Archives of Razi Institute, Vol. 74, No. 3 (2019) 313-320

Copyright © 2019 by
Razi Vaccine & Serum Research Institute

Short Communication

Phylogenetic Analysis of *bor* gene in an *Escherichia coli* Strain χ1378 (O78:K80) Isolated from an Avian Colibacillosis Case in Tehran, Iran

Nayeri Fasaei [1], B., Zahraei Salehi [1], T., Salari [2,] *, S., Ranjbar [3], M.M., Yousefi [4], A.R.

1. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran
3. Department of FMD, Razi Vaccine and Serum Research Institute, Agricultural Research, Education, and Extension Organization (AREEO), Karaj, Iran
4. Department of Pathology and Experimental Animals, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

Received 03 March 2018; Accepted 11 September 2018
Corresponding Author: saeedsalari@uoz.ac.ir

ABSTRACT

Colibacillosis is known as a fatal bacterial disease resulting in a high level of commercial loss worldwide. This study amid to elucidate the sequence, genetic characteristics, and phylogeny of the *bor* gene in *Escherichia coli* (*E. coli*) strain χ1378 (O78:K80) isolated from avian colibacillosis in Iran and develop a rapid and optimal polymerase chain reaction (PCR) molecular-based technique with specific primers to detect this gene in *E. coli*. A virulent avian *E. coli* (i.e., laboratory designation *E. coli* strain χ1378) isolated from a chicken with systemic colibacillosis from a broiler farm in Tehran, Iran, in 2004 was used as a source of the *bor* gene. After DNA extraction, PCR method was used to amplify the *bor* gene. A 658 bp fragment of the *bor* gene was amplified, sequenced, blasted, and phylogenetically studied. The most similar sequences to the *bor* gene in *E. coli* strain χ1378 were *E. coli* APEC O78, Enterobacteria phage HK630, and *Escherichia coli* BW2952, respectively. There was a high similarity between the *bor* gene in *E. coli* bacteria with their phage and plasmid. Moreover, a high similarity was observed between the *bor* and *iss* genes (approximately 92%) showing that they were homologous genes. In addition, the similarity analysis of different bacterial species, as well as their plasmid and bacteriophage, to the *bor* gene indicated that the highest similarity to O78:K80 was related to *Paracoccidioides brasiliensis*, *Bacillus thuringiensis* CT43 plasmid pBMB0558, and *Salmonella enterica* subsp. enterica serovar Kentucky strain CVM29188 plasmid, respectively. Altogether, the results of the present study confirmed the presence of the *bor* gene in the studied isolates and clarified its sequence, phylogenetic relationship, and similarities of *E. coli* strain χ1378 (O78:K80) isolated from avian colibacillosis.

Keywords: *E. coli*, Colibacillosis, Avian, Identification

Analyse phylogénétique du gène bor dans une souche χ1378 (O78:K80) d'*Escherichia coli* isolée d'un cas de colibacillose aviaire à Téhéran, en Iran

Résumé: La colibacillose est connue comme une maladie bactérienne mortelle entraînant un niveau élevé de pertes commerciales dans le monde entier. Cette étude visait à élucider la séquence, les caractéristiques génétiques et la phylogénie du *gène bor* de la souche d'*Escherichia coli* (*E. coli*) χ1378 (O78:K80) isolée de la colibacillose aviaire en Iran ainsi qu'à développer une technique moléculaire rapide et optimale de réaction en chaîne de la polymérase (PCR) avec des amorces spécifiques dédiées à la détection de ce gène dans E. coli. La souche d'*E. coli* aviaire virulente (nommée souche χ1378 d'*E. coli*) isolé d'un poulet à la colibacillose systémique d'une ferme de poulets de chair à Téhéran, en Iran, en 2004, a été utilisé comme source du gène *bor*. Après extraction de l'ADN, le gène *bor* a été amplifié par PCR. De ce fait, un fragment de 658 pb du gène *bor* a

été amplifié, séquencé, explosé et étudié phylogénétiquement. Les séquences les plus similaires au gène bor dans la souche χ1378 d'*E. coli* étaient respectivement *E. coli* APEC O78, Enterobacteria phage HK630 et *E. coli* BW2952. Il y avait une grande similitude entre le gène bor dans la bactérie *E. coli* avec leur phage et leur plasmide. De plus, une grande similarité a été observée entre les gènes bor et iss (environ 92%), montrant qu'il s'agissait de gènes homologues. En outre, l'analyse de similarité du gène bor appartenant à différentes espèces bactériennes, ainsi que de leurs plasmides et bactériophages, indique que la similarité la plus élevée avec O78:K80 est liée *Paracoccidioides brasiliensis*, *Bacillus thuringiensis* CT43, *plasmide* pBMB0558, et *Salmonella enterica* subsp, plasmide de la souche CVM29188 d'enterica sérovar Kentucky, respectivement. En somme, les résultats de la présente étude ont confirmé la présence du gène *bor* dans les isolats étudiés et révélé sa séquence, sa relation phylogénétique et les similitudes de la souche *E. coli* χ1378 (O78:K80) isolée de la colibacillose aviaire.

Mots-clés: *E. coli*, Colibacillose, Aviaire, Identification

INTRODUCTION

The extra pathogenic *Escherichia coli* (ExPEC) strains are responsible for diseases in both humans and animals. Although serotype/virulence factors are related to these differences, the full understanding of the molecular mechanism behind this diversity has not been elucidated up to now. Better elucidation of virulence mechanisms in avian pathogenic *Escherichia coli* (APEC), one of the most important ExPEC strains and causative agents in poultry colibacillosis, is urgently necessary for developed treatments and preventative measures regarding ExPEC infections (Russo and Johnson, 2006). To date, according to the literature reviews, no studies have undertaken to sequence and characterize the *bor* gene in avian isolates, particularly the O78 serotype, which has been associated with avian colibacillosis in the majority of the flocks in Iran (Vandekerchove et al., 2004; Zahraei et al., 2004). The *bor* is the identified gene on phage lambda expressed in lysogeny processes and whose product shows close similarity to bacterial virulence proteins (Johnson et al., 2008). The *bor* gene can be detected in many *Escherichia coli* (*E. coli*) strains. It has been shown that the *bor* gene has a close relationship with the *iss* locus of plasmid ColV, I-K94, which helps with bacterial resistance to serum complement killing in vitro and virulence in animals (Johnson et al., 2008). Similar in vitro effect has been reported for the *bor* gene (Barondess and Beckwith, 1995). The *bor* gene is a specific phage gene represented to affect the serum sensitivity of a lysogenic host (Barondess and Beckwith, 1995). The *bor* gene codes a lipoprotein located in the outer membrane of *E. coli*, and antigenically related known proteins are expressed by the lysogens of lambdoid coliphages in cells carrying the cloned *iss* gene, as well as in several clinical isolates of *E. coli*. The *bor* is a widespread gene known as the starting point in the mechanistic analysis of *bor*-mediated serum resistance. The *iss* gene similarity to the *bor* gene is 93% at the DNA level, and it encodes a protein of 102 amino acids with 79% similarity to the *bor* gene. Applying *iss* as a marker for ExPEC, Bekal et al. (2003) reported that the false-positive reactions they obtained for the *iss* gene, a well-studied virulence locus, could be attributed to its high similarity to the *bor* gene. It promotes an approximately 20-fold increase in *E. Coli* K-12 survival time in animal sera in vitro and an approximately 100-fold increase in the animal virulence of a non-K12 *E. coli* strain (Bekal et al., 2003). The *bor* gene has the same in vitro effect and function as the *iss* gene causing about a 20-fold survival increase in animal serum on *E. coli* K-12 lysogens. Closely related structures of these proteins underlie a functional similarity. The selective benefits dictated by the *bor* gene in wild-type environments could be important for the phage and its lysogenic host (Barondess and Beckwith, 1995). It is required to investigate the prevalence of the clinical strain of APEC harboring *bor* gene or lambda

bacteriophage, especially in Iran, and the present study started to address these issues. In addition, this study focused on the survey of the molecular characterization of the *bor* gene in APEC isolates and determination of the genetic relationship between Iranian APECs and those of other countries. The aims of the present study were 1) to elucidate the sequence and genetic characteristics of the *bor* gene in *E. coli* strain χ1378 (O78:K80) isolated from avian colibacillosis in Iran, 2) to contribute to the understanding of the genetic status and the genetic differentiation of the *bor* gene in *E. coli* populations and other bacteria worldwide, 3) to further analyze the phylogenetic relationships, based on the *bor* gene, between the Iranian populations of *E. coli* and other populations of *E. coli*, as well as their phages and plasmids isolated and sequenced from different part of world, 4) to develop a rapid and optimal polymerase chain reaction (PCR) molecular-based technique with specific primers for the detection of this gene in the Iranian strains of *E. coli*.

MATERIAL AND METHODS

Bacterial strains, media, and reagents. In the present study, *E. coli* strain χ1378 isolated from a chicken diagnosed systemic colibacillosis in Tehran (Zahraei et al., 2004) was applied as the source of the *bor* gene. The obtained strain was biochemically characterized and finally identified as O78:K80 serotype (MAST serotyping kit; MAST Group Ltd, Merseyside, UK). In this study, *E. coli* DH5α (Fermentas, Vilnius, Lithuania) and *Salmonella Typhimurium* LT2 were used as positive and negative controls, respectively. In addition, *E. coli* strain χ1378 was used as the source of the *iss* gene. The bacterial isolates were grown on Luria-Bertani broth and agar (Difco Laboratories, USA).

Preparation of chromosomal DNA. Chromosomal DNA from *E. coli* strain χ1378, *E. coli* DH5α (Fermentas, Vilnius, Lithuania), and *Salmonella Typhimurium* LT2 were extracted using the UltraClean® Microbial DNA Isolation Kit (MO BIO Laboratories, USA) as recommended by the manufactures.

Amplification protocol. Sequences of Enterobacteria phage lambda (accession no.: J02459.1) and Bacteriophage lambda *bor* gene (Accession No. X55792.1) were retrieved from the GenBank database, and the primers were synthesized. The *bor* gene sequence of native *E. coli* strain χ1378 was amplified using *bor*-upper: 5'- CTCGATGCAAAATACACGAA GGAGTTAGCT-3' and *bor*-lower: 5'- TAATTTTCT ACACATACGATTCTGCGAACT-3' primers. The PCR amplification of the *bor* gene was performed using Cinnagene PCR set system. The DNA molecular weight was visualized on 1% agarose gel after ethidium bromide staining, and DNA concentrations were estimated by spectrophotometer at 260 nm. The final approved PCR reaction were carried out in 25 µl reaction volume containing 2.5 µl of 10X AMS PCR buffer (CinnaGene, 1 ml), 0.8 µl of 50 mM MgCl2 (CinnaGene, 500 µl), 0.5 µl of 10 mM deoxynucleoside triphosphates (CinnaGene, 100 µl), 1.2 µl of each of the upstream and downstream primers (10 pmol), 1.0 U of expand high fidelity polymerase (Fermantase, 2.5 u/µl), and 3 µl of DNA template (50 ng). The PCR program was carried out through 35 cycles in a thermocycler (TC 512 Techne) at 94 °C for 30-sec denaturation, 55 °C for 30-sec annealing, and 72 °C for 40-sec extension. Then, it was followed by the denaturing of DNA for 5 min at 94 °C and extention for 7 min at 72 °C.

Polymerase chain reaction product purification using agarose gel electrophoresis. Fragment of *bor* generated (658bp) from annealing temperature of 55 °C was purified using rapid PCR product purification kit (Molecular Biological System Transfer, MBST). The PCR products were analyzed in 1% agarose gel for 90 min at 80 V in 1X TAE buffer and then stained with ethidium bromide (25 µg ml^{-1}, CinnaClone) for 15 min. GeneRuler 1 kb (Fermentas, Canada) was loaded as the DNA ladder. The amplified PCR product was

extracted and purified by the agarose gel using a Gel extraction kit (Fermentas Co., Canada).

Direct Sequencing of polymerase chain reaction product. The PCR product was sequenced by the application of ABI PRISMcc BigDyeTM terminator cycle sequencing kits and reads using an ABI PRISMcc 3730XL automated sequencer (Macrogen Inc., Seoul, South Korea). Two directional sequencings by forward and reverse primers were used for the reading of the same samples.

Sequence registration. Sequence data were edited using the BioEdit software (version 7.9). Then the *bor* gene sequence was registered by Sequin online software on National Center for Biotechnology Information (NCBI) with accession number of KC253896.

Blasting, retrieving, aligning, and editing sequences. The edited *bor* gene sequences were directed to a nucleotide BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for the evaluation of similarity to the sequences previously registered in NCBI. Then, the closest hits were chosen and summarized in informative Tables 1. Moreover, the nucleotide sequences of the *bor* gene from different *E. coli* strains and other bacteria, as well as plasmid and phage, were retrieved from the GenBank database. The obtained sequences were aligned using ClustalW, analyzed, and trimmed in CLC sequence viewer software (version 6.6.2). Eventually, very short stretches of sequences or regions with ambiguities were excluded from the analyses.

Construction of phylogenetic tree. Prepared, aligned, and edited sequences were used for phylogenetic reconstruction by MEGA software package (version 5.3). The phylogenetic analysis was separately followed on the two sets of aligned sequences, including the genomic ones, as well as plasmid and phages containing the *bor* gene sequences. Furthermore, out-groups were added to each of the datasets. Trees were constructed using the neighbor-joining (NJ) algorithm under the gap removal option and Kimura's two-parameter substitution model. Repeatability of phylogenetic branches was estimated by bootstrap analysis with 1,000 replications (the percentages were shown near the branches).

RESULTS AND DISCUSSION

Nucleotide sequence statistics of polymerase chain reaction product. Table 1 tabulates some general sequence analysis of the *bor* gene in *E. coli* strain χ1378 (O78:K80) isolated from avian colibacillosis in Tehran, Iran.

Blasting results. *E. coli* strain χ1378 (O78:K80) isolated from avian colibacillosis in Tehran was used as the entry data to search GenBank to confirm the presence of the *bor* gene amplified in PCR and sequences with the most similar identity to the sequence of the present study. Blast results revealed that most similar sequence to the *bor* gene in *E. coli* strain χ1378 (O78:K80) were APEC O78, *Enterobacteria phage* HK630, and *E. coli* BW2952, respectively (Table 2). These results showed that there was a high similarity between the *bor gene* in *E. coli* bacteria, as well as their phage and plasmid, and this gene is conserved in *E. coli* genomic DNA, as well as their phage and plasmid. Nucleotide blast showed a high similarity between the *bor* and *iss* genes (approximately 92%); therefore, it seems that they are homologous genes. Similarity analysis of the *bor* gene to different bacterial species, their plasmid, and bacteriophage with the *bor* gene in *E. coli* strain χ1378 (O78:K80) isolated from avian colibacillosis in Iran by BioEdit software showed that the closest similarity to O78:K80 were *Paracoccidioides brasiliensis*, *Bacillus thuringiensis CT43 plasmid pBMB0558*, and *Salmonella enterica* subsp. enterica serovar Kentucky strain CVM29188 plasmid, respectively (Table 3).

Phylogenetic tree. For phylogenetic analysis, the sequences of the genomic (32 strains) of *E. coli*, as well as plasmid and phages, obtained from GenBank, along with the *bor* gene in *E. coli* strain χ1378 (O78:K80) isolated from avian colibacillosis in Iran were included in the present study. The phylogenetic analysis

included most of all available *bor* sequences from GenBank and present study (figures 1 and 2).

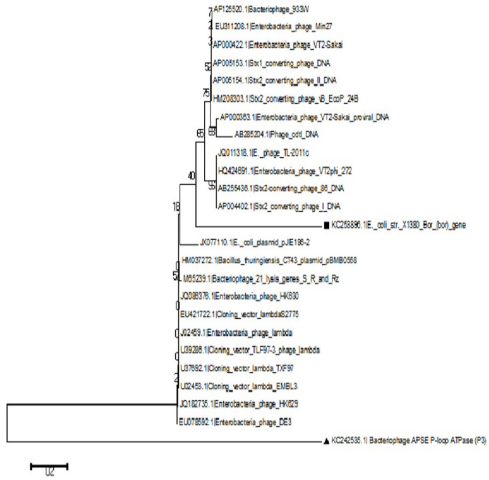

Figure 1. Phylogenetic tree constructed with MEGA software package (version 5.3) illustrating the relationships with *bor* gene among *Escherichia coli* (*E. coli*) strains; the *bor* gene in *E. coli* strain X1380 (O78:K80) isolated from avian colibacillosis in Iran marked by ■ and out-group (*Cronobacter sakazakii*) by ▲ in tree; tree constructed using the neighbor-joining algorithm based on differences in *bor gene* in different strains of *E. coli*; units at the bottom of the tree indicating the number of substitution events; the length of each pair of branches representing the distance between sequence pairs; the dataset was resampled 1,000 times using the bootstrap method; the sequence information at the tips of the branches, including accession numbers of the sequences and strain name for each sequence; phylogenetic trees created based on data from nucleotide sequences**.**

The sequences were compared using CLC sequence viewer software (version 6.6.2) and MEGA software package (version 5.3) with 1,000 bootstrap resampling and NJ method. Two different trees were constructed on the basis of the blast results. No stretch of branches showed high similarity and conservation of the *bor* gene in some part of the phylogenetic tree (Figure 1). In the evaluation of relationships in the *bor* gene among *E. coli* strains, the phylogenetic tree was divided into two main groups in which *E. coli* strain χ1378 (O78:K80) was located in group 1 close to APEC strain. As it is shown in Figure 1, the members of group 1 had more differences among taxa than group 2, and group 2 has full similar taxa. As it is shown in Figure 2,

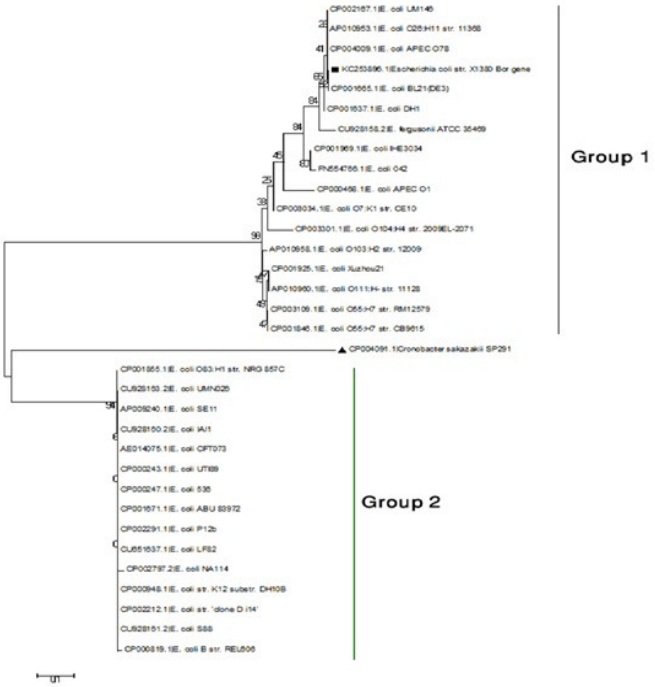

Figure 2. Phylogram of the present study genomic *bor* gene in comparison to *bor* gene on plasmids and phages; ■ indicating sequences obtained in the present study and ▲ showing out-group (bacteriophage APSE P-loop ATPase [p3] in tree).

there was a high similarity and relationship between this gene in the different hosts (i.e., bacterial species) and phages. Moreover, X1380 is in the middle of the tree with more divergence than others. The *bor* gene was initially reported by Barondess and Beckwith (1995) as a virulent gene with complement resistance potential. Then, it was reported in other studies in different *E. coli* strains (Lynne et al., 2007; Johnson et al., 2008) and appeared similar to the *lom* gene that helps in binding the host to eukaryotic cells and is also known as lysogenic conversion genes (i.e., accessory genes that are expressed from the prophage and change the host in some of its characteristics) (Casjens and Hendrix, 2015). The results of this study help to better understand *bor* sequence characteristics and similarities as a virulent gene and resistant to some of the immune

system factors, as well as phylogeny, similarity, and evolutionary relationships. In addition, it paves the way for immuno-bioinformatics studies (Ranjbar et al., 2015). The NCBI nucleotide blast results for the *bor* gene in *E. coli* strain χ1378 (O78:K80) isolated from avian colibacillosis in Iran showed that this gene is fully conserved among different *E. coli* strains. Moreover, avian *bor* is very similar to the *bor* from a human *E. coli* isolate. This result was previously confirmed for the *bor* and *iss* genes (Horne et al., 2000). Similarity and phylogenic analysis of the *bor* gene in different bacterial species, as well as their plasmid and bacteriophage to *E. coli,* denoted the evolutionary relationships and the possibility of transferring from a genus or species to another. Furthermore, the homologous plasmid-encoded *iss* gene product is similar to the bor and some other proteins in virulence, evolution, and suppressive effects on the immune system (Chuba et al., 1989; Wooley et al., 1992; Barondess and Beckwith, 1995). Furthermore, this characteristic has been observed in some other bacterial species (Doorduijn et al., 2016; Ataei Kachooei et al., 2017). Moreover, there has been a report on the identification of virulence genes of APEC for use as a rapid diagnostic tool (Johnson et al., 2008). Comparisons of gene/protein sequences in phylogenetic results can help with deep insights into their functionality and evolutionary background. In Iran, many pathogenic strains of *E. coli* are present, and there is not much data on the virulent gene sequences

Table 1. Sequence information and nucleotide distribution

Organism (name and description)	Sequence type	Length	Weight (double-stranded)	Nucleotide	
				N. Count	N. Frequency
Bacteria (*bor* gene in *E. coli* strain X1380 [O78:K80] AC: KC253896	DNA	653 bp	403.497 kDa	A:174	0.266
				C:157	0.240
				G:145	0.222
				T:177	0.271
				G+C=302	0.462
				A+T=351	0.538

Table 2. National Center for Biotechnology Information nucleotide blast results of *bor* gene in *E. coli* strain X1380 (O78:K80) isolated from avian colibacillosis in Iran (ranking of sequences is based on their Max Score, Identity, and E. Value arranged ascending to descending, respectively)

Ranking	Name (description)	Max Score	Identity	E.Value	Accession Number
1	*Escherichia coli* APEC O78	1206	100%	0.0	CP004009.1
2	*Enterobacteria* phage HK630	1206	100%	0.0	JQ086376.1
3	*Escherichia coli* BW2952	1206	100%	0.0	CP001396.1
4	Cloning vector lambda S2775	1206	100%	0.0	EU421722.1
5	Enterobacteria phage lambda	1206	100%	0.0	J02459.1
6	Cloning vector TLF97-3, phage lambda lacZ translational fusion vector	1206	100%	0.0	U39286.1
7	Cloning vector lambda EMBL3, right arm	1206	100%	0.0	U02453.1
8	*Escherichia coli* P12b	1201	99%	0.0	CP002291.1
9	Enterobacteria phage HK629	1195	99%	0.0	JQ182735.1
10	*Escherichia coli* BL21(DE3)	1184	99%	0.0	AM946981.2
11	*Escherichia coli* O103:H2 strain 12009 DNA	1162	99%	0.0	AP010958.1
12	*Escherichia coli* DH1 (ME8569) DNA	1157	99%	0.0	AP012030.1
13	*Escherichia coli* strain K12 substrain DH10B	1157	99%	0.0	CP000948.1
14	*Escherichia coli* Xuzhou21	1151	98%	0.0	CP001925.1
15	*Escherichia coli* O55:H7 strain RM12579	1151	98%	0.0	CP003109.1

of these bacteria. Sequences of many of these genes are still unknown, and it is required to perform further studies in this regard. The results of the present study confirmed the presence of the *bor* gene and clarified its sequence, phylogenetic relationship, and similarities in *E. coli* strain χ1378 (O78:K80) isolated from avian colibacillosis in Iran. Analysis of microbial sequences showed that a part of the genome of some bacteria was correlated to prophage DNA. In addition, the investigation of bacteriophages showed that they have attracted a medical and ecological focus (Serra-Moreno et al., 2006). Phage lambda DNA integrates into host DNA by the homologous recombination processes of recombinases at one preselected site in *E. coli* (Serra-Moreno et al., 2006). Release of bacteriophage from its cell-host is through a process of induction. Lysogens refers to the cells carrying a prophage that can be activated due to the effect of inductive factors. In addition, this activated prophage can lead to the lysis of the host cell. The protein coded by these virulent genes may have important effects on the host bacterium, which can have its phenotype modified by the expression of genes encoded by the prophage. These alterations range from protection against further phage infection to increase the virulence of a pathogenic host. The presence or absence of prophages can be evaluated for much of the variation among individuals within a bacterial species. Furthermore, phages are known as one of the major ways for the horizontal transfer of genetic information between bacteria (Serra-Moreno et al., 2006). The lambda prophage is more transcriptionally active than it has been assumed for a long time and suggests that lysogeny may generally have a role in bacterial survival in animal hosts and perhaps in pathogenesis (Barondess and Beckwfth, 1990). In the present study, sequence analysis approach was applied to further characterize the identification and evolution of the bacteriophage lambda *bor* gene.

Table 3. Similarity analysis of *bor* gene in different bacterial species, as well as their plasmid and bacteriophage to *E. coli* (numbering of sequences is based on their Max Score, Identity, and E. Value (not shown in Table) arranged ascending to descending, respectively).

Number	Name	Max Score	Identity	Length	Accession number
1	*Paracoccidioides brasiliensis*	916	99%	466	AF441251
2	*Bacillus thuringiensis* CT43 plasmid *pBMB0558*	757	98%	559	HM037272.1
3	*Salmonella enterica* subsp. *enterica* serovar *Kentucky* strain CVM29188 plasmid	559	89%	551	CP001122.1
4	*Shigella sonnei* Ss046	355	93%	245	CP000038.1
5	*Shigella boydii*	137	88%	257	CP000036.1
6	*Shigella sonnei* bacteriophage	121	91%	210	AJ279086.1
7	*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* strain LT2	101	86%	260	AE006468.1
8	*Salmonella typhimurium* phage ST64B	101	86%	260	AY055382.1
9	*Salmonella enterica* subsp. *enterica* serovar *Choleraesuis* strain	101	86%	260	AE017220.1
10	*Salmonella enterica* subsp. *enterica* serovar *Newport* strain SL254	99.6	89%	261	CP001113.1
11	*Salmonella enterica* subsp. *enterica* serovar *Dublin* strain CT_02021853	99.6	89%	261	CP001144.1
12	*Salmonella enterica* subsp. *enterica* serovar *Gallinarum* strain 91/287	99.6	89%	261	AM933173.1
13	*Salmonella enterica* subsp. *enterica* serovar *Enteritidis* strain P125109	99.6	89%	261	AM933172.1
14	*Salmonella enterica* subsp. *enterica* serovar *Paratyphi C* strain RKS4594	99.6	89%	261	CP000857.1
15	*Salmonella enterica* subsp. *enterica* serovar *Weltevreden* strain 1-3289-60-2007	99.6	89%	261	FR775213.1
16	*Salmonella enterica* subsp. *enterica* serovar *Gallinarum/pullorum* strain RKS5078	99.6	89%	261	CP003047.1

Sequence information related to specific genes in wider aspects can be useful in certain clinical contexts for the precise prediction of likely bacteria phenotype and its virulence (Kant, 1995). Moreover, the results of this study showed that a simple PCR-based technique in comparison to complex techniques is more sufficient for the reliable identifying and sequencing of genes using new and optimal primer pairs designed, specifically to amplify a portion of interest. Identifying and sequencing of virulence genes, such as *bor*, help prevent and control colibacillosis in affected Iranian avian flock regularly and could answer to some evolutionary functional questions and organism pathogenesis.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Support

This study was supported by University of Zabol (UOZ/GR/9618/154).

Acknowledgment

The authors gratefully acknowledge the research council of the Zabol University.

References

Barondess, J.J., Beckwfth, J., 1990. A bacterial virulence determinant encoded by lysogenic coliphage lambda. Nature 346, 6287, 871-874.

Barondess, J.J., Beckwith, J., 1995. *bor* gene of phage lambda, involved in serum resistance, encodes a widely conserved outer membrane lipoprotein. J Bacteriol 177, 5, 1247-1253.

Bekal, S., Brousseau, R., Masson, L., Prefontaine, G., Fairbrother, J., Harel, J., 2003. Rapid identification of *Escherichia coli* pathotypes by virulence gene detection with DNA microarrays. J Clin Microbiol 41, 5, 2113-2125.

Casjens, S.R., Hendrix, R.W., 2015. Bacteriophage lambda: early pioneer and still relevant. Virology 479, 310-330.

Chuba, P.J., Leon, M.A., Banerjee, A., Palchaudhuri, S., 1989. Cloning and DNA sequence of plasmid determinant iss, coding for increased serum survival and surface exclusion, which has homology with lambda DNA. Mol Gen Genet MGG 216, 2, 287-292.

Doorduijn, D.J., Rooijakkers, S.H., van Schaik, W., Bardoel, B.W., 2016. Complement resistance mechanisms of Klebsiella pneumoniae. Immunobiology 221, 10, 1102-1109.

Horne, S.M., Pfaff-McDonough, S.J., Giddings, C.W., Nolan, L.K., 2000. Cloning and sequencing of the iss gene from a virulent avian *Escherichia coli*. Avian Dis, 44, 1, 179-184.

Johnson, T.J., Wannemuehler, Y.M., Nolan, L.K., 2008. Evolution of the iss gene in *Escherichia coli*. Appl Environ Microbiol 74, 8, 2360-2369.

Kant, J., 1995. Direct DNA sequencing in the clinical laboratory. Clin Chem 41, 10, 1407-1409.

Lynne, A.M., Skyberg, J.A., Logue, C.M., Nolan, L.K., 2007. Detection of Iss and Bor on the surface of *Escherichia coli*. J Appl Microbiol 102, 3, 660-666.

Ranjbar, M.M., Ahmadi, N.A., Ghorban, K., Ghalyanchilangeroudi, A., Dadmanesh, M., Amini, H.-R., 2015. Immnoinformatics: Novel view in understanding of immune system function, databases and prediction of immunogenic epitopes. Koomesh 17, 1, 18-26

Russo, T.A., Johnson, J.R., 2006. Extraintestinal isolates of *Escherichia coli*: identification and prospects for vaccine development. Expert Rev Vaccines 5, 1, 45-54.

Serra-Moreno, R., Acosta, S., Hernalsteens, J.P., Jofre, J., Muniesa, M., 2006. Use of the lambda red recombinase system to produce recombinant prophages carrying antibiotic resistance genes. BMC Mol Biol 7, 31.

Vandekerchove, D., De Herdt, P., Laevens, H., Pasmans, F., 2004. Colibacillosis in caged layer hens: characteristics of the disease and the aetiological agent. Avian Pathol 33, 2, 117-125.

Wooley, R.E., Spears, K.R., Brown, J., Nolan, L.K., Fletcher, O.J., 1992. Relationship of complement resistance and selected virulence factors in pathogenic avian *Escherichia coli*. Avian Dis, 36, 3, 679-684.

Zahraei, S., Rajabi, Z., Modirsanei, M., Bokaei, S., 2004. Evaluation of the immunogenicity of endemic *Escherichia coli* serotypes isolated from poultry in Iran. J Vet Res 59, 2, 189-195.