Construction of an *iss* deleted mutant strain from a native avian pathogenic *Escherichia coli* O78: K80 and in vitro serum resistance evaluation of mutant

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Abstract:

BACKGROUND: Colibacillosis, caused by different serotypes of avian pathogenic *Escherichia coli* (APEC), is one of the important diseases in poultry industry. The isolate O78 is the most prevalent serotype of APEC in Iran. One of the APEC virulence factors, increased serum survival (*iss*) gene, is related to serum resistance. The usual form of colibacillosis in avian is extraintestinal, and serum resistance is applied one way by APEC to reach internal organs; hence, it appears that the control of colibacillosis in poultry regarding the deletion of *iss* and the construction of a serum sensitive APEC strain is beneficial. Additionally, the knowledge about APEC serum resistance could be extended using mutant strains. **OBJECTIVES:** The present study was an attempt to generate an *iss* mutant strain from native APEC-O78 strain χ1378 and to study the level of serum resistance of native APEC-O78 strain χ1378 in comparison with its mutant (APEC-O78 strain χ1378Δ*iss*). **METHODS:** The lambda red recombinase system was utilized to delete *iss* gene in native APEC-O78 strain χ1378. This strain was first transformed with the plasmid pkD46 to introduce the lambda red recombinase system and then the PCR product with sequence homology to the *iss* gene and a kanamycin resistance marker was transformed into the APEC-O78 strain χ1378. Serum sensitivity of mutant and wild type strain was investigated by microtiter test. **RESULTS:** The generation of mutant was successful and the *iss* was replaced with kanamycin resistance cassette. Also, it was observed that the mutant was sensitive to serum. However, serum sensitivity of *iss* deleted mutant was not statistically different from its parents. **CONCLUSIONS:** Application of lambda red recombination could be a simple and useful technique for production of a precisely defined gene deletion. Also, there may be some genes that compensate the activity of *iss* gene.

Introduction

Colibacillosis is still considered the most important bacterial disease affecting the poultry industry (Barnes et al., 2008; Derakhshandeh et al., 2009; Kariyawasam et al., 2006; Kariyawasam and Nolan, 2009). This disease occurs by many serotypes of Avian Pathogenic *Escherichia coli* (APEC), such as O1, O2, and O78 (La Ragoine et al., 2000; Mellata et al., 2003; Nayeri Fasaei et al., 2009; Stordeur et al., 2004; Vandekerchove et al., 2004; Zahraei Salehi et al., 2004); however, it is usually the result of serotype

O78 in Iran (Zahraei Salehi et al., 2004). Clinical form of colibacillosis in avian, in contrast with mammalian colibasillosis, is extraintestinal, while intestinal form is more prevalent in mammals (Barnes and Gross, 2005; Gross, 1994). The use of prevalent pathogenic serotypes, in each country, is advisable to prepare vaccines and to control the vaccination strategies (Zahraei Salehi et al., 2004). Therefore, to this time, researches about the native APEC-O78 strain χ1378, along with other methods of colibacillosis control such as the use of antibiotics, farm sanitation, and other management practices, could be helpful. Farm sanitation and management practices are arduous and expensive, and also the cost involved in the treatment, undesirable side effects, appearance of apparent transferable antibiotic resistance, and public concerns over the use of antibiotics limit the use of antibiotics (Derakhshandeh et al., 2009; Gomis et al., 2003; La Ragione et al., 2001; La Ragione et al., 2004). Therefore, control of the disease through vaccination, especially with live attenuated vaccines, is thought to be a logical and desirable approach (Zahraei Salehi et al., 2004). It is worthwhile to consider native and frequent strains for studies of control and vaccine approaches (Zahraei Salehi et al., 2004). Live *E. coli* vaccine has been found to induce a more marked level of immunity (Kwaga et al., 1994). It is known that a properly delivered live bacterial vaccine will be more effective since all of the relevant antigens will be present to stimulate both cellular immunity and humoral immunity at the appropriate site, while these antigens may be absent or altered in killed bacteria. Also, the preparations of subunit vaccines are not cost effective (Kwaga et al., 1994). Many different live attenuated vaccines have been used to control colibacillosis; however, there is no vaccine currently available that is economical, functional in farms, and also effective against different strains of APEC (Derakhshandeh et al., 2009; Nolan et al., 2003; Nolan et al., 1992; Vidotto et al., 1990). One approach to develop attenuation and vaccination can be achieved through inactivation of one or more virulence factors (Nayeri Fasaei et al., 2009). In addition, identification and characterization of virulence factors in APEC, as a spectacular research area, could be done by construction of different mutants (Sharan et al., 2009). The role of different virulence factors related to serum resistance in mammalian *E. coli* and avian *E. coli* has been investigated; however, the knowledge about the serum resistance mechanism of APEC-O78 is ill-defined (Mellata et al., 2003). Increased serum survival (*iss*) gene, as a conserved virulence gene, has a role related to serum resistance and frequently occurs in avian *E. coli*, especially in avian pathogenic *E. coli* (Skyberg et al., 2008). Also, *iss* is located in different serotypes of APEC (Derakhshandeh et al., 2009). In addition, the ability of APEC to resist the host protective effects of serum plays a significant role in the development of APEC in body fluids and internal organs (Mellata et al., 2003). Since there is no report about the mechanism of serum resistance in native APEC-O78 strain χ1378, it seems worthwhile to delete *iss* and investigate the contribution of *iss* gene to serum resistance to have a better understanding of how to control colibacillosis.

Materials and Methodes

A. Construction of native APEC-O78 strain χ1378 Δ*iss*: The bacterial strains used in this study were routinely cultured on Luria-Bertani (LB) agar and broth, containing the appropriate antibiotics. The primers used in this study are listed in table1. Deletion of *iss* gene was carried out in a virulent wild native strain of APEC-O78 χ1378, isolated from a chicken with systemic colibacillosis in Iran, as described previously (Datsenko and Wanner, 2000; Derakhshandeh et al., 2009; Horne et al., 2000; Lynne et al., 2007b; Nayeri Fasaei et al., 2009; Skyberg et al., 2008). Briefly, after serotyping of native APEC-O78 strain χ1378 (MAST serotyping kit; MAST Group Ltd, Merseyside, UK), the presence of *iss* gene (760bp) was detected by PCR using *iss* upper (F) and *iss* lower (R) primers. Then, *iss* gene was sequenced in APEC-O78 strain χ1378 (Derakhshandeh et al., 2009). The PCR was carried out on a total volume of 25 µL containing 1x PCR buffer, 1.6 mM MgCl2, 0.2 mM dNTPs, 0.5 µM of each primer, 1.25 U of Taq DNA polymerase and 10 µg of template DNA (PCR Set System, Sinaclon, Tehran, Iran). The amplification program was used at 94°C for 5 min, 30 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec, and 72°C for 7 min (Techne Thermocylcer, TC-512). Temperature sensitive plasmid pKD46, encoding the lambda Red recombinase (Nature Technology

Corporation, England), was transformed into electro-component native APEC-O78 strain χ1378 by electroporation. Plasmids were purified using commercial kit (Plasmid Isolation Kit Minipreparation, MBST, Tehran, Iran). L-Arabinose was used for the induction of the Lambda Red genes expression at 10 mM final concentration (BBL, USA). Overnight bacterial cultures of native APEC-O78 strain χ1378 were diluted 1: 100 into 8 mL of fresh SOB medium (each liter containing 20 g tripton, 0.5 g yeast extract, and 1 mL of 0.25 M KCl) and incubated at 37°C, while shaking, until they reached an OD600nm of 0.6. Culture was then concentrated by centrifugation at 3200 ×g for 15 min at 4°C. From this step, everything was maintained on ice. After discarding the supernatant, cells were then re-suspended in 4, 2 and 1 mL of ice-cold 10% glycerol (Merck, Germany) and centrifuged at 3200, 17900 and 17900 ×g for 15, 2 and 2 min, respectively, at 4°C. After these 3 washing steps, the cells were suspended in 80 µL ice-cold 10% glycerol and used immediately in 40 µL aliquots for electroporation step. Electroporation was carried out using Gene Pulser® II Electroporation System and cold Gene Pulser® 0.2 Cm gap Cuvettes (Bio-Rad® Laboratories Inc., Richmond, CA) at 2.5 kV with 25 µF and 200 Ω by adding 50 ng of pKD46 to native APEC-O78 strain χ1378 electrocompetent cells. Also, a control reaction, without adding plasmid, was electro-porated. Immediately after electroporation, cells were resuspended in 1 mL of cold LB and incubated for 1.5 h at 30°C. Five hundred microliters of the mixture were plated on LB containing 100 µg/mL ampicillin, and the plates were incubated at 30°C overnight. Ampicillin-resistant, temperature-sensitive colonies were selected for transformation by kanamycin cassette flanked by 5' and 3' sequences of the *iss* gene. Primers for - mut - *iss* and rev - mut - *iss*, and pKD4 as template, were used to amplify the kanamycin cassette flanked by homolog regions of 3' and 5' end of *iss* gene (1.6 kb). High fidelity PCR amplification was conducted using 15 reactions of AccuPower™ HF PCR PreMix (BIONEER) containing 1 µL of pKD 4 template, 1 µL of each primer (10 µM) and 17 µL of distilled water. PCR conditions were as follows: 94°C for 3 min, followed by 30 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 30 sec min and then an extension period of 72°C for 7 min (Techne Thermocycler, TC-512). The PCR product was purified from the agarose gel by manufacture's protocol (Gene Jet Gel Extraction Kit, Fermantase), then confirmed by agarose gel. A single fresh colony of native APEC-O78 strain χ1378 containing pKD46 was placed into 5 mL of LB-ampicillin and shaken at 30 °C overnight. Subsequently, 500 µL of this culture was mixed with 50 mL SOB containing 100µg/mL ampicillin and L-arabinose was added to a final concentration of 10 µm. The mixture was incubated at 30 °C with shake. At an OD_{600nm} of 0.6, the cells were made electrocompetent following this protocol. Cells were concentrated by centrifugation at 3200 ×g for 15 min, 4°C. Then, 4 washing steps, at 4°C, carried out by 10, 5, and 2.5 mL of cold 10% glycerol and centrifugation at 3200 ×g for 15 min for each step. Then pellet were mixed with 250 µL of cold 10% glycerol. 100 µL of electrocompetent cells (APEC-O78 strain µL378 containing pKD46) were mixed with 300 ng of purified PCR product and this mixture was electroporated as described above and then was spread on LB agar plates containing 50 µg/mL kanamycin. After kanamycin selection, the expected deletions in mutants were verified by PCR protocol targeting the new antibiotic resistance cassette junction fragment (1.8kb) through the use of the *iss* upper (F) and *iss* lower (R) primers on the kanamycin resistant colonies. The PCR reaction was performed in 25 µL reaction volume containing 2.5 µL of 10X PCR buffer, 1.6 mM of MgCl2, 0.2 mM of deoxynucleoside triphosphates, 0.5 mM of each of the upstream and downstream primers (10 pmol), 1.25 U of Taq DNA polymerase and 50 ng of DNA template (PCR Set System, Sinaclon, Tehran, Iran). PCR amplification involved 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 30 sec in a thermocycler (Techne Thermocylcer, TC-512). The reaction mixture was held at 94°C for 5 min before and 72°C for 7 min after the reaction.

B. In vitro serum resistance assay of native APEC-O78 strain χ1378 and its mutant by microtiter method: Tow hours culture of APEC-O78 strain χ1378, APEC-O78 strain χ1378Δ*iss*, and serum sensitive DH5α (control), grown in 3 mL Peptone Glucose (PG) broth (Difco), were adjusted to 0.5 McFarland standard (0.5 mL of 0.048 M BaCl2 (1.17% w/v BaCl2.2H2O) to 99.5 mL of 0.18 M

H2SO4 (1% v/v)). Then, 10-fold serial dilutions of each culture have been prepared and 100 µL of 10-3 dilution (104 CFU), verified by viable count was dispensed into flat-bottomed 96-well microtiter plates and mixed with 100 µL of 50% chicken serum diluted in phosphate-buffered saline. The experiment was repeated using PG broth instead of serum. Also, as medium control, 100 µL of serum mixed with 100 µL PG broth also included. Plates were incubated at 37^{o}C and the growth was monitored using a microplate reader set at 490 nm (Stat fax-2100, UK) every 30 min for 4 hr. The experiment was repeated three times. The results are the average of three experiments. A one-way ANOVA was used to test the null hypothesis of equal mean growth rates among the strains. A post hoc test, Fisher LSD, was used to identify differences between strains with P-value 0.05 (Lee et al., 1991; Lynne et al., 2007a; Wooley et al., 1991).

Results

The *iss* gene was deleted in native APEC-O78 strain χ1378 to generate a mutant (APEC-O78 strain χ1378Δ*iss*) as described by Datsenko and Wanner (Datsenko and Wanner, 2000). At first, by PCR, with specific primers for *iss* in native APEC-O78 strain χ1378, the 760 bp PCR product was detected (Figure 1) and sequenced. The sequence was submitted to GenBank by Derakhshandeh et al. (2009) with the assigned accession number FJ416147. Then, the pKD46 electroporated to electrocompetent native APEC-O78 strain χ1378, and this strain was ampicillin resistante and sensitive to temperature higher than 30°C. The process of native APEC-O78 strain χ1378Δ*iss* construction is shown in Figure1. This figure shows 1.6 kb purified PCR product of the kanamycine cassette flanked with *iss* homology. Also, there are different sizes of band with the PCR protocol targeting the new antibiotic resistance cassette junction fragment in wild-type and mutant strain after the replacement of kananamycin cassette (Figure 1). The resistance of three strains to serum, native APEC-O78 strain χ1378 (wild), native APEC-O78 strain χ1378Δ*iss* (mutant), and DH5α (serum sensitive) were investigated using microtiter test. The results show that the growth of wild type, mutant strain, and DH5α were not statistically different in PG broth ($p<0.05$; Figure 2). DH5α grew significantly lower in chicken serum than it did in wild type and mutant strain (Figure 3; $p<0.05$). Also, in comparison with wild type, the growth of mutant in chicken serum decreased; however, it was not significant (Figure 3; $p<0.05$).

Discussion

Colibacillosis, an *Escherichia coli* infection, is a major problem for the poultry industry (Barnes et al., 2008; Derakhshandeh et al., 2009; Kariyawasam et al., 2006; Kariyawasam and Nolan, 2009). O1, O2, and O78 serotypes of APEC are the major etiological agent for colibacillosis in poultry (Vidotto et al., 1990); however, in Iran the majority of colibacillosis is related to O78 serotype (Zahraei Salehi et al., 2004). Prevention and control of colibacillosis by vaccination is one of the inquiries of researchers, and the use of native strain is advised in vaccine preparation (Zahraei Salehi et al., 2004). The present study investigated native APEC serotype O78. The pathogenesis of avian *E. coli* is different from mammalian *E. coli*, since in mammals intestinal form is more prevalent while the extraintestinal form occurs more in avian (Nolan et al., 2003). In addition, serum resistance is at least one of the mechanisms used by APEC to reach internal organs of chickens (Mellata et al., 2003). With regard to the act of *iss* gene, which increased serum resistance, it seems that deletion of *iss* from native APEC-O78 strain χ1378 could be profitable in controlling colibacillosis. On the other hand, there have been few investigations about the mechanism of serum resistance in APEC-O78 (Mellata et al., 2003). In the present study, the *iss* gene was deleted from native APEC-O78 strain χ1378 by lambda red recombineering (Datsenko and Wanner, 2000). *iss* gene was replaced with kanamycin cassette by homologous recombination due to red recombinase enzymes, produced by pKD46; as a result, the mutant strain was kanamaycin resistant and also the PCR result shows 1.8 kb of the PCR product. This confirms the deletion of *iss* from native APEC-O78 strain χ1378. Lynne et al. (2007) deleted *iss* from APEC-O2 by pSKY5000 (Lynne et al., 2007b), while in the present study pKD46 was used. The lambda Red recombinase was expressed by pSKY5000 rather than pKD46. pSKY5000 is a

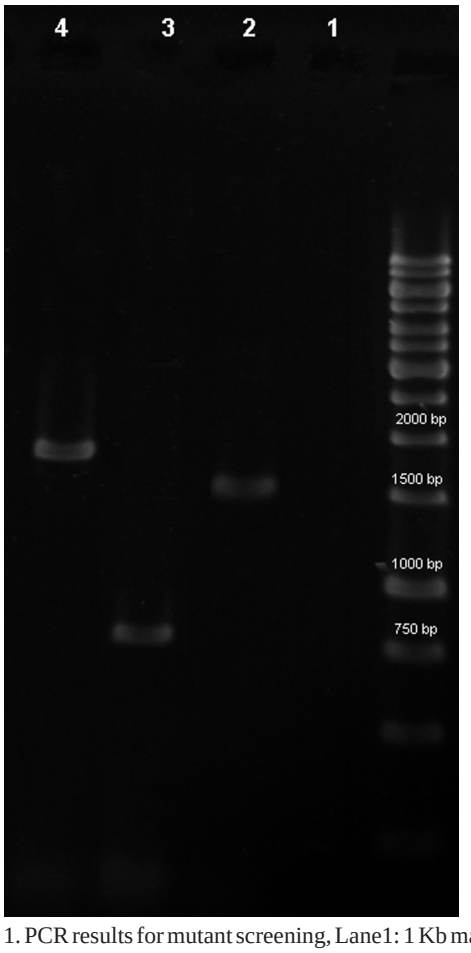

Figure 1. PCR results for mutant screening, Lane1: 1 Kb marker; Lane 2 & 3: PCR results by for-mut-*iss* and rev-mut *iss* primers (no band in wild type, 1.6 kb in mutant); Lane 4 & 5: PCR results by *iss* upper and *iss* lower primers (760 bp in wild type, 1.8 kb in mutant).

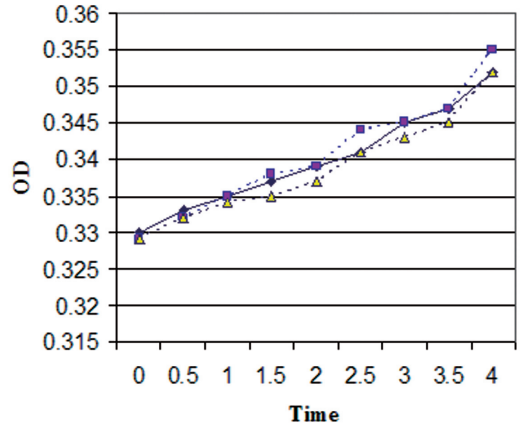

Figure 2. The growth rate of APEC-O78 strain χ1378, APEC-O78 strain χ1378Δ*iss* and DH5α in PG broth at different times. APEC-O78 strain χ 1378 DH5α APEC-O78 strain χ 1378 Δ *iss*

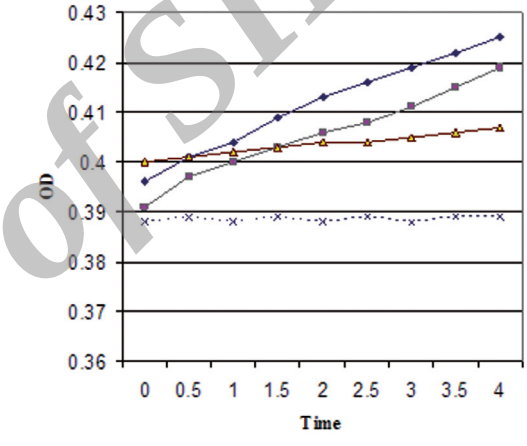

Figure 3. The growth rate of APEC-O78 strain χ1378, APEC-O78 strain χ1378Δ*iss* and DH5α (sensitive to serum) in chicken serum at different times. APEC-O78 strain χ 1378 DH5α APEC-O78 strain χ 1378 Δ *iss* Control

chloramphenicol resistant derivative of pKD46. Our results show that the method of mutagenesis, used in the present study, may be more comfortable than the suicide vector. The findings of Heiat et al. (2012), Nayeri et al. (2009) and Zare et al. (2008), that deleted different genes from different strains, confirmed this subject (Heiat et al., 2012; Nayeri Fasaei et al., 2009; Zare et al., 2008). This process is not difficult or expensive. In methods that use suicide vectors, there are two recombination steps (Herring et al., 2003). In addition, designing and construction of these vectors are labor intensive and depend on employment of several enzymes, while in the method used in our study, applied by little time and effort, and successfully, the mutant was generated by one step recombination. Moreover, in Datsenko and Wanner method, temperature sensitive helper plasmids could be easily eliminated by high temperature in mutant strain (Herring et al., 2003; Tischer et al., 2001). The mutant presented in this study could be examined in alternative or next researches, such as invivo evaluation of immunization, colonization, and invasion or used to construct multiple deletions. In the present study, the resistance of native APEC-O78 strain χ1378Δ*iss* to chicken serum, in comparison with wild type, was investigated by microtitr assay (Lynne et al., 2007a). Results demonstrated that there is no significant difference between mutant and wild type strain ($p<0.05$). Mellata et al. (2003) investigated the role of K1 capsule, P fimbriae, and O78 LPS in APEC in resistance to serum and demonstrated that the K1 capsule is probably required to prevent serum effect

Table 1. Primers used in this study.

Primers name	Sequences (5' to3')	Ref.
iss upper(F)	GTGGCGAAAACTAGTAAAACAGC	Derakhshandeh et al., 2009; Lynne et al., 2007a
iss lower(R)	CGCCTCGGGGTGGATAA	
for- mut- *iss*	TATTCATTTCCCATGATTCTGAGTACCTACCAAGTCTGAGTGTGTAGGCTGGAGCTGCTT	Lynne et al., 2007a
rev-mut- *iss*	AAAAACAACTGTAGGGAGCCCAGAAGTATATTAATGAACACATATGAATATCCTCCTTAG	

in particular strains, such as O1 and O2, but is not needed to protect O78. In addition, they showed that P fimbriae has not a significant role in serum resistance in O2 strain but maybe has a role in other serotypes and need to be investigated. Their study also implicated that in O1 strains, in addition to LPS, there may be other factors related to serum resistance. In their study, the presence of *iss* and *traT* were demonstrated in all mutant and wild type strains. Also, the mutant strains of APEC O78:K80 and APEC O2:K1 contained *iss* and *traT* genes but had lost the K1, or the O serotype was not protected against the bactericidal effect of serum. However, these mutant strains were more resistant than the control strains. The control strains were wild type and *iss* and *traT* negative (Mellata et al., 2003). Nolan et al. (2003) reported that *iss* might have a more important role in birds than mammals to produce virulence and resistance to serum. These differences may be due to the rout of initial entry and forms of clinical disease in these hosts (Nolan et al., 2003). Lynne et al. (2007) studied the contribution of *iss* and *bor* gene to *E. coli* serum resistance. They showed that *iss* contributes more to serum resistance than *bor* (Lynne et al., 2007a). Chuba and Colleagues reported that the effect of *iss* on serum resistance was not gene dosage dependent (Chuba et al., 1986). Skyberg et al. (2008) investigated the role of *iss*, *tsh*, *iutA*, *iroN*, *sitA* and *cvaB* genes in virulence of APEC-O2. They deleted these genes and mutants were compared to the wild type (APEC-O2) for lethality to chick embryos and growth in human urine. No significant differences between the mutants and the wild type were detected, and they reasoned that insensitivity of the virulence assays or other factor could have obscured changes in the virulence of the mutants (Skyberg et al., 2008). These finding showed that the serum resistance of native APEC-O78 strain χ 1378 is multifactorial. Moreover, the effects of single gene deletion might be obscured by some compensatory mechanism. Hence, deletion of more genes related to serum resistance is advisable to achieve serum sensitive native strain. Additionally, when differences in virulence between the mutants and the wild type were not detected, follow-up studies to determine if the genes are differentially expressed in native APEC-O78 strain χ1378 in serum is necessary (Skyberg et al., 2008). The mutant showed little, but not significant, growth in serum compared to the wild-type parent, showing that there are probably other genes to compensate the act of *iss*. Chuba et al. (1986) noted that *traT* gene, located on Col-V plasmid, like *iss* but less, was involved in serum resistance (Chuba et al., 1986). The finding of lynne et al. (2007) demonstrated that *iss* appears to play a major role in the serum resistance associated with pAPEC-O2-ColV (Lynne et al., 2007a). Chuba et al. (1989) showed that *iss* had significant homology to *bor* gene of lysogen bacteriophage lambda (Chuba et al., 1989). In our later work, *bor* gene in native APEC-O78 strain χ1378 have been identified and sequenced and 90% homology with *iss* has been observed (data have not published), therefore deletion of *bor* gene from native APEC-O78 strain χ1378Δ*iss* could be advisable. Our work added to the current understanding of serum resistance of native APEC-O78 strain χ1378. There are not so much data on virulent genes sequences of this bacterium, and the sequences of these genes are still unknown.

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ایجاد جهش در سویه بومی χ1378 اشریشیا کلی بیماریزای طیور O78:K80 با حذف ژن *iss* و ارزیابی مقاومت سرمی موتان در شرایط آزمایشگاهی

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چکیده

زمینه مطالعه: کلی باسیلوز یکی از بیماریهای مهم در صنعت طیور است. سروتیپ O78 اشریشیا کلی بیماریزای طیور (APEC-O78) شایع ترین عامل این بیماری در ایران است. از جمله عوامل حدت اشریشیا کلی بیماریزای طیور، ژن افزایش دهنده بقای سرمی (*iss*) است که در مقاومت به سرم نقش دارد. مقاومت به سرم یکی از رهیافت های سیستمیک شدن اشریشیا کلی بیماریزای طیور می باشد. با توجه به این که غالب ترین شکل بالینی کلی باسیلوز طیور به صورت خارج روده ای می باشد و احتمالاً این فرم از بیماری به مقاومت سرمی باکتری ارتباط دارد، در نتیجه به نظر می رسد که احتمالاً با حذف ژن *iss* رهیافتی در کنترل بیماری کلی باسیلوز ایجاد گردد. علاوه بر این، اطلاعات کمی در مورد مکانیسم مقاومت سرمی APEC-O78 وجود دارد. **هدف:** در مطالعه حاضر، سویه موتان با حذف ژن *iss* از سویه بومی (χ1378) اشریشیا کلی بیماریزای طیور O78 ایجاد گردید و سویه موتان از نظر مقاومت به سرم با سویه وحشی مورد ارزیابی قرار گرفت. **روش کار:** در این مطالعه، سیستم lambda red recombinase برای حذف ژن *iss* در سویه بومی (χ1378) اشریشیا کلی بیماریزای طیور O78 مورد استفاده قرار گرفت. ابتدا پلاسمید pKD46 (تولیدکننده آنزیم های سیستم lambda red recombinase) وسپس محصول PCR که دارای توالی مشابه اطرافی با ژن *iss* و نشانگر مقاومت به کانامایسین بود به داخل سویه بومی (χ1378) اشریشیا کلی بیماریزای طیور O78 ترانسفورمه گردید. حساسیت به سرم نیز به روش میکروتیتر در سویه وحشی و موتان مورد ارزیابی قرار گرفت. **نتایج:** نتایج نشان داد که سویه جهش یافته با موفقیت ایجاد شده و به جای ژن *iss*، کاست مقاومت کانامایسین جایگزین شد. همچنین، اگرچه، سویه موتان به سرم حساس بود اما تفاوت معنی داری از نظر مقاومت به سرم بین سویه موتان و وحشی وجود نداشت. **نتیجه گیری نهایی:** روش lambda red recombination به عنوان یک روش ساده و مفید برای حذف ژن قابل استفاده است. همچنین احتمالاً ژن های دیگری در جبران فعالیت ژن *iss* نقش دارند.

واژه های کلیدی: ژن افزایش دهنده بقای سرمی، lambda red recombineering، اشریشیا کلی بیماریزای طیور O78، مقاومت سرمی

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