determination of Minimum Inhibitory Concentration (Mic): Minimum inhibitory concentration refers to the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. The MIC of green tea extract for the urine samples was determined using the broth dilution method in sterile tubes according to Clinical and Laboratory Standards Institute.

A stock solution of 12.5% green tea extract was prepared by dissolving 10 gm. green tea in 100 ml distilled water.

4 milliliters of this solution was pipette into an empty test tube that labeled as 1.

2 ml of nutrient broth was pipette into 5 other test tubes.

Subsequently 2 ml green tea extract solution from test tube 1 was transferred to test tube 2 containing nutrient broth to prepare two fold serial dilutions. The tubes were inoculated with 100µl (105 CFU) of an overnight culture of test organisms. After 24 hours incubation, on the basis of turbidity observed in tubes, the MIC was determined as the lowest concentration of green tea extract to show on inhibitory effect on growth of the bacteria.

Table II: showing the number of isolated organisms from the fresh urine samples

URINE SAMPLES	ISOLATED ORGANISMS			
	E.coli	***Pseudomonas***	***S.aureus***	***Candida***
Sample No: 1	Present	Present	present	Present
Sample No: 2	—	—	—	—
Sample No: 3	Present	Present	present	—
Sample No: 4	Present	—	present	Present
Sample No: 5	Present	—	present	Present
Sample No: 6	Present	—	—	----
Sample No: 7	Present	—	—	----
Sample No: 8	Present	Present	present	Present
Sample No: 9	—	Present	—	----
Sample No :10	Present	—	—	----
Sample No: 11	—	—	present	----
Sample No : 12	—	—	present	----
Sample No: 13	Present	—	—	---
Sample No: 14	—	—	—	Present

Table III: showing the antimicrobial activity test results in percentages

Test Organisms	Resistant	Intermediate	Sensitive
E.coli	77.77%	—	22.22%
Pseudomonas	50%	25%	25%
S.aures	42.85%	14.28%	42.85%
Candida	60%	—	40%

activity on different bacteria. Agar well diffusion was performed to check the sensitivity zones of isolates. MIC was also performed. No zone of inhibition observed from E.coli which was obtained from sample number 6, 7, 10 and 13. Likewise S.aureus and candida which were obtained from sample number 12 and 14 respectively show resistivity against green

Table IV: showing the antibacterial effect of green tea extract against urine isolates through agar diffusion method

URINE SAMPLES	TEST ORGANISMS	ZONE OF INHIBITION	NATURE OF ORGANISIMS
NUMBER 1	E.coli	19 mm	Sensitive
	Pseudomonas	16 mm	Intermediate
	S.aureus	13 mm	Intermediate
	Candida	23 mm	Sensitive
NUMBER 2	E.coli	----	Resistant
	Pseudomonas	24 mm	Sensitive
NUMBER 3	S.aureus	10 mm	Resistant
NUMBER 4	E.coli	10 mm	Resistant
	Candida	10 mm	Resistant
	S.aureus	24 mm	Sensitive
NUMBER 5	E.coli	20 mm	Sensitive
	Candida	----	Resistant
	S.aureus	----	Resistant
NUMBER 8	E.coli	----	Resistant
	Pseudomonas	----	Resistant
	S.aureus	25 mm	Sensitive
	Candida	30 mm	Sensitive
NUMBER 9	Pseudomonas	----	Resistant
NUMBER 11	S.aureus	25 mm	Sensitive

RESULTS:

14 fresh urine samples were collected and inoculated on four different specific agar respective to the required bacteria. Positive results were observed on all four specific agars. In which 9 were E.coli, 4 were Pseudomonas, 7 were S.aureus and 5 were candida. E.coli is the mostly isolated organism in these samples. (Table 1.1 and 1.2). The antibacterial activity of green tea extract was checked on isolates. Green tea extract showed different antibacterial tea extract. The other results of antibacterial activity are discussed in table 2.1. Antimicrobial activity test results in percentages are discussed. In table no 3.1 antibacterial activity against green tea extract is determined by MIC by making concentration 6.25mg/ml – 100mg/ml.

DISCUSSION

Total 27 isolates were isolated from 14 urine samples. According to the results the pat hogens that were isolated from the urine samples of

Table VI: showing MIC percentages

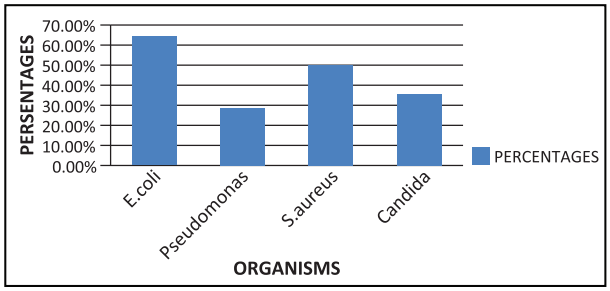
ORGANISMS	MIC (conc. In micro gram)				
	100	50	25	12.5	6.25
E.coli	55.55%	22.22%	66.66%	66.66%	44.44%
Pseudomonas	50%	50%	75%	50%	25%
S.aureus	57.14%	42.85%	57.14%	57.14%	14.28%
Candida	60%	60%	40%	40%	-

Table V showing the antibacterial activity against green tea extract by MIC by making concentration 6.25mg/ml –100mg/ml. TABLE 3.1

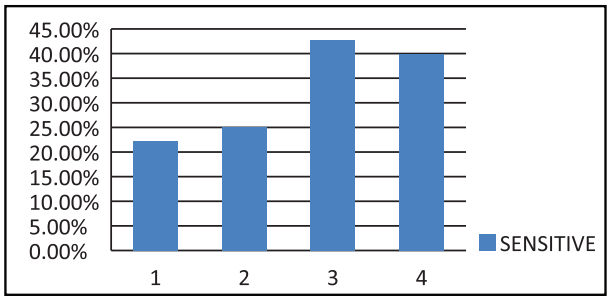
URINE SAMPLES	ISOLATED ORGANISMS	MIC (conc. In micro gram)				
		100	50	25	12.5	6.25
NUMBER 1	E.coli	+	+	+	---	---
	S.aureus	+	+	+	---	---
	Pseudomonas	+	+	---	---	+
	Candida	---	---	+	++	++
NUMBER 3	E.coli	+	+	---	---	---
	Pseudomonas	++	+	+	---	---
	S.aureus	+	+	++	++	++
NUMBER 4	S.aureus	---	---	---	---	---
	E.coli	+	+	---	---	+
NUMBER 5	S.aureus	---	+	---	---	---
	Candida	---	---	---	+	++
	E.coli	---	---	---	---	+
NUMBER 8	Pseudomonas	++	+	++	+++	+++
	S.aureus	---	---	---	+	+
	Candida	---	---	---	---	+
	E.coli	+	+	++	++	++
NUMBER 6	E.coli	---	+	---	---	+
NUMBER 7	E.coli	---	---	---	++	+++
NUMBER 9	Pseudomonas	---	---	---	++	++
NUMBER 10	E.coli	---	+	---	---	+
NUMBER 11	S.aureus	---	+	++	++	++
NUMBER 12	S.aureus	---	----	---	---	+
NUMBER 13	E.coli	+	+	++	+++	+++
NUMBER 14	Candida	---	---	---	---	++

UTI patients, show inhibition against the green tea extract by agar well diffusion method and broth dilution method to performed minimum inhibitory concentration (MIC). The MIC of green tea against all the 27 isolates revealed positive result at 12.25 mg/ml in which 66.66%

GRAPHICAL REPRESENTATION



Fig#1: showing graphical representation of table 1.2



Fig#2: showing the graphical representation of table 2.2

was E.coli, S.aureus was 57.14%, Pseudomonas 50% and Candida was 40%. In contrast only Candida and S.aureus are the most sensitive isolates inhibited by agar well diffusion technique. Inhibition of these isolates inhibited by agar plate was determined by zone of inhibition in mm. The results of this study show that green tea can have antimicrobial effect on the pathogens that cause UTIs. It concluded that green tea extract can be used to treat the infections which caused by drug resistant pathogens

Fig. 4: showing zone of inhibition of different isolates

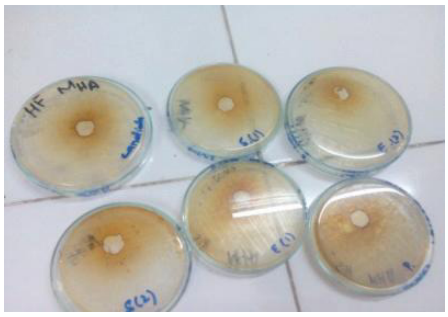


Fig. 4: Zone of Inhibition (Agar Well Diffusion Method)

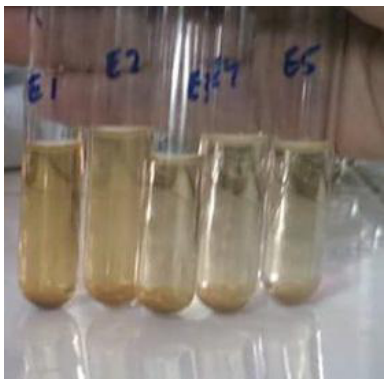


Fig. 5 showing MIC of different isolates

CONCULUSION:

The results of this study show that green tea can have antimicrobial effect on the pathogens that cause UTIs. It can be used as complimentary medicine in treating diseases caused by multi drug resistant strains of bacteria.

REFERENCES:

Payam Behzadi, Elham Behzadi, and Reza Ranjbar, Urinary tract infections and Candida albicans, Cent European J Urol. 2015; 68(1): 96–101.

Barbara W. Trautner, MD, PhD, Management of Catheter-Associated Urinary Tract Infection (CAUTI), Curr Opin Infect Dis. 2010 Feb; 23(1): 76–82

Tem Isikgoz Tasbakan, Raika Durusoy,2 Husnu Pullukcu,1 Oguz Resat Sipahi,1 and Sercan Ulusoy, Hospital-acquired urinary tract infection point prevalence in Turkey: Differences in risk factors among patient groups, Ann Clin Microbiol Antimicrob. 2013; 12: 31

Johnson. Virulence factors in Escherichia coli urinary tract infection, Clin Microbiol Rev. 1991 Jan; 4(1): 80–128.

Petra Tielen, Nathalie Rosin, Ann-Kathrin Meyer, Ktrin Dohnt, Isam Haddad, Lothar

Jänsch, Johannes Klein, Maike Narten, Claudia Pommerenke, Maurice Scheer, Max Schobert, Dietmar Schomburg, Bernhard Thielen, and Dieter Jahn. Regulatory and Metabolic Networks for the Adaptation of Pseudomonas aeruginosa Biofilms to Urinary Tract-Like Conditions, Published online 2013 Aug 13. doi: 10.1371/journal.pone.0071845

Madeleine G. Sowash and Anne-Catrin Uhlemann. Community-Associated Methicillin-Resistant Staphylococcus aureus Case Studies, Methods Mol Biol. 2014; 1085: 25–69.

Jonas Marschall, internist and infectious disease specialist, Christopher R Carpenter, internist and emergency medicine specialist, Susan Fowler, medical librarian, and Barbara W Trautner, internist and infectious disease specialist for the CDC Prevention Epicenters Program, Antibiotic prophylaxis for urinary tract infections after removal of urinary catheter: meta-analysis, BMJ. 2013; 346: f3147.

Erin C. Hagan, Amanda L. Lloyd, David A. Rasko, Gary J. Faerber, Harry L. T. Mobley, PLoS Pathog. 2010 November; 6(11): e1001187. Published online 2010 November 11. doi: 10.1371/journal.ppat.1001187, PMCID: PMC2978726

R Mittal, S Aggarwal, S Sharma, S Chhibber… - Journal of infection and …, 2009 – Elsevier

Das R N, Chandrashekhar T S, Joshi H S, Gurung M, Shrestha N, Shivananda P G. Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal, Singapore Med J 2006; 47(4) : 281

Karina A. Top, Amanda Buet, Susan Whittier, Adam J. Ratner, Lisa Saiman J. Predictors of Staphylococcus aureus Rectovaginal Colonization in Pregnant Women and Risk for Maternal and Neonatal Infections. Pediatric Infect Dis Soc. 2012 March; 1(1): 7–15. doi: 10.1093/jpids/pis001

François L. Mayer, Duncan Wilson & Bernhard Hube. Candida albicans pathogenicity mechanisms. Virulence. 2013 Feb 15;4(2):119-28. doi: 10.4161/viru.22913. Epub 2013 Jan 9.

Afsaneh Noormandi, Fatemeh Dabaghzadeh J, Effects of green tea on Escherichia coli as a uropathogen. Tradit Complement Med. 2015 January; 5(1): 15–20. Published online 2014 December 16. doi: 10.1016/j.jtcme.2014.10.005

Wanda C. Reygaert. The antimicrobial possibilities of green tea. Front Microbiol. 2014; 5: 434. Published online 2014 August 20. doi: 10.3389/fmicb.2014.00434.

Kim Ann Zimmermann. Urinary System: Facts, Functions & Diseases, Live Science Contributor | January 15, 2015 10:19 pm ET

C. Dugdale, David (16 September 2011). "Female urinary tract". MedlinePlus Medical Encyclopedia.

Maton, Anthea; Jean Hopkins; Charles William McLaughlin; Susan Johnson; Maryanna Quon Warner; David LaHart; Jill D. Wright (1993). Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall. ISBN 0-13-981176-1.

Caldwell HK, Young WS III (2006). "Oxytocin and Vasopressin: Genetics and Behavioral Implications" (PDF). In Lajtha A, Lim R. Handbook of Neurochemistry and Molecular Neurobiology: Neuroactive Proteins and Peptides (3rd ed.). Berlin: Springer. pp. 573–607. ISBN 0-387-30348-0.

Nielsen S, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA (February 1995). Proc. Natl. Acad. Sci. U.S.A. 92 (4): 1013–7. doi:10.1073/pnas.92.4.1013. PMC 42627. PMID 7532304.

Tim Taylor, Anatomy and Physiology Instructor

Jerry R. Balentine, DO, FACEP, Melissa Conrad Stöppler, MD. Urinary Tract Infections (UTIs)

Urinary tract infections in adults. National Institute of Diabetes and Digestive and Kidney Diseases. Accessed April 15, 2015.

Brett White, MD, Oregon Health and Science University, Portland, Oregon. Diagnosis and Treatment of urinary tract infection in children. American family physician;volume 83, number 4: February 15,2010

Betsy Foxman, PhD. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. The American journal of medicine-volume-113 (1A) 7S.JULY 8,2002

MD. Hamza saber, Lovely Barail, J Ashraful Haq, MD. Shariful Alam Jilani, Mrs Jaheda Begum. The pattern of organism causing urinary tract infection in diabetic and non diabetic patients in Bangladesh. Bangladesh J Med Microbiol 8,2010; 04(01): 6

Gulfareen Haider, Nishat Zehra, Aftab Afroze Munir, Ambreen Haider. Risk factors of urinary tract infection in pregnancy. J Pak Med Assoc. Vol. 60, No. 3, March 2010

Thana Khawcharoenporn, Shawn Vasoo, and Kamaljit Singh, "Urinary Tract Infections due to Multidrug-Resistant Enterobacteriaceae: Prevalence and Risk Factors in a Chicago Emergency Department," Emergency Medicine International, vol. 2013, Article ID 258517, 7 pages, 2013. doi:10.1155/2013/258517