Original Article | Iran J Med Microbiol. 2022; 16(6): 581-586

10.30699/ijmm.16.6.581

Iranian Journal of Medical Microbiology | ISSN:2345-4342

Evaluation of the Virulence Genes in Quinolone and Fluoroquinolones-resistant Uropathogenic *Escherichia coli* Isolates

Leili Shokoohizadeh[1], Mojgan Rabiei[1], Amir Baharifar[2], Fariba Keramat[3], Liaqat Ali[4], Mohammad Yousef Alikhani[1,3*]

1. *Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Department of Pharmacology, Hamadan University of Medical Sciences, Hamadan, Iran*
3. *Brucellosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*
4. *Department of Biological Sciences, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan*

ABSTRACT

Background and Aim: Uropathogenic *Escherichia coli* (UPEC) is the most prevalent causative agent of urinary tract infections (UTIs) in both community and hospital settings. Annually about 150 million people globally develop UTIs, resulting in increased healthcare costs. The current study examined the identification and the frequency distribution of virulence factors among fluoroquinolones-resistant (FQs-R) and fluoroquinolones-susceptible (FQs-S) UPEC strains in Hamadan hospitals, west of Iran.

Materials and Methods: One hundred-seventy urine samples were collected consecutively from inpatients at three hospitals in Hamadan from March to September 2018. The UPEC isolates were identified using biochemical tests and polymerase chain reaction (PCR). The disk diffusion and the broth microdilution methods determined the antimicrobial susceptibility and the minimum inhibitory concentration (MIC) of Ciprofloxacin. The multiplex-PCR method investigated the prevalence of *pap*, *aer*, and *hly* genes.

Results: Among 170 urine samples collected from inpatients, *E. coli* was the most common isolate, with a frequency of 125 (73.5%). Resistance to Nalidixic acid and fluoroquinolones, including Ciprofloxacin, Norfloxacin, and Ofloxacin, was detected in 88.8%, 71.2%, 70.4%, and 68.8% of UPEC isolates, respectively. The prevalence of *hly* and *pap* genes in FQs-R strains was significantly lower than in FQs-S strains.

Conclusion: The high-level antibiotic resistance to quinolones & fluoroquinolones and heterogeneity of virulence genes among clinical UPEC isolates need strong attention.

Keywords: Uropathogenic *Escherichia coli*, Virulence Factors, Quinolone-Resistant, Fluoroquinolone-Resistant

Received: 2022/03/14; **Accepted**: 2022/06/29; **Published Online:** 2022/09/09

Corresponding Information: **Mohammad Yousef Alikhani**, *Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran* Email: alikhani@umsha.ac.i alikhani43@yahoo.com

Shokoohizadeh L, Rabiei M, Baharifar A, Keramat F, Liaqat A, Alikhanli M Y. Evaluation of the virulence genes in quinolone and fluoroquinolones- resistant Uropathogenic *Escherichia coli* isolates. Iran J Med Microbiol. 2022; 16 (6):581-6.

Download citation: BibTeX | RIS | EndNote | Medlars | ProCite | Reference Manager | RefWorks

Send citation to: Mendeley Zotero RefWorks

1. Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections in the clinical setting, and Uropathogenic *Escherichia coli* (UPEC) is the major causative pathogen **(1)**. First-line antibiotics for UTI treatment include quinolones (Q_s) and fluoroquinolones (FQ_s) **(2)**. Recent studies showed that the frequency of isolation of UPEC strains resistant to FQs (FQ-R) has increased in recent years **(3)**. UPEC strains can produce various types of virulence factors **(4)**. Studies also suggest that Q-R & FQ-R UPEC strains show reduced virulence and are less capable of causing urinary tract infections than susceptible strains **(5)**. The Qs & FQs have been used to treat a broad spectrum of infections, such as UTI_s. A significant increase in Q_S & FQ_s- resistant *E. coli* isolates have been reported **(1)**. The mechanisms of resistance

to Q and FQs in *E. coli* include alterations (mutations) in genes that encoded subunits of DNA gyrase (*gyr*A & *gyr*B genes), and topoisomerase IV (*par*C and *parE* genes) as the Q_s and FQ_s targets, efflux pump systems and acquisition of plasmid–carrying resistance genes (*qnr*A, *qnr*B, *qnr*S) **(6, 7)**. It has been shown that Q-R, and FQ-R UPEC strains developed a reduced prevalence of virulence factors (VF_s) and pathogenesis of UTIs than susceptible strains **(8)**. *E. coli* expressed three of the major VF_s: aerobactin (*aer*), hemolysin (*hly*), and P fimbriae (*pap*). P fimbriae are the second common virulence factor for some pathogenic strains and are encoded by the *papA-K* operons, which are carried on pathogenicity islands (PAI_s). These fimbriae recognize and bind to *Galα* **(1-4)** *Gal* receptors on renal epithelial cells with papG adhesion **(9)**. The most critical secreted VF_s for UPEC is α – hemolysin (*hly*A), which may cause tissue damage and facilitate bacterial dissemination and release of host nutrients. Hemolysin assists in acquiring iron to regulate the expression of virulence factors **(10)**. Aerobactin is a virulence factor of UPEC strains and other pathogen organisms. Iron acquisition systems utilize siderophores to attract iron for growth and colonize in host iron-poor niches **(11).** Considering the lack of proper information about the prevalence of virulence factors and fluoroquinolones resistance pattern of UPEC strains in Hamadan city of Iran, these subjects were the main aims of the current research.

2. Materials and Methods

E. coli Identification

One hundred- seventy urine samples were collected consecutively from patients aged 12 to 89 years with an average age of 47 (42 males & 128 females) admitted to three hospitals in Hamadan city, west of Iran. Among 170 urine samples, 125 (73.5%) *E. coli* strains were isolated. The *E. coli* isolates were identified by biochemical tests **(12)** and confirmed by the *rpoB* gene using PCR **(13)**.

Antimicrobial Susceptibility Testing

The Kirby-Bauer disk diffusion method detected the susceptibility of the UPEC isolates to antibiotics, and the results were interpreted according to the CLSI recommendations **(14)**. antibiotics used in this study belonged to four antimicrobial classes (Beta-lactams, Aminoglycosides, Quinolones, and Macrolides), including Amikacin (30 µg), Cefotaxime (30 µg), Doxycycline (30 µg), Imipenem (10 µg), Piperacillin (30 µg), Trimethoprim/sulfamethoxazole (1.25/23.75 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Nitrofurantoin (100 µg), Ciprofloxacin (5µg), Norfloxacin (5 µg), Ofloxacin (5 µg), and Nalidixic acid (30 µg), (MAST Group Ltd., UK). *E. coli* ATCC 25922 was used as a control strain for susceptibility testing. Ciprofloxacin MIC of FQs-R strains was determined by the broth microdilution method and results were interpreted according to the CLSI guidelines **(14)**.

Molecular Detection of Virulence Genes

Genomic DNA of UPEC isolates were extracted using the boiling method. The presence of *pap, hly, aer* genes among UPEC isolates was investigated by Multiplex PCR using specific primers, as described previously **(15)**. PCR reaction was performed in a final volume of 25µl, containing 3µl template DNA, 2X Taq premix 12.5µl (Ava gene Co, Iran), 2 µl of each primer (Takapouzist Co, Iran), and 3.5 µl ddH_2O. The following program was used for amplification of *pap, hly, aer* genes: initial denaturation at 94˚C for 1 min followed by 30 cycles of denaturation at 94˚C for 1 min, the annealing temperature of 63˚C for the 30s, and extension at 72˚C for 90s, followed by a final extension of 72˚C for 5 min. Detection of PCR products was performed along with a 100 bp DNA ladder on 1.5% agarose gel electrophoresis. The size of amplified fragments of *pap*, *hly,* and *were* 336, 1177, and, 602 bp, respectively. The PCR amplicons were sequenced by Bioneer Company (Korean Biotechnology Co), and the result of the sequences was analyzed by NCBI - BLAST.

The data of this study were analyzed using SPSS software version 16.0 (Chicago, SPSS Inc., IBM, USA). $P \leq 0.05$ was considered statistically significant.

3. Results

Among all urinary tract microbial pathogens, *E. coli* was the most common isolate, with a frequency of 125 (73.5%) from hospitalized patients. The frequencies of other causative agents of urinary tract infections were *Acinetobacter spp*. (8.8%), *Klebsiella pneumonia* (7.1%), *Staphylococcus aureus* (4.7%), *Pseudomonas aeruginosa* (3%), and *Proteus spp*. (2.9%).

The frequencies of resistance to commonly tested antibiotics for *E. coli* were as follows: Amikacin (26.4%), Cefotaxime (59.2%), Doxycycline (88.0%), Imipenem (55.2%), Piperacillin (54.4%), Trimethoprim/sulfamethoxazole (77.6%), Ceftriaxone (65.6%), Ceftazidime (55.2%), and Nitrofurantoin (25.6%). More than half (54.4%) of the clinical isolates of *E. coli* showed multi-drug resistant patterns (resistance to three or more classes of antibiotics). The Ciprofloxacin MIC values ranged from 8 to 256 µg/ml (Table 1). Among the 125 UPEC clinical isolates, 111(88.8%) strains were resistant to Nalidixic acid, 89 (71.2%), 88 (70.4%), and 86 (68.8%) isolates were resistant to Ciprofloxacin, Norfloxacin, and Ofloxacin, respectively.

Table 1. Ciprofloxacin MIC values

Breakpoint for Ciprofloxacin	Sensitive	Intermediate	Resistance						
	1µg/mL	2µg/mL	≥4 µg/Ml						
MIC(µg/mL)	———	1	256	128	64	32	16	8	4
Isolates NO (%)	36	1	10(11.3)	32(36.3)	23(26.1)	15(17.0)	7(7.9)	1(1.1)	0(0.0)
Total NO (%)	36(28.8)	1(1.12)	88(70.4)						

The results of the amplification of virulence factor genes are shown in Figure 1. The frequency of virulence factors among susceptible and resistant to quinolones and fluoroquinolones UPEC isolates were compared and shown in Table 2. There was a difference between the frequency distribution of virulence genes among resistance and susceptible strains. The prevalence of *hly* and *pap* genes in FQ-R and Q-R strains was lower than FQ-S and Q-S strains; however, the prevalence of *aer* gene was higher in FQ-R and Q-R strains. Statically analysis using the Chi-square test showed a significant difference (P= 0.009) in *hly* gene distribution between FQ-R and FQ-S strains. Our results also showed a significant difference between the distribution of *aer* (P= 0.013*)* and *hly* (P= 0.042) and Q-R and Q-S isolates (Table 2).

Table 2. The frequency of virulence factors among isolates susceptible and resistance to quinolones and fluoroquinolones

	pap + No (%)	*pap* - No (%)	*P* value	*aer* + No (%)	*aer* – No (%)	*P* value	*hly* + No (%)	*hly* – No (%)	*P* value
Fluoroquinolone Susceptible (n=36)	16(44.44)	20(55.55)	0.102	19(52.77)	17(47.22)	0.352	9(25.0)	27(75.0)	0.009
FluoroquinoloneResistant (n=89)	26(29.21)	63(70.78)		55(61.79)	34(38.20)		7(7.86)	82(92.13)	
***Quinolones* Susceptible (n=14)**	6(42.85)	9(64.8)	0.354	4(28.57)	10(71.42)	0.013	4(28.57)	10(71.42)	0.042
***Quinolones* Resistant (n=111)**	34(30.6)	77(69.36)		70(63.0)	41(36.93)		11(9.9)	100(90.09)	

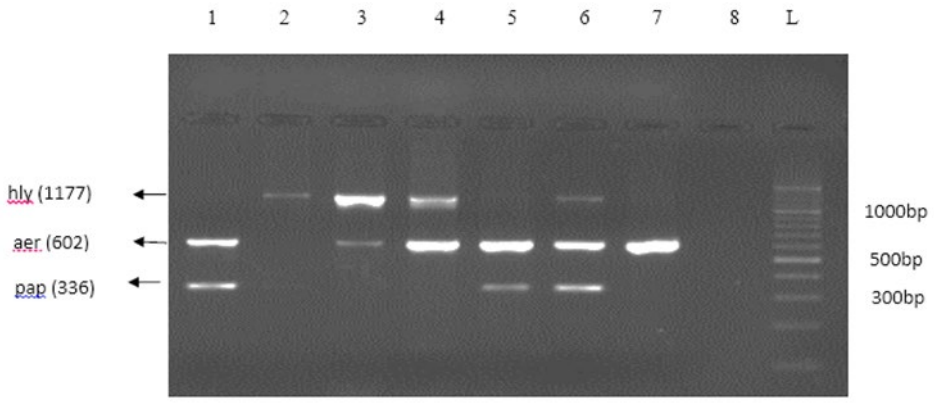

Figure 1. Representative PCR results for virulence genes in UPEC strains. L: Ladder 100bp, Lane 1-5 &7: Clinical isolates (*pap*: 336 bp, *aer*: 602 bp, *hly*: 1177 bp), Lane 6: Positive control, Lane 8: Negative control

4. Discussion

In this study, we investigated the prevalence of resistance to different antibiotics classes and virulence factors, including *hly*, *pap,* and *aer* genes, among UPEC isolates in Hamadan hospitals. Our study shows uropathogenic *E. coli* is the most common uropathogenic bacteria with high-level resistance rate to most used antibiotics, especially fluoroquinolones. Low-level resistance to Amikacin and Nitrofurantoin was detected among UPEC isolates. Amikacin and Nitrofurantoin have known as effective antibiotics to treat urinary tract infections and according to our results, we can consider these antibiotics as choice drugs to treat urinary tract infections due to *E. coli* strains. The high-level resistance to quinolones and fluoroquinolones was also detected, an alarming challenge in treating UTI patient in hospitals. In the previous studies, high-level resistance to most antibiotics as well as fluoroquinolones was reported in UPEC strains in Hamadan hospitals and other regions of Iran **(16-19)**.

Another factor that has been studied in our research was the detection and comparison of urovirulence genes *hly*, *pap,* and *aer* among FQ-R and FQ-S UPEC strains. We focused on the distribution of virulence factors in Q and FQ-resistant UPEC strains as previously described that antimicrobial agents, e.g., Ampicillin, and aminoglycosides, do not significantly correlate with virulence factors **(20-23).** Here we confirmed the result of previous studies that reported a lower prevalence of α-hemolysin and pap adhesion of FQs-R compared to FQs-S *E. coli* strains. As previously reported, the prevalence of *pap* and *hly* genes was lower in Q-R and FQs-R strains and *aer* gene was higher in FQs-R UPEC strains **(5, 23)**. Some reports have suggested that quinolone-resistant *E. coli* have a minor role in upper urinary tract infection and have low VFs than quinolone-susceptible *E. coli* **(24).** Oliveria et al., showed the lower prevalence of virulence genes in UPECs, the frequency of virulence genes in 204 strains isolated from urinary tract infections as follows: *aer*: 41%, *sfa*: 26%, *pap*: 25%, *hly*: 5% **(24)**. Malekzadegan et al. have suggested the association between antibiotic resistance with the isolates harboring certain virulence genes, such as *sfa* or the presence of *iut*A, *pap* GII and PAI marker in UPEC strains and the importance of some urovirulence genes (e.g., *iut*A, *pap* GII, and *hly*A) as a marker for developing of symptomatic UTIs **(18)**.

Kawamura et al. demonstrated that 113 strains were resistant to quinolones, 64.6% of strains were resistant to quinolones and 81.6% of strains were resistant to fluoroquinolones. The frequency of virulence factors *pap* gene and *hly* gene is lower, and the *aer* gene is higher in FQs-R strains, they also, have compared the prevalence of virulence gene among susceptible and resistance to quinolones, and fluoroquinolones resistant strains, and reported it as follows: in the susceptible strains to quinolones: (*hly*: 4%, *aer*: 54.1%, *pap*: 62.8%), among resistant strains to quinolones: (*pap*: 17.8%, *hly*: 18.2%, *aer*: 90%), and the FQs-R isolates: (*pap*: 5%, *hly*: 0%, *aer*: 75.3%) **(25)**. One of the interesting findings in our study was the detection of more prevalence of *aer* gene in FQ-R and Q-R strains compared to FQ-S and Q-S strains. Therefore, the *aer* gene can be considered a crucial virulence factor in resistant UPEC strains. “There is a suggestion that the higher incidence of VFs among susceptible *E. coli* isolates would depend on their phylogenetic distribution and that VFs would be intrinsic bacterial characteristics”. Some studies have related quinolone resistance and low virulence with phylogenetic origin **(22)**. Horcajada et al. have demonstrated that Q-R UPEC strains from phylogenetic group B2 have fewer virulence factors than Q-S strains. Overall, these observations indicate that the differences in phenotypes, including quinolone resistance and carriage of virulence genes, are associated with the complex context of genetic background **(22)**.

5. Conclusion

In conclusion, our findings indicate a high level of resistance to different classes of antibiotics as well as quinolones and fluoroquinolones in UPEC strains isolated from UTIs in hospitalized patients in Hamadan hospitals. Furthermore, our findings show that *aer* gene should also be considered a crucial virulence factor in both fluoroquinolone and quinolone-resistant strains. More consideration is required for a rational prescription of antibiotics as well as fluoroquinolones to treat UTIs. Further studies also are required to understand the cause of the difference in the frequency distribution of virulence factors among antibiotic-susceptible and resistant UPEC isolates.

Acknowledgment

We would like to thank all members of the microbiology laboratory of Hamadan University of medical science and the staff of the Microbiology laboratory of Sina Farshchian hospital.

Funding

This research was supported by the Vice Chancellor for Research & Technology of Hamadan University of Medical Sciences, Hamadan, IRAN (Grant no: 9304101833).

Conflict of Interest

The authors declare no conflict of interest.

Reference

1. Nöllmann M, Crisona NJ, Arimondo PB. Thirty years of Escherichia coli DNA gyrase: From in vivo function to single-molecule mechanism. Biochimie. 2007;89(4):490-9. [DOI:10.1016/j.biochi.2007.02.012] [PMID]
2. Andersson MI, MacGowan AP. Development of the quinolones. J Antimicrob Chemother. 2003; 51(suppl_1):1-11. [DOI:10.1093/jac/dkg212] [PMID]
3. Da Silva G, Mendonça N. Association between antimicrobial resistance and virulence in Escherichia coli. Virulence. 2012;3(1):18-28. [DOI:10.4161/viru.3.1.18382] [PMID]
4. Hoban DJ, Nicolle Le Fau - Hawser S, Hawser S Fau - Bouchillon S, Bouchillon S Fau - Badal R,

Badal R. Antimicrobial susceptibility of global inpatient urinary tract isolates of Escherichia coli: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program: 2009-2010. Diagn Microbiol Infect Dis. 2011;70(4):507-11. [PMID] [DOI:10.1016/j.diagmicrobio.2011.03.021]

5. Velasco M, Horcajada Jp Fau - Mensa J, Mensa J Fau - Moreno-Martinez A, Moreno-Martinez A Fau - Vila J, Vila J Fau - Martinez JA, Martinez Ja Fau - Ruiz J, et al. Decreased invasive capacity of quinolone-resistant Escherichia coli in patients with urinary tract infections. Clin Infect Dis. 2001;33(10):1682-6. [DOI:10.1086/323810] [PMID]
6. Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. J Antimicrob Chemother. 2005;56(3):463-9. [DOI:10.1093/jac/dki245] [PMID]
7. Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. J Antimicrob Chemother. 2003;51(5):1109-17. [DOI:10.1093/jac/dkg222] [PMID]
8. Drews SJ, Poutanen SM, Mazzulli T, McGeer AJ, Sarabia A, Pong-Porter S, et al. Decreased Prevalence of Virulence Factors among Ciprofloxacin-Resistant Uropathogenic Escherichia coli Isolates. J Clin Microbiol. 2005;43 (8):4218-20. [PMID] [PMCID] [DOI:10.1128/JCM.43.8.4218-4220.2005]
9. Melican K, Sandoval R, Kader A, Josefsson L, Tanner G, Molitoris B, et al. Uropathogenic Escherichia coli P and Type 1 fimbriae act in synergy in a living host to facilitate renal colonization leading to nephron obstruction. PLOS Pathog. 2011;7(2):e1001298. [PMCID] [DOI:10.1371/journal.ppat.1001298] [PMID]
10. Mobley HL, Green DM, Trifillis AL, Johnson DE, Chippendale GR, Lockatell CV, et al. Pyelonephritogenic Escherichia coli and killing of cultured human renal proximal tubular epithelial cells: role of hemolysin in some strains. Infect Immun. 1990;58(5):1281-9. [PMCID] [DOI:10.1128/iai.58.5.1281-1289.1990] [PMID]
11. Crosa JH. Genetics and molecular biology of siderophore-mediated iron transport in bacteria. Microbiol Rev. 1989;53(4):517-30. [PMID] [PMCID] [DOI:10.1128/mr.53.4.517-530.1989]
12. Mahon C, Lehman D, Manuselis G. Textbook of diagnostic microbiology-E-Book: Elsevier Health Sciences; 2014.
13. Majlesi A, Kakhki RK, Mozaffari Nejad AS, Mashouf RY, Roointan A, Abazari M, et al. Detection of plasmid-mediated quinolone resistance in clinical isolates of Enterobacteriaceae strains in Hamadan, West of Iran. Saudi J Biol Sci. 2018;25(3):426-30. [PMID] [DOI:10.1016/j.sjbs.2016.11.019] [PMCID]
14. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. PA: Clinical and Laboratory Standards Institute; 2018.
15. Yamamoto S, Tsukamoto T, Terai A, Kurazono H, Takeda Y, Yoshida O. Distribution of Virulence Factors in Escherichia coli Isolated from Urine of Cystitis Patients. Microbiol Immunol. 1995;39(6): 401-4. [PMID] [DOI:10.1111/j.1348-0421.1995.tb02219.x]
16. Shenagari M, Bakhtiari M, Mojtahedi A, Atrkar Roushan Z. High frequency of mutations in gyrA gene associated with quinolones resistance in uropathogenic Escherichia coli isolates from the north of Iran. Iran J Basic Med Sci. 2018;21(12): 1226-31.
17. Badamchi A, Javadinia S, Farahani R, Solgi H, Tabatabaei A. Molecular Detection of Plasmid Mediated Quinolone Resistant Genes in Uropathogenic E coli from Tertiary Referral Hospital in Tehran Iran. Arch Pharm Ther. 2019;1(1):19-24. [DOI:10.33696/Pharmacol.1.005]
18. Malekzadegan Y, Khashei R, Sedigh Ebrahim-Saraie H, Jahanabadi Z. Distribution of virulence genes and their association with antimicrobial resistance among uropathogenic Escherichia coli isolates from Iranian patients. BMC Infect Dis. 2018;18(1):1-9. [DOI:10.1186/s12879-018-3467-0] [PMID] [PMCID]
19. Dehbanipour R, Khanahmad H, Sedighi M, Bialvaei AZ, Faghri J. High prevalence of fluoroquinolone-resistant Escherichia coli strains isolated from urine clinical samples. J Prev Med Hyg. 2019;60(1):E25-E30.
20. Piatti G, Mannini A, Balistreri M, Schito Anna M. Virulence Factors in Urinary Escherichia coli Strains: Phylogenetic Background and Quinolone and Fluoroquinolone Resistance. J Clin Microbiol. 2008;46(2):480-7. [DOI:10.1128/JCM.01488-07] [PMID] [PMCID]
21. Johnson James R, Owens K, Gajewski A, Kuskowski Michael A. Bacterial Characteristics in Relation to Clinical Source of Escherichia coli Isolates from Women with Acute Cystitis or Pyelonephritis and Uninfected Women. J Clin

Microbiol. 2005;43(12):6064-72. [PMCID] [PMID] [DOI:10.1128/JCM.43.12.6064-6072.2005]

22. Horcajada JP, Soto S Fau - Gajewski A, Gajewski AF, Smithson A, Smithson A Fau - Jiménez de Anta MT, Jiménez de Anta Mt Fau - Mensa J, et al. Quinolone-resistant uropathogenic Escherichia coli strains from phylogenetic group B2 have fewer virulence factors than their susceptible counterparts. J Clin Microbiol. 2005; 43(6):2962-4. [PMID] [PMCID] [DOI:10.1128/JCM.43.6.2962-2964.2005]
23. Moreno E, Prats G, Sabaté M, Pérez T, Johnson JR, Andreu A. Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic Escherichia coli. J Antimicrob Chemother. 2006; 57(2):204-11. [DOI:10.1093/jac/dki468] [PMID]
24. Oliveira FA, Paludo Ks Fau - Arend LNVS, Arend Ln Fau - Farah SMSS, Farah Sm Fau - Pedrosa FO, Pedrosa Fo Fau - Souza EM, Souza Em Fau - Surek M, et al. Virulence characteristics and antimicrobial susceptibility of uropathogenic Escherichia coli strains. Genet Mol Res. 2011;10 (4):4114-25. [DOI:10.4238/2011.October.31.5] [PMID]
25. Kawamura-Sato K, Yoshida R, Shibayama K, Ohta M. Virulence genes, quinolone and fluoroquinolone resistance, and phylogenetic background of uropathogenic Escherichia coli strains isolated in Japan. Jpn J Infect Dis. 2010; 63(2):113-5. [DOI:10.7883/yoken.63.113] [PMID]