ORIGINAL PAPER

URINARY TRACT INFECTIONS

Microarray long oligo probe designing for *Escherichia coli:* an *in-silico* DNA marker extraction

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Introduction Urinary tract infections are predominant diseases which may be caused by different pathogenic microorganisms, particularly *Escherichia coli* (*E.coli*). DNA microarray technology is an accurate, rapid, sensitive, and specific diagnostic tool which may lead to definite diagnosis and treatment of several infectious diseases. DNA microarray is a multi-process method in which probe designing plays an important. Therefore, the authors of the present study have tried to design a range of effective and proper long oligo microarray probes for detection and identification of different strains of pathogenic *E.coli* and in particular, uropathogenic *E.coli* (UPEC).

Material and methods *E.coli* O26 H11 11368 uid41021 was selected as the standard strain for probe designing. This strain encompasses the largest nucleotide sequence and the most number of genes among other pathogenic strains of *E.coli.* For performing this *in silico* survey, NCBI database, GReview Server, PanSeq Server, Oligoanalyzer tool, and AlleleID 7.7 were used to design accurate, appropriate, effective, and flexible long oligo microarray probes. Moreover, the genome of *E.coli* and its closely related microorganisms were compared.

Results In this study, 15 long oligo microarray probes were designed for detecting and identifying different strains of *E.coli* such as UPEC. These probes possessed the best physico-chemical characteristics. The functional and structural properties of the designed probes were recognized by practical tools and softwares.

Conclusions The use of reliable advanced technologies and methodologies for probe designing guarentees the high quality of microarray probes and makes DNA microarray technology more flexible and an effective diagnostic technique.

Key Words: urinary tract infection ‹› *E.coli* ‹› microarray ‹› probe designing

INTRODUCTION

Escherichia coli (*E.coli*) is a potential uropathogenic bacterium which may cause a wide range of urinary tract infections (UTIs) including asymptomatic and/or symptomatic bacteriuria, cystis, and pyelonephritis both in children and adults. UTIs caused by UPEC are the most spread infections in the world. According to previous surveys, community acquired UTIs caused by UPEC ranks first in comparison with other pathogenic agents and UPEC related nosocomial UTIs are in second place, worldwide [1–8].

The presence of several virulence factors enables UPEC to cause different types of UTIs in human hosts. Adhesins, different types of fimbrial and afimbrial structures, haemolysins, and cytotoxic necrotizing factors are the most important and well-known virulence factors in UPEC strains. Although identification of virulence factor genes is possible through Polymerase Chain Reaction (PCR) based molecular techniques in simple levels, for fine detection and

Table 1. *61 Reported identified Escherichia coli (E.coli) genomes via NCBI FTP site [11, 20, 21]*

E.coli strains	Complete genome/RefSeq accessio no	Sequence length (bp)	Total genes
E.coli 042 uid161985	NC_017626	5,241,977	5,392
E.coli 536 uid58531	NC_008253	4,938,920	4,816
E.coli 55989 uid59383 Removed from NCBI RefSeq	NC_011748	5,154,862	Not mentioned
E.coli ABU 83972 uid161975	NC_017631	5,131,397	5,083
E.coli APEC O1 uid58623	NC_008563	5,082,025	5,572
E.coli APEC O78 uid187277	NC_020163	4,798,435	4,810
E.coli ATCC 8739 uid58783	NC_010468	4,746,218	4,644
E.coli BL21 DE3 uid161947	NC_012971	4,558,953	4,530
E.coli BL21 DE3 uid161949	NC_012892	4,558,947	4,530
E.coli BW2952 uid59391	NC_012759	4,578,159	4,564
E.coli B REL606 uid58803	NC_012967	4,629,812	4,573
E.coli CFT073 uid57915 Removed from NCBI RefSeq	NC_004431	5,231,428	Not mentioned
E.coli DH1 uid161951	NC_017625	4,630,707	4,578
E.coli DH1 uid162051	NC_017638	4,621,430	4,573
E.coli E24377A uid58395	NC_009801	4,979,619	5,301
E.coli ED1a uid59379 Removed from NCBI RefSeq	NC_011745	5,209,548	Not mentioned
E.coli ETEC H10407 uid161993	NC_017633	5,153,435	5,411
E.coli HS uid58393 Removed from NCBI RefSeq	NC_009800	4,643,538	Not mentioned
E.coli IAI1 uid59377 Removed from NCBI RefSeq	NC_011741	4,700,560	Not mentioned
E.coli IAI39 uid59381	NC_011750	5,132,068	Not mentioned
E.coli IHE3034 uid162007	NC_017628	5,108,383	5,132
E.coli JJ1886 uid226103	NC_022648	5,129,938	5,397
E.coli KO11FL uid162099	NC_017660	5,021,812	4,963
E.coli KO11FL uid52593	NC_016902	4,920,168	5,037
E.coli K 12 substr DH10B_uid58979 Removed from NCBI RefSeq	NC_010473	4,686,137	Not mentioned
E.coli K 12 substr MDS42 uid193705	NC_020518	3,976,195	3,872
E.coli K 12 substr MG1655 uid57779	NC_000913	4,641,652	Not mentioned
E.coli K 12 substr W3110 uid161931 Removed from NCBI RefSeq	NC_007779	4,646,332	Not mentioned
E.coli LF82 uid161965 Removed from NCBI RefSeq	NC_011993	4,773,108	Not mentioned
E.coli LY180 uid219461	NC_022364	4,835,601	4,789
E.coli NA114 uid162139	NC_017644	4,971,461	5,039
E.coli O103 H2 12009 uid41013	NC_013353	5,449,314	5,689
E.coli O104 H4 2009EL 2050 uid175905	NC_018650	5,253,138	5,529
E.coli O104 H4 2009EL 2071 uid176128	NC_018661	5,312,586	5,475
E.coli O104 H4 2011C 3493 uid176127	NC_018658	5,273,097	Not mentioned
E.coli O111 H 11128 uid41023	NC_013364	5,371,077	6,034
E.coli O127 H6 E2348 69 uid59343 Removed from NCBI RefSeq	NC_011601	4,965,553	Not mentioned
E.coli O157 H7 EC4115 uid59091	NC_011353	5,572,075	5,924
E.coli O157 H7 EDL933 uid57831 Removed from NCBI RefSeq	NC_002655	5,528,445	Not mentioned
E.coli O157 H7 TW14359 uid59235	NC_013008	5,528,136	5,820
E.coli O157 H7 uid57781	NC_002695	5,498,450	Not mentioned
E.coli O26 H11 11368 uid41021	NC_013361	5,697,240	6,125
E.coli O55 H7 CB9615 uid46655	NC_013941	5,386,352	5,526
E.coli O55 H7 RM12579 uid162153	NC_017656	5,263,980	5,495
E.coli O7 K1 CE10 uid162115	NC_017646	5,313,531	5,443
E.coli O83 H1 NRG 857C uid161987	NC_017634	4,747,819	Not mentioned

Table 1. *61 Reported identified Escherichia coli (E.coli) genomes via NCBI FTP site [11, 20, 21]*

E.coli strains	Complete genome/RefSeq accessio no	Sequence length (bp)	Total genes
E.coli PMV 1 uid219679	NC_022370	4,984,940	Not mentioned
E.coli S88 uid62979 Removed from NCBI RefSeq	NC_011742	5,032,268	Not mentioned
E.coli SE11 uid59425	NC_011415	4,887,515	5,207
E.coli SE15 uid161939	NC_013654	4,717,338	4,786
E.coli SMS 3 5 uid58919	NC_010498	5,068,389	5,164
E.coli UM146 uid162043	NC_017632	4,993,013	5,082
E.coli UMN026 uid62981	NC_011751	5,202,090	Not mentioned
E.coli UMNK88 uid161991	NC_017641	5,186,416	5,863
E.coli UTI89 uid58541	NC_007946	5,065,741	5,171
E.coli W uid162011	NC_017635	4,900,968	5,023
E.coli Xuzhou21 uid163995	NC_017906	5,386,223	5,651
E.coli BL21 Gold DE3 pLysS AG uid59245	NC_012947	4,570,938	4,560
E.coli clone D i14 uid162049	NC_017652	5,038,386	4,959
E.coli clone D i2 uid162047	NC_017651	5,038,386	4,958

identification of diverse *E.coli* strains in genomic scale, high-throughput techniques, including microarray as a next generation sequencing (NGS) technology, are needed. Therefore, pangenomics and comparative genomics are appropriate means to gain this goal [6–15]. Despite knowing the fact that *E.coli* is a natural member of human gastro-intestinal tract microflora, the extraintestinal pathogenic *E.coli* (ExPEC) strains may cause different infections, such as UTIs, in their human hosts. The use of rapid, accurate, cost effective, sensitive, specific, and advanced diagnostic methods enables us to have a reliable diagnosis and definite treatment [5, 10–18].

Due to the importance of *E.coli* in association with different infectious diseases such as UTIs, several genomes relating to different strains of *E.coli* are identified and reported to genome databases. Currently there are 61 recorded whole genomes pertaining to *E.coli* strains which are reported by the Genomes OnLine Database (GOLD, http://www.genomesonline.org/) and deposited in NCBI FTP site (ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/). These strains are indicated in table 1 [11, 20, 21].

One of the most important purposes of comparative genomics is to detect and identify the unique genomic regions which may be used for appropriate microarray probe designing. In accordance with several recorded investigations, there are close relationships between *E.coli*, *Salmonella enterica,* and *Shigella (Sh.) sonnei, Sh.flexneri, Sh.dysenteriae* and *Sh.boydii* [22, 23].

The main goal of this original article is to design several effective and proper long oligo microarray probes for detection and identification a diversity of *E.coli* strains, such as UPEC pathotypes, from the other close related bacterial genera.

MATERIAL AND METHODS

In the present *in silico* research, 61 recorded complete genomes belonging to different strains of *E.coli* were studied via NCBI ftp (ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/) and the RefSeq complete genome pertaining to each strain was detected through the related files of GeneMark-2.5m. Then, each RefSeq complete genome was retrieved from NCBI (http://www.ncbi.nlm.nih.gov/nuccore) to study the sequence length and total genes involved in a complete genome. The data is shown in Table 1 [11, 12, 24].

The *E.coli* strain of O26 H11 11368 uid41021 ranks first for encompassing the most number of genes and nucleotides (Table 1); hence it was presumed as the standard criterion for comparative genomic analysis and long oligo probe designing. Although the aforementioned strain is known as Enterohaemorrhagic *E.coli* (EHEC), it contains a huge number of virulence factor genes including different types of adhesins, which are common in UPEC and EHEC strains in order to have a successful colonization and infection [5, 7, 11, 13, 25].

The .gbk file regarding *E.coli* O26 H11 11368 uid41021 and .fna files relating to *Sh.sonnei, Sh.flexneri, Sh.dysenteriae, Sh.boydii,* and *Salmonella enterica* were downloaded from the NCBI FTP site.

These files were uploaded to GView Server (https://server.gview.ca/) to have a schematic view of comparative genomes in association with *E.coli* O26 H11 11368 uid41021, *Sh.sonnei, Sh.flexneri, Sh.dysenteriae, Sh.boydii,* and *Salmonella enterica.* The unique regions of genome as the analysis type and other default parameters represented by the GView Server (1e-10 for value cutoff, bacteria for genetic code, 100 for

alignment length cutoff and 80 for percentage identity cutoff) were applied for comparing *E.coli* O26 H11 11368 uid41021with other bacterial strains in this research (Figure 1) [24].

Furthermore, the pangenomic sequence analysis was achieved by PanSeq Server (https://lfz.corefacility.ca/panseq/analyses/#userNovel) [24]. The analysis was done via novel region detector. *E.coli* O26 H11 11368 uid41021 was added to the selected query and other strains including *Salmonella enterica* subsp. enterica serovar enteritidis str. P125109 chromosome complete genome, *Shigella boydii* CDC 3083-94 chromosome complete genome, *Shigella dysenteriae* Sd197 complete genome, *Shigella flexneri* 2a str. 2457T complete genome, and *Shigella sonnei* Ss046 chromosome complete genome were also added to selected reference. The other parameters (including "Minimum novel region size: 500", "Nucmer values b: involving 200, c: 50, d: 0.12, g: 100, and l: 20", "Percent Sequence Identity Cutoff:90", "Fragmentation Size: 500", "Core Genome Threshold: 3", and "Blast Word Size: 20") were selected as suggested by the server.

The identified unique genomic regions pertaining to *E.coli* O26 H11 11368 uid41021 were then blasted via NCBI BLAST tool software [7].

The confirmed unique genomic regions were analyzed by alleleID 7.7 software for designing appropriate long oligo microarray probes. In the following, the unique sequences were added to the new sequence page of the microarray tab and the probe designing was done via probe search in the analyze tab. For determining the size of probe, the button of probe length with 55-64 nucleotides was selected, as the software suggested. The produced and designed probe was processed for further analyses [7].

The designed microarray probes were rechecked by NCBI BLAST tool software and their physicochemical and other characteristics such as ΔG, T_m, ΔH, and hairpins were assessed by the online tool of oligoanalyzer 3.1 (https://eu.idtdna.com/calc/analyzer). Other default parameters including target type: DNA, oligo Conc: 0.25 μM, Na^+ Conc: 50 mM, Mg^{++} Conc: 0 mM, dNTPs Conc: 0 mM, Nucleotide type: DNA, Sequence type: linear, Temperature: 25°C, Max foldings: 20, Suboptimality: 50%, Start position: 0, and Stop position: 0 were included in this study.

At the end, the accurate and standard long oligo probes were selected to be used in designing diagnostic microarray chip [7].

RESULTS

The results from GReview server indicated a close relationship between *E.coli* O26 H11 11368 uid41021 and other selected bacteria such as *Sh.sonnei*, *Sh.flexneri*, *Sh.dysenteriae*, *Sh.boydii*, and *Salmonella enterica* (Figure 1).

Figure 1, which was directly taken from the GView server, shows the common sequences in color while the white areas assign the loss of sequences in different studied strains.

The PanSeq server identified the unique genomic regions via comparison of the pan-genomes belonging to *E.coli* O26 H11 11368 uid41021, Salmonella enterica subsp. enterica serovar enteritidis str. P125109 chromosome complete genome, *Sh.boydii* CDC 3083-94 chromosome complete genome, *Sh.dysenteriae* Sd197 complete genome, *Sh.flexneri* 2a str. 2457T complete genome, and *Sh.sonnei* Ss046 chromosome complete genome. The application of Gview and PanSeq servers, NCBI BLAST tool, and AlleleID 7.7 software resulted in 15 appropriate long oligo microarray probes, which are shown in Table 2.

DISCUSSION

There are many scientific researches that confirm the wide range of UTIs caused by different strains of ExPEC. ExPEC are important bacterial agents causing up to 90% of community acquired UTIs, over 80% of bacteriuria, more than 60% of recurrent cystitis, over 70% of uncomplicated UTIs, and up to 50% of complicated UTIs [4, 5, 7, 26–31].

E.coli is an extraordinary bacterium which possesses a huge number of virulence genes. Therefore, different strains of *E.coli* are considered as potentially pathogenic bacterial agents. Despite several categorizations pertaining to *E.coli*, there are still remark-

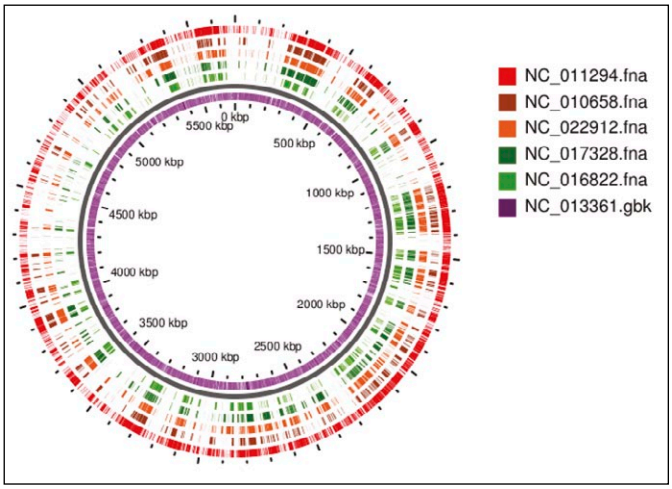

Figure 1. *A comparison between Escherichia coli (E.coli) O26 H11 11368 uid41021 (gbk file) and other bacteria including Shigella sonnei (pale green, .fna file), Shigella flexneri (dark green, fna file), Shigella dysenteriae (orange, fna file), Shigella boydii (red-brown, .fna file), and Salmonella enterica (red, .fna file).*

Table 2. *15 different microarray probes designed by AlleleID 7.7*

Microorganism	Long oligo Microarray Probe	length	oligoanalyzer	Location and gene product
E.coli	GATCAGTCGATATGCTCACGATGAACCTTCCATTCAGATTGTATTGATGCTTCTGTT	57	✓	4854732-4854788 Na+/H+
E.coli	CTGAACTCATGGCTTTGGTTAATAAATCCGACGAAGACATTGATTACAGCGACATTC	57	✓	5629045-5629101 hypothetical protein
E.coli	TGTTCAGTTAATGGTGGGCATGGGAATGAATACAACATTACACTATTTGCTGATAGGT	58	✓	4827729-4827786 type I restriction modification DNA specificity domain protein
E.coli	AAAACTTTACTTTCTTTGATGAACAAATTGCCAAACTCGGTGATGACTTGATCCCCTT	58	✓	3820187-3820244 hypothetical protein
E.coli	CTAAATGTGTCAAGCTACTCATAGAGCAGGGATTTCATATTCATCTGGATAAAGCCGC	58	✓	692085-692142 HNH endonuclease
E.coli	ATCAATAAAAACGGTCGCCAGATTGTGAGAACTGAAGGAACGGCAAATACCACTGTG	57	✓	2460848-2460904 1441 bp at 5' side: dGTPase975 bp at 3' side: 50S ribosome-binding GTPase family protein
E.coli	CATCTGACATAATACCAACTGAACGCCATTTACCACAAAGCTGATTCTTAATTGCCG	57	✓	4847093-4847149 hypothetical protein
E.coli	ATTTACTCTCTTAGCTTACTCTGGCAAATCCTTTACGTTACTCTCTGATGACTTCTT	57	✓	2278419-2278475 hypothetical protein
E.coli	CACTCATTGCAGAGTTCACTATGGCTAAGCACAGTTGGTTATTAATCGCCGCATTAC	57	✓	4763454 to 4763510 hypothetical protein
E.coli	CTTCTCTTGAAGATATCCTGCTTGAGATAACCTCGCTGGTAGATAACGCATTGGATC	57	✓	4368609-4368665 hypothetical protein
E.coli	CGATGTGTTGATGTATCTCGAAGAAGACGATACCCGTGATGTTGGTGTGATAATGAC	57	✓	623791-623847 inner membrane protein
E.coli	AATGCGGCTATCGTGATGGTTAATGCGGTATGCGAGAAATAAGGATATTTAGATGAAA	58	✓	4808962-4809019 fimbrial assembly proteinfimbrial protein
E.coli	AACAGGAAAGGGACATCAAGCAAGTTCATCACTGGTGATAATCCGGCAAAGTTATTC	57	✓	5060219-5060275 predicted transporter
E.coli	CTCTATTATGACGAGAATTTCAGTGACTATTACTATGGCATTTCAGAGAGTGAGTCCCGT	60	✓	4607597-4607656 putative outer membrane protein
E.coli	GCGGCCCTGGGTGGCATATTTATTGTCAGTGGTTTAATTCTGTTAATGAACTATAAC	57	✓	4763030 to 4763086 membrane protein

able overlaps between virulence factors of *E.coli* strains within different groups in their human hosts. According to previous investigations, UPEC encompasses a collection of virulence genes which may lead to UTIs. At the same time, other strains, such as EHEC, possess the key virulence genes which may also lead to UTIs [5, 6, 7, 25, 32–36].

Table 1 shows that *E.coli* O26 H11 11368 uid41021 (EHEC) bears the highest number of genes (6125 genes) and nucleotide sequences (5,697,240 bps). Hence, this strain was selected as standard sample for designing long oligo microarray probes in the present study.

Annually, governments spend a lot of money treating patients with UTIs. A survey estimates an overall cost of 236€ per patient a year with UTIs [37, 38]. Thereby, an accurate, rapid, sensitive, and specific diagnostic tool results in a definite treatment which may lead to a reduction in the unnecessary medical costs around the world.

DNA microarray is an advanced molecular diagnostic technology which provides a reliable diagnosis for detecting and identifying microbial agents causing different infectious diseases [14, 16, 17, 18, 19, 39, 40, 41].

DNA microarray technology consists of several stages, with probe designing being one of the most important. Today, there is a vast range of databases, servers, tools, and softwares which can be used for designing DNA microarray probes [14, 16, 17, 39, 41]. GViewer Server was used to show clear similarities among *E.coli*, *Shigella spp.* and *Salmonella enterica.* On the other hand, NCBI guaranteed appropriate support for GenBank data and BLAST tools. Finally, PanSeq server provided a suitable result for genomic unique regions. By the help of these technologies, the obtained outcomes were usable for AlleleID 7.7 software to retrieve invaluable raw data in the form of designed DNA microarray probes. In parallel with aforementioned possibilities, the oligoanalyzer tool determined the physico-chemical properties belonging to the designed probes [7, 14].

Microarray probe designing is a multi-science process with a wide range of facilities and potencies. Long oligo microarray probes are suitable choices

for an appropriate and proper diagnosis and definite treatment; thus, this group of probes was designed in the present survey. Probes can also be designed in general or specific forms. As the presence of multi-drug resistant microorganisms is a complicated and considerable problem in traditional medicine and environmental microbiology, the microarray technology may open a new way to incredible approaches for detecting and identifying normal virulence or multi-drug resistance genes [7, 14, 16, 41, 42, 43].

CONCLUSIONS

The use of progressive and advanced technologies enables us to design and produce tens and hundreds of different microarray probes with a wide range of diversity and quality. Simultaneously, the application of practical methodologies has an important role in the physico-chemical charactersitics belonging to designed DNA microarray probes. Therefore, in this investigation, we tried to design 15 long oligo microarray probes with the best functional and structural properties. These probes are proper candidates to be used in diagnostic microarray chip for detecting and identifying different strains of *E.coli*, such as UPEC. All in all, the practical databases, servers, tools, and softwares relating to microarray probe designing give DNA microarray technology a great opportunity to be more flexible, reliable, reproducible, and effective as a pioneer diagnostic technique.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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References

1. Farshad S, Ranjbar R, Japoni A, Hosseini M, Anvarinejad M, Mohammadzadegan R. Microbial susceptibility, virulence factors, and plasmid profiles of uropathogenic Escherichia coli strains isolated from children in Jahrom, Iran. Arch Iran Med. 2012; 15: 312-316.
2. Momtaz H, Karimian A, Madani M, et al. Uropathogenic Escherichia coli in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Ann Clin Microbiol Antimicrob. 2013; 12: 1-12.
3. Ranjbar R, Haghi-Ashtiani M, Jafari NJ, Abedini M. The prevalence and antimicrobial susceptibility of bacterial uropathogens isolated from pediatric patients. Iranian J Publ Health. 2009; 38: 134-138.
4. Farshad S, Ranjbar R, Anvarinejad M, Shahidi MA, Hosseini M, eds. Emergence of multi drug resistant strains of Eschetichia coli isolated from urinary tract infection. The Open Conf Proc J. 2010; 1: 192-196.
5. Behzadi P, Behzadi E. Environmental Microbiology, 1st edn, Tehran: Niktab, 2007; Chapts 3 and 9, pp. 59, 169-199.
6. Kaper JB, Nataro JP, Mobley HL. Pathogenic escherichia coli. Nat Rev Microbiol. 2004; 2: 123-140.
7. Jahandeh N, Ranjbar R, Behzadi P, Behzadi E. Uropathogenic Escherichia coli virulence genes: invaluable approaches for designing DNA microarray probes. Cent European J Urol. 2015; 68: 452-458.
8. Behzadi P, Behzadi E, Ranjbar R. Urinary tract infections and Candida albicans Cent European J Urol. 2015; 68: 96-101.
9. Dormanesh B, Dehkordi FS, Hosseini S, et al. Virulence Factors and O-Serogroups Profiles of Uropathogenic Escherichia Coli Isolated from Iranian Pediatric Patients. Iran Red Crescent Med J. 2014; 16: e14627.
10. Mirzarazi M, Rezatofighi SE, Pourmahdi M, Mohajeri MR. Occurrence of genes encoding enterotoxins in uropathogenic Escherichia coli isolates. Braz J Microbiol. 2015; 46: 155-159.
11. Lukjancenko O, Wassenaar TM, Ussery DW. Comparison of 61 sequenced Escherichia coli genomes. Microb Ecol. 2010; 60: 708-720.
12. Vernikos G, Medini D, Riley DR, Tettelin H. Ten years of pan-genome analyses. Curr Opin Microbiol. 2015; 23: 148-154.
13. Willenbrock H, Hallin PF, Wassenaar TM, Ussery DW. Characterization of probiotic Escherichia coli isolates with a novel pan-genome microarray. Genome Biol. 2007; 8: R267.
14. Najafi A, Ram M, Ranjbar R. Microarray: Principles & Applications, 1st edn, Tehran: Persian Science & Research Publisher, 2012; Chapts 1-6, pp. 1-59.
15. Behzadi P, Behzadi E, Ranjbar R. Basic Modern Molecular Biology, 1st edn, Tehran: Persian Science & Research Publisher, 2014; Chapt 1, pp. 1-4.
16. Behzadi P, Behzadi E, Ranjbar R. The application of Microarray in Medicine. ORL.ro. 2014; 24: 24-26.
17. Behzadi P, Najafi A, Behzadi E, Ranjbar R. Detection and Identification of Clinical Pathogenic Fungi by DNA Microarray. Infectio.ro. 2013; 35: 6-10.
18. Behzadi P, Ranjbar R, Alavian SM. Nucleic Acid-Based Approaches for Detection of Viral Hepatitis. Jundishapur J Microbiol. 2015; 8: e17449.
19. Behzadi P, Behzadi E, Yazdanbod H, Aghapour R, Cheshmeh MA, Omran DS. A survey on urinary tract infections associated with the three most common uropathogenic bacteria. Maedica (Buchar). 2010; 5: 111-115.
20. NCBI FTP Site National Center for Biotechnology Information, U.S. National Library of Medicine, 8600 Rockville Pike, BethesdaMD, 20894USA: NCBI; 2015 [cited 2015 17/06/2015].
21. NCBI. Nucleotide National Center for Biotechnology Information, U.S. National Library of Medicine, 8600 Rockville

Pike, Bethesda MD, 20894 USA: NCBI; 2015 [cited 2015 17/06/2015].

22. Gordienko EN, Kazanov MD, Gelfand MS. Evolution of pan-genomes of Escherichia coli, Shigella spp., and Salmonella enterica. J Bacteriol. 2013; 195: 2786-2792.

23. Richards VP, Lefébure T, Bitar PDP, et al. Genome Based Phylogeny and Comparative Genomic Analysis of Intra-Mammary Pathogenic Escherichia coli. PloS One. 2015; 10: e0119799.

24. Sankarasubramanian J, Vishnu US, Sridhar J, Gunasekaran P, Rajendhran J. Pan-Genome of Brucella Species. Indian J Microbiol. 2015; 55: 88-101.

25. Toval F, Schiller R, Meisen I, Putze J, Kouzel IU, Zhang W, et al. Characterization of urinary tract infection-associated Shiga toxin-producing Escherichia coli. Infect Immun. 2014; 82: 4631-4642.

26. Doumith M, Day M, Ciesielczuk H, et al. Rapid identification of major Escherichia coli sequence types causing urinary tract and bloodstream infections. J Clin Microbiol. 2015; 53: 160-166.

27. Farshad S, Anvarinejad M, Tavana AM, et al. Molecular epidemiology of Escherichia coli strains isolated from children with community acquired urinary tract infections. Afr J Microbiol Res. 2011; 5: 4476-4483.

28. Anvarinejad M, Farshad S, Alborzi A, Ranjbar R, Giammanco GM, Japoni A. Integron and genotype patterns of quinolones-resistant uropathogenic Escherichia coli. Afr J Microbiol Res. 2011; 5: 3736-3770.

29. Ranjbar R, Goudarzi MM, Jounaidi N. Vaginal Lactobacilli and pap Operon Expression in Uropathogenic Escherichia coli. Biomed & Pharmacol J. 2015; 8: 91-97.

30. Ranjbar R, Ahmadnezhad B, Jonaidi N. The Prevalance of Beta Lactamase Producing Escherichia coli Strains Isolated from the Urine Samples in Valiasr Hospital. Biomed & Pharmacol J. 2014; 7: 425-431.

31. Habibian R, Khameneie MK, Sedighian H, Daneshi F, Moghadam MB, Mahboobi M. Virulence Factor Diversity Between Imipenem Resistant and Imipenem Susceptible Strains of Escherichia coli Isolated from Hospitalized Patients with Severe Urinary Tract Infections. Biosc Biotech Res Asia. 2014; 11: 469-477.

32. Smith JL, Fratamico PM, Gunther NW. Extraintestinal pathogenic Escherichia coli. Foodborne Pathog Dis. 2007; 4: 134-163.

33. Toval F, Köhler C-D, Vogel U, et al. Characterization of Escherichia coli isolates from hospital inpatients or outpatients with urinary tract infection. J Clin Microbiol. 2014; 52: 407-418.

34. Köhler C-D, Dobrindt U. What defines extraintestinal pathogenic Escherichia coli? Int J Med Microbiol. 2011; 301: 642-647.

35. Le Gall T, Clermont O, Gouriou S, et al. Extraintestinal virulence is a coincidental by-product of commensalism in B2 phylogenetic group Escherichia coli strains. Mol Biol Evol. 2007; 24: 2373-2384.

36. Nowrouzian FL, Adlerberth I, Wold AE. Enhanced persistence in the colonic microbiota of Escherichia coli strains belonging to phylogenetic group B2: role of virulence factors and adherence to colonic cells. Microbes Infect. 2006; 8: 834-840.

37. Ciani O, Grassi D, Tarricone R. An economic perspective on urinary tract infection: the 'costs of resignation'. Clin Drug Investig. 2013; 33: 255-261.

38. Kucheria R, Dasgupta P, Sacks S, Khan M, Sheerin N. Urinary tract infections: new insights into a common problem. Postgrad Med J. 2005; 81: 83-86.

39. Behzadi P, Behzadi E, Ranjbar R. Microarray Data Analysis. Alban Med J. 2014; 4: 84-90.

40. Mao B-H, Chang Y-F, Scaria J, et al. Identification of Escherichia coli genes associated with urinary tract infections. J Clin Microbiol. 2012: 50: 449-456.

41. Behzadi P, Behzadi E, Ranjbar R. Microarray probe set: Biology, bioinformatics and biophysics. Alban Med J. 2015; 2: 78-83.

42. Behzadi P, Behzadi E, Ranjbar R. Multidrug-Resistant Bacteria. Infectio. ro. 2014; 39: 29-31.

43. Tajbakhsh E, Khamesipour F, Ranjbar R, Ugwu IC. Prevalence of class 1 and 2 integrons in multi-drug resistant Escherichia coli isolated from aquaculture water in Chaharmahal Va Bakhtiari province, Iran. Ann Clin Microbiol Antimicrob. 2015; 14: 37. ■