Research Article

# Microbial Infections of Urinary Tract in Females with Toxoplasmosis

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**ABSTRACT**

This study was done to identify the impact of acute *Toxoplasma gondii* infection on the prevalence of urinary tract microorganisms in women and antimicrobial resistance genes. This study includes 200 females were divided in two groups 100 toxoplasmosis and 100 non-Toxoplasma females. Urine samples were collected for microbial recognition by standard microbiology techniques. Antibiotic susceptibility tests were performed on predominant bacterial strains detected and confirmed by PCR. In Toxoplasma as well as non-Toxoplasma females, the result of urine samples showed that *E. coli* was the most prevalent bacterial strains. It was found that *E. coli* isolates showed resistance to Ceftriaxon (100%), Amoxyclav (100%), Naldixic acid (39.58%), Nitrofurantoin (31.25%), Doxycycline (33.33%) and Gentamicin (20.83%) in Toxoplasma patients. However, in non-Toxoplasma female showed resistance to Ceftriaxon (53.33%), Amoxyclav (83.33%), Naldixic acid (33.33%), Nitrofurantoin (20%), Doxycycline (10%) and Gentamicin (10%). The prevalence of resistance genes for each antibiotic were 46.67%, 10%, 10%, 30%, 20% and 76.67% for blaCMY-2, aac(3)-IV, tetA, qnrB3, nfsA, TEM, respectively in non-Toxoplasma patients. While, in patients with toxoplasmosis, the outcomes of positive genes were significantly different ($p<0.05$) and they were 95.83%, 18.75%, 29.17%, 35.42%, 29.17% and 93.75% for blaCMY-2, aac(3)-IV, tetA, qnrB3, nfsA, TEM, respectively. Antimicrobial resistance might be higher in Toxoplasma females than non-Toxoplasma females.

**Keywords:** Toxoplasmosis, *E. coli,* antimicrobial resistance, UTI, urine.

## INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by a parasite *Toxoplasma gondii*[1]. If women infected with toxoplasmosis during early pregnancy, placental transmission of the pathogen to the fetus is probable with possible severe effects, such as, miscarriage, malformations and eye damage[2]. In Iraq, it has been observed that females demonstrated 30.63% of infection[3].Urinary tract infection (UTI) is extending from the asymptomatic presence of bacteria in the urine to extreme disease of the kidney with resultant sepsis[4]. UTIs are classified into either lower tract contamination, situated in the bladder and urethra (cystitis and urethritis), and upper tract contamination, situated in the ureters, collecting system, and parenchyma (pyelonephritis)[5].

Any anomalies in the urinary tract that postpone the urinary stream can increase the susceptibility to UTI [5].The majority of urinary tract infections (90%) are caused by Gram negative bacteria such as *Escherichia coli*, *Klebsiella spp.*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*[6]. There are two major means of bacterial access into the urinary tract: hematogenous and ascending routs[7]. The common pathogens pass through the blood are *Staphylococcus aureus* and *Candida* species [4]. The majority of UTI cases result from bacterial ascending from the perineum[5]. Since the invention and consequent use of antibiotics, resistance of bacteria has become a big worry[8]. A variety of bacterial species have developed mechanisms that render bacteria resistant to some or nearly all antibiotics There are two main ways of antimicrobial resistance. One is concerned with specific biochemical mechanisms utilized by the bacteria. The other is the development, acquisition and spread of the resistance gene itself [9].

Acquired bacterial antibiotic resistance results from regulatory or structural gene mutation and/or the acquisition of a foreign resistance gene[10]. This study tries to explore any influence of *Toxoplasma gondii* on the antibiotic resistance genes in urinary tract bacteria.

## MATERIALS AND METHODS

This study was conducted *at Al Diwaniyah Maternary and Children Teaching Hospital* and approved by the appropriate administrative committee. Two hundred female patients were targeted during a period from November 2015 to July 2016. Preceding to enrollment, all patients (n=200) signed a composed consent permitting the use of their data for research purposes. Their ages ranged from (19-40) years old, who were presented to the clinic seeking management for toxoplasmosis. All females with other illnesses as diabetes mellitus, hypertension, and obstetric problems were excluded. Patients treated with oral, parenteral or with local application of antibiotics for at least one month before attendance at the hospital were also excluded.

Study design One hundred women were diagnosed as toxoplasmosis. The other hundred were negative to Toxoplasma. Microbial recognition of urine samples was then done for each patient and identified by cultural,

morphological and biochemical analysis. Antibiotic susceptibility tests were later done for predominant bacterial strains. Resistant strains will be compared to standard strains using standard genetic for gene sequencing of polymerase chain reactions (PCR).

**Toxoplasma Analysis**

Serum was used for detection of toxoplasmosis using ELISA kits [11]. Serum blood was collected under sterile conditions from females by venipuncture. After clotting of blood for 1 hour at room temperature, the tubes were centrifuged at 1600 rpm for 15min at 4ºC. Serum was isolated prompt and 3mL was subjected to the centrifugation on a Beckman rotator at 16000 rpm for 60 min at 4ºC. Obtained deposit were delicately resuspended with micropipette in 0.5ml and put away at –80ºC till the tests were prepared for ELISA.

**Microbial Analysis**

Clean voided midstream urine samples were collected in sterile container. All the patients were instructed on how to collect the sample aseptically and taken to the laboratory immediately for culture. Urine samples were tested within 30 minutes on Blood agar and Mac Conkey's agar. All isolates were diagnosed by standard microbiology techniques[12].

**Antimicrobial Susceptibility Tests**

According to the standard procedures, antimicrobial susceptibility tests were done on Mueller-Hinton agar (Oxoid, England) using Kirby Bauer disk diffusion method[13]. The antimicrobial agents tested were: Amoxyclav (20/10µg), Imipenem (10 µg), Nitrofurantion (300µg), Naldixic acid (30 µg), Doxycycline (30 µg), Ciprofloxacin (30 µg), Gentamicin (10 µg), Ceftriaxone (30 µg), (Oxoid, England). Resistance data were interpreted according to the National Committee for Clinical laboratory Standards (NCCLS) [14].

**Molecular Analysis**

***DNA Extraction***

Genomic DNA was extracted from *E coli* strains by Genomic DNA Mini Bacteria Kit, as in manufactures instructions.

PCR Assay, Primers and Gene Sequences

The presence of genes associated with resistance to Ceftriaxone (blaCMY-2), Gentamicin (aac(3)IV), Doxycycline (tetA), Naldixicacid (qnrB3), Nitrofurantion (nfsA), and Amoxyclav (TEM) was determined by PCR and the set of primers used for each gene is shown in Table 1.

PCR reactions were performed in a total volume of 20 µl, including MgCl2, KCl, Tris-HCl (pH 9.0), dNTP, 1.5µl primers, 12.5µl Taq DNA polymerase, and 5 µl of DNA. Amplification reactions were carried out using a DNA thermocycler (MyGene, Bioneer. Korea) as follows: 5 min at 95 °C, 30 cycles each consisting of 30secat 95 °C, 30secat ~55 °C and 1 min at 72 °C, followed by a final extension step of 5 min at 72 °C.

Amplified samples were analyzed by electrophoresis in 1.5% agarose gel and stained with ethidium bromide. A molecular weight marker with 100 bp increments (100 bp DNA ladder) was used as a size standard.

The PCR product performs the DNA sequencing by Macrogen, South Korea. The obtained nucleotide sequence genes were processed through Bio Edit software and analyzed using NCBI-Blast alignment, identification (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) The genetic divergence was performed between sequences of antibiotic resistance genes from Toxo plasma patients and non Toxo plasma patients of *E. coli* isolates by molecular evolutionary genetics analysis (Pair wise Distance) using (Mega 6.0 version).

**Table 1:** PCR primer sequences for antibiotic resistance genes.

| Gene | Sequence (3`-5`) | Amplicon | Genbank |
|---|---|---|---|
| blaCMY-2 | TATTCCGGGTATGGCCGTTG | 581bp | DQ355981.1 |
| | CGTCAAGTTGTCCCGGAGAA | | |
| aac(3)-IV | CCTGCCAAATGTAAAGCGCA | 149bp | AJ493438.1 |
| | GTACCTGCCCATCGAGTTCA | | |
| tetA | TGCCGATATCACTGATGGCG | 452bp | KJ850490.1 |
| | CTACAGGGCCGGTGATCATT | | |
| qnrB3 | AGTCGTGCGATGCTGAAAGA | 253bp | DQ303920.1 |
| | AACGGTTTTCCCACAGCTCA | | |
| nfsA | GATCTGTCCGGATGCTCAGC | 358bp | NC000913.3 |
| | ATTATTGCTGCCACGGGTGA | | |
| TEM | GGGAACCGGAGCTGAATGAA | 317bp | AY307100.1 |
| | CATAGTTGCCTGACTCCCCG | | |

**blaCMY-2**: ceftriaxone, **aac(3)-IV:** Gentamicin, **tetA:** Tetracycline **qnrB3:**Naldixic acid, **nfsA:** Nitrofurantoin, **TEM:** Amoxyclav

**Statistical Analysis**

Data were collected and analyzed by using SPSS19 (Statistical Package for Social Studies) for descriptive statistics involving means and slandered error of mean (SEM). T-test was used to compare between groups.

## RESULTS

**Patient Demographics and Baseline Characteristics**

In Toxoplasma patients the results of parameters were: mean age was 26.9 $\pm$ 2.69 years. There were 26 (26%) pregnants and 74 (74%) non pregnants. 49 patients were from rural area while 51 patients were from urban areas. Miscarriage was reported in 58 patients and the rest (42) were having a clear history of miscarriage. 77 patients were found to be housewife and 23 women were employees.

On the other hand, the control population (non-Toxoplasma patients), their mean age were 25.4 $\pm$ 2.24 years. There were 41 (41%) pregnants and 59 (59%) non

pregnants. For those who came from rural area were 56 patients while 44 were coming from urban areas. Miscarriage was reported in 50 patients out of 100. In addition 56 patients were housewife and 44 women were employees.

### Microbial Isolation

In Toxoplasma patients, the result showed to be as follows: *E. coli* was the most prevalent strain during this study (48%).Other isolates were shown as *Enterobacter* (12%), *Pseudomonas.* spp. (14%), *Staph. epidermedis* (9%), *Staph.aureus* (8%), *Proteus* spp. (6%)and no growth (3%).On the other hand, in non Toxoplasma group the bacterial isolates were found as follows: *E. coli* was also the most frequent strain (30%). Other isolates were shown as *Enterobacter* (9%), *Pseudomonas.* spp. (8%), *Staph. epidermedis* (16%), *Staph. aureus* (8%), *Proteus* spp. (15%), *Klebsiella* spp. (2%) and no growth (12 %) as it is shown in Figure 1

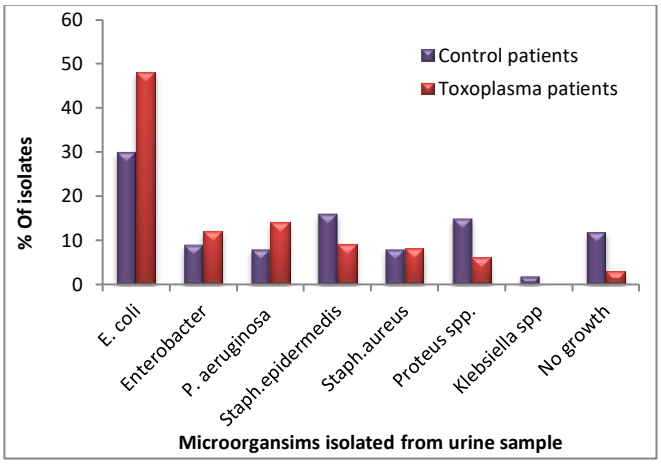


**Figure 1:** Distribution of organisms isolated from urine cultures

### Antimicrobial Susceptibility Test

It was found that antimicrobial susceptibility among *E. coli* strains differed by nature of antimicrobials. The rate of resistant strains to every antimicrobial appears in Figure 2.

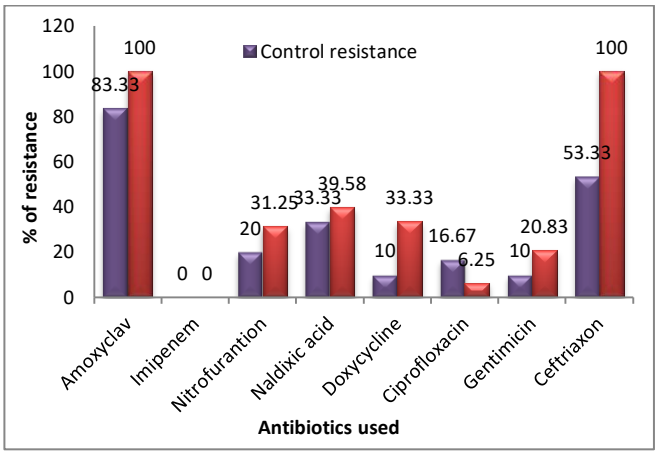


**Figure 2:** Percentage of resistant *E.coli* isolates from urine to antimicrobials

### Prevalence of Antibiotic Resistance Genes

Antibiotic and their resistance genes were constructed in Figures (3 and 4). The prevalence of resistance genes for each antibiotic were 46.67%, 10%, 10%, 30%, 20% and 76.67% for blaCMY-2, aac (3)-IV, tetA, qnrB3, nfsA, TEM, respectively in non-Toxoplasma patients. While, in patients with toxoplasmosis, the outcomes of positive genes were significantly different ($p<0.05$) and they were 95.83%, 18.75%, 29.17%, 35.42%, 29.17% and 93.75% for blaCMY-2, aac(3)-IV, tetA, qnrB3, nfsA, TEM, respectively.

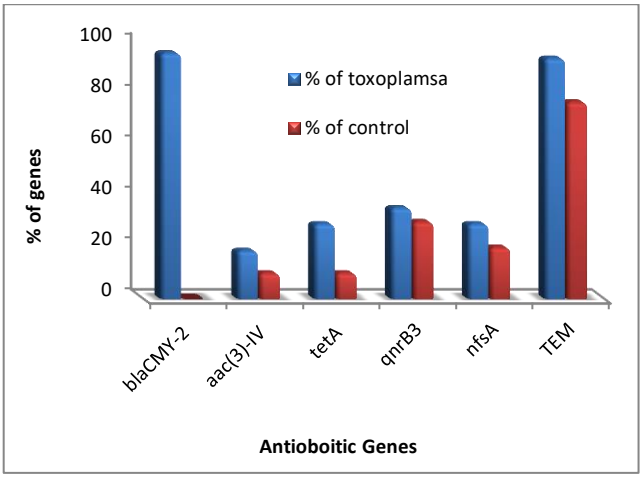


**Figure 3:** Percentage (%) of antibiotics resistant genes in *E coli* isolates from urine samples.

### DNA Sequencing

The *E.coli* antibiotic resistance genes sequence from the Toxoplasma patient (S1) and patient without toxoplasmosis (S2) for Ceftriaxone: blaCMY-2 gene, Gentamicin: aac(3)-IV gene, Doxycycline: tetA gene, Naldixic acid: qnrB3 gene, Nitrofurantoin: nfsA gene, and Amoxicillin: TEM gene shows a close relation to Global NCBI-GenBank records Antibiotic resistance same genes at (97%, 96%, 98%, 97%, 96%, and 97% distance identity) respectively as shown in Figure (5)

### Estimation of Genetic Divergence

The distance identity substitutions divergence per site between sequences of Ceftriaxone: blaCMY-2 gene, Gentamicin: aac(3)-IV gene, Doxycycline: tetA gene, Naldixic acid: qnrB3 gene, Nitrofurantoin: nfsA gene, and a: TEM gene in *E. coli* of patient toxoplasmosis (S1) and patient without toxoplasmosis (S2) were shown (0) no distance differences in all genes except Gentamicin: aac(3)-IV which shows (20.83) distance differences. Whereas the distance identity substitutions divergence with Global NCBI-GenBank was shown in S1 and S2 isolates (3.66 and 3.73), (0.91 and 21.83), (8.65 and 8.81), (3.56 and 3.56), (7.05 and 16.02) and (9.22 and 9.23), respectively.

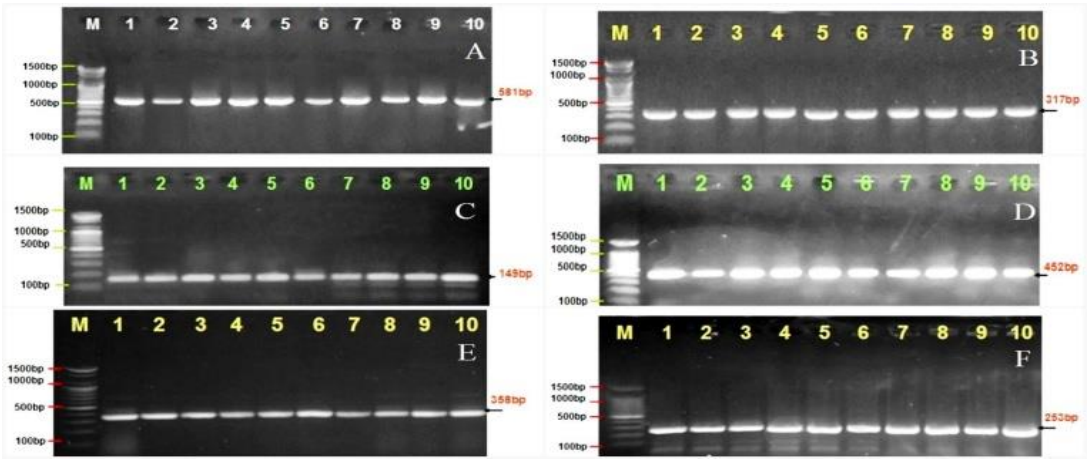


**Figure 4:** Agarose gel electrophoresis images that demonstrate the PCR product examination of antibiotic resistance gene in *Escherichia coli* positive isolates. Where M: marker (1500-100bp), lane (1-10) some positive *Escherichia coli*.

A(blaCMY-2 gene)for Ceftriaxone, B(TEM gene)for Amoxyclav, C(aac(3)-IV gene)for Gentamicin, D(tetA gene)for Doxycycline, E(nfsA gene)for Nitrofurantion, F(qnrB3 gene)for Naldixic acid

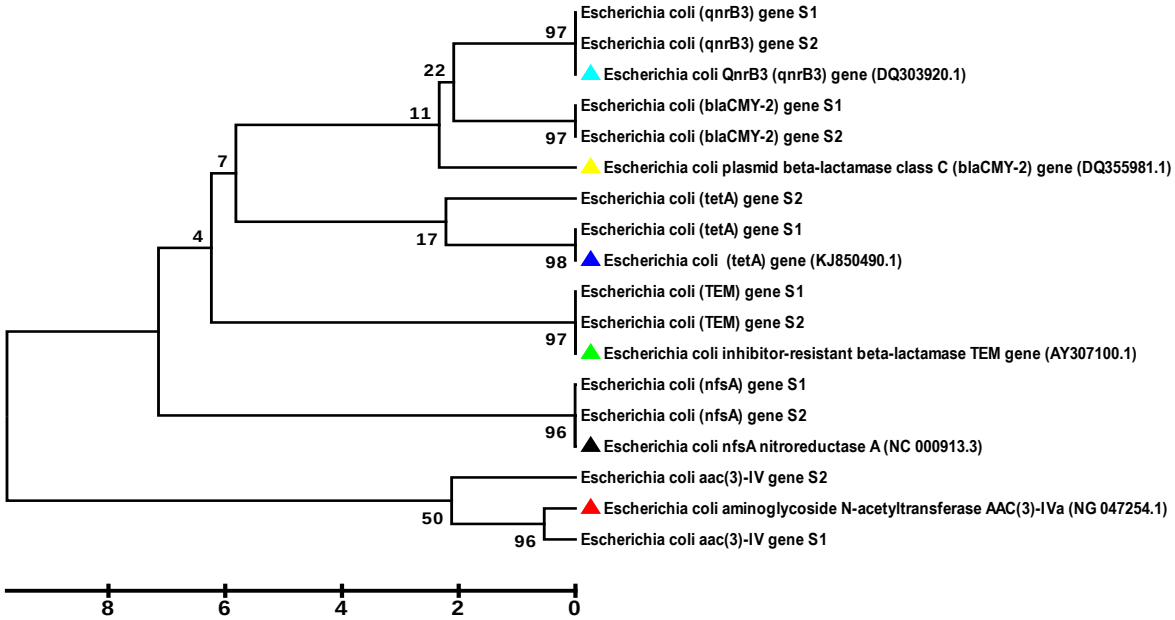


**Figure 5:** Phylogenetic tree analysis based on *E.coli* antibiotic resistance gene, partial sequence that used for confirmative detection of Local *E.coli* Antibiotic resistance genes by NCBI-Genbank standard Antibiotic resistance genes. The phylogenetic tree was constructed using the unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version)

## DISCUSSION

Our data states a frequent occurrence (26%) of *Toxoplasma. Gondii* in the tested sample of pregnant women. A wide discrepancy in the frequency of Toxoplasmosis occurrence among pregnant women was noticed obviously in different populations, for instance, a low level (4%) of endemicity of Toxoplasmosis was reported among pregnant women in Kinshasa City, Democratic Republic of Congo [15]. A moderate sero-prevalence level (34.4%) of Toxoplasmosis was noticed among Iranian pregnant patients [16]. Conversely, high level (81.1%) of endemicity of Toxoplasmosis has been remarked among pregnant women in Jimma town, Southwestern Ethiopia[17].

Toxoplasmosis is correlated with high level of abortion. The present data do support the correlation between abortion and sero-positivity of Toxoplasmosis and it was in a good accordance with previous finding stating a significant statistical correlation between abortion and sero-positivity among pregnant women in Yemen[18]. The present finding verifies that the residency issue did not impose any significant consequences on the frequent occurrence of sero-prevalence of Toxoplasmosis. Dissimilar to different past finding expressed that the sero-positivity rate of *T. gondii*-specific antibodies were higher among pregnant women from the urban than those from rural groups (41.5% versus 22.0%, respectively) in Tanzania [19].

Conversely, Toxoplasma sero positivity in women living in urban and rural areas was 29.1 and 47.5%, (P<0.001), respectively among Iranian pregnant females [16].

The result is in discordance with a previously reported finding stating no statistical significant association could be found between Toxoplasmosis infection and woman's career [15]. On the contrary, the present findings are in a good agreement with that of Zemene *et al*. 2012 who reported high sero-prevalence (82.7%) of Toxoplasmosis among pregnant housewives comparing to those having careers in southern Ethiopia.

In the present study, the most frequent agents are the Entero bacteriaceae amongst them *E. coli* was the most prevalent isolates. Those in agreement with different studies, like Ronald2003[20]; and Mollick *et al*2016 [21] whose found that *E. coli* has been the most important pathogen connected with symptomatic urinary tract infections. This could be clarified that uropathogenic *E. coli* (UPEC) that is available inside bowel flora has virulence factors that allow adherence and colonization of the lower urinary tract causing UTI. Adherence of this microorganism depends its own adhesive tool, the reactive components of the urothelium and the liquid that is available between both surfaces. Adhesions found on the surface of the bacterial layer are responsible for initial attachment onto urinary tract tissues forming a bio film. With bio film development, bacteria participate with each other to stay viable [22].

The present study intends to examine the distribution of antimicrobial resistance genes among *E.coli* isolates from urine samples. Various statistical associations were seen among resistance genes. Positive associations might be the result of the co-area of resistance genes in a single portable genetic component, for example, a plasmid, a transposon, or an integron. All inclusive, two groups of associations appear to rise up out of our outcomes. For *E.coli*, the first group of association for those antibiotic belonging to the same class such as ceftriaxone and Amoxyclav. However, the other group of association among those Gentamicin, Doxycycline, Naldixic acid and Nitrofurantoin.

## CONCLUSION

The present study highlights a high sero-prevalene of Toxoplasmosis among pregnant women in Iraq. Antimicrobial resistance might be higher in Toxoplasma females than non-Toxoplasma females and it was confirmed by genotypic technique.

## REFERENCES


1. Hussien JH, Mohammed A-A, Aljorani RH. Study the Effect of Acute Toxoplasmosis Infection on Some Hormones and the Phagocytic Activity of Neutrophils in Pregnant and Non-pregnant Women Before and After Treatment. Int J Curr Microbiol App Sci. 4(10), 2015, 459-65.
2. Dubey JP. Toxoplasmosis–a waterborne zoonosis. Veterinary Parasitology 126(1), 2004, 57-72.
3. AL-Shikhly MA. Early detection of toxoplasmosis percentage in pre-marital females by immunological methods [M.Sc. Thesis]: College of Science. University of Baghdad; 2010.
4. Tanagho, Emil A, Mcaninch, W. J. Bacterial Infections of the genitourinary tract. Smith's General Urology. United States of America: McGraw-Hill companies Inc; 2004. p. 203-27.
5. Heffner VA, Gorelick MH. Pediatric urinary tract infection. Clinical Pediatric Emergency Medicine. 9 (4), 2008, 233-7.
6. Lazarevic G, Petreska D, Pavlovic S. Antibiotic sensitivity of bacteria isolated from the urine of children with urinary tract infections from 1986 to 1995. Srpskiarhivzacelokupnolekarstvo. 126 (11-12), 1997,423-9.
7. Alper Brian S, Curry Sarah H. Urinary tract infection in children. Am Fam Physician. 72 (12), 2005,2483-8.
8. Lipsitch M, Singer RS, and Levin BR. Antibiotics in agriculture: When is it time to close the barn door? Proceedings of the National Academy of Sciences.99 (9), 2002, 5752-4.
9. Dzidic S, Suskovic J, Kos B. Antibiotic resistance mechanisms in bacteria: biochemical and genetic aspects. Food Technology and Biotechnology.46 (1), 2008, 11-21.
10. Harbottle H, Thakur S, Zhao S, White D. Genetics of antimicrobial resistance. Animal biotechnology. 17 (2), 2006, 111-24.
11. Sudan V, Jaiswal AK, Shanker D. Recent trends in the diagnosis of toxoplasmosis. Clin Rev Opin.5, 2013, 11-7.
12. Zaria LT, Raufu IA, Mohammed HS. Isolation and Antibiotic Sensitivity of *Escherichia coli*from Pregnant and Nonpregnant Women Attending the University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria. International Journal of Biomedical and Health Sciences. 6(3), 2010, 159-64.
13. Hombach M, Zbinden R, Böttger EC. Standardization of disk diffusion results for antibiotic susceptibility testing using the sirscan automated zone reader. BMC microbiology. 13(1), 2013, 1.
14. Wayne P. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing.17, 2007, 100-17.
15. Doudou Y, Renaud P, Jacqueline F, Hypolite S, Hypolite M, Patrick M. Toxoplasmosis among pregnant women: high seroprevalence and risk factors in Kinshasa, Democratic Republic of Congo. Asian Pacific journal of tropical biomedicine. 4(1), 2014, 69-74.

16. Babaie J, Amiri S, Mostafavi E, Hassan N, Lotfi P, Rastaghi ARE. Seroprevalence and risk factors for Toxoplasma gondii infection among pregnant women in Northeast Iran. Clinical and Vaccine Immunology. 20 (11), 2013,1771-3.

17. Zemene E, Yewhalaw D, Abera S, Belay T, Samuel A, Zeynudin A. Seroprevalence of Toxoplasma gondii and associated risk factors among pregnant women in Jimma town, Southwestern Ethiopia. BMC infectious diseases. 12 (1), 2012, 1.

18. Saif N, Al Ameeri G, Alhweesh M, Alkadasi M, Zaid AA. Sero Prevalence of Toxoplasmosis in Pregnant Women in Taiz-Yemen. Int J Curr Microbiol App Sci. 3(7), 2014, 680-90.

19. Mwambe B, Mshana SE, Kidenya BR, Massinde AN, Mazigo HD, Michael D. Sero-prevalence and factors associated with Toxoplasma gondii infection among pregnant women attending antenatal care in Mwanza, Tanzania. Parasites & vectors. 6(222), 2013, 2-3.

20. Ronald A. The etiology of urinary tract infection: traditional and emerging pathogens. Disease-a-Month.49 (2), 2003, 71-82.

21. Mollick S, Dasgupta T, Hasnain MJ, Ahmed M. Isolation and Characterization of Pathogens Responsible for Urinary Tract Infection in Bangladesh and Determination of their Antibiotic Susceptibility Pattern. Journal of Applied Pharmaceutical Science.6 (04), 2016, 072-6.

22. Nicolle LE. Uncomplicated urinary tract infection in adults, including uncomplicated pyelonephritis. Urologic Clinics of North America.35 (1), 2008, 1-12.

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