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Original article

# Frequency of *iss* and *irp2* genes by PCR method in *Escherichia coli* isolated from poultry with colibacillosis in comparison with healthy chicken in poultry farms of Zabol, South East of Iran

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## Abstract

There is no special trait for differentiation of Avian Pathogenic *Escherichia coli* from Avian Fecal *Escherichia coli*. This investigation is aimed, as a case control study, to evaluate and compare the frequency of *iss* and *irp2* in 43 AFEC strains and also 40 and 56 *E. coli* strains isolated from the liver and kidney of chickens with colibacillosis, respectively, farmed in Zabol, as a border region of Iran, by PCR. 86.9% and 37.2% of isolates collected from chickens with colibacillosis and feces samples obtained from healthy chickens were positive for *iss* gene, respectively ( $P < 0.05$ ). On average, 59.3% of *E. coli* strains isolated from colibacillosis have *irp2* gene while 27.9% of isolates from the feces of healthy birds were positive ( $P < 0.05$ ). 52.15% of isolates from colibacillosis and 19.62% of isolates from healthy chicken feces were positive for both genes, with statistical significant difference ( $p < 0.05$ ). This marked difference in the distribution of *iss* and *irp2* genes makes these two genes good markers to differentiate AFEC and APEC strains especially in Sistan region to improve colibacillosis control measurements.

**Key words:** AFEC, APEC, *irp2*, *iss*, Zabol

## Abbreviations

Extraintestinal Pathogenic *Escherichia coli* (EXPEC); Avian Pathogenic *Escherichia coli* (APEC); Avian Fecal *Escherichia coli* (AFEC); increased serum survival (*iss*); Iron Responsive Element protein 2 (*irp2*); *Escherichia coli* (*E. coli*); Polymerase Chain Reaction (PCR)

## Introduction

There are increased concerns that Avian Pathogenic *Escherichia coli* (APEC) strains, the causative agent of colibacillosis, are becoming more resistant to antimicrobial agents (Foley et al. 2000, Ghanbarpour and Salehi 2010, Nateghi et al. 2010). On the other hand, some evidence indicates that APEC strains play major role as a convenient source to transmit food-borne disease and poultry products considered as a common source of Extraintestinal Pathogenic *Escherichia coli* (EXPEC) causing disease in humans (Deb and Harry 1978, Bolin and Jensen 1987, Delicato et al. 2003, Ghanbarpour and Salehi 2010). Therefore, colibacillosis control will be serious problem in the future and the control will be beneficial for human or animal health. Due to increased resistance to antibiotics and the risk of drug residues in poultry production, attention is more focused on vaccine development. This goal requires characterization of major and common virulence genes and determine their abundance in each geographic location (Kwaga et al. 1994, Jeong et al. 2012). Full understanding and use of the unique characteristics of APEC as targets for vaccination or diagnosis result in the planning criteria in order to control colibacillosis effectively (Boerlin and White 2006, Moulin-Schouleur et al. 2006). Most studies indicated that 5 genes, including *hlyf*, *iroN*, *ompT*, *iss* and *iutA*, could be considered to differentiate Avian Fecal *Escherichia coli* (AFEC) from APEC and investigation based on these genes is valuable as well as genotyping of 46 virulence genes (Gyimah and Panigrahy 1998, Gibbs et al. 2003, Monroy et al. 2005). Although numerous studies have shown that the majority of strains of avian pathogenic *E. coli* have contained *irp2*, but according to our literature review, this issue in Iran requires further researches (Dozois et al. 1992). This study aimed to determine and compare the prevalence of two important virulence genes, *iss* and *irp2*, in *E. coli* isolated from colibacillosis and healthy poultry farmed in Zabol as a border region of Iran to establish the design and implementation of appropriate preventive and treatment methods against colibacillosis.

## Materials and Methods

Fifty cloacal swabs from healthy chickens, 50 and 50 samples from the liver and the kidney (respectively) of poultry with colibacillosis (totally, one-hundred) were collected from poultry farms located in different sites of Zabol, Sistan and Baluchistan province of Iran, during December 2014 to May 2015. Sick birds have been killed based on protocol approved by a Local Ethics Commission (Larjani et al. 2005). Isolates were identified as *E. coli* (Quinn et al. 2002). DNA was extracted from bacterial cultures by boiling method and all isolates were evaluated for presence of *iss* and *irp2* genes via Polymerase Chain Reaction (PCR) with some modifications through primers designed by Nateghi et al. (2010). Data were analyzed via SPSS by K-square and Fisher exact tests.  $p < 0.05$  was considered as significant level.

## Results

Among the diseased birds, the frequency of *iss* in isolates recovered from the liver (82.5%) was comparatively low in comparison with isolates recovered from the kidney (91.3%). It is not a statistical different ( $p > 0.05$ ). Concisely, 86.9% of *E. coli* yielded from poultry with colibacillosis were significantly positive for *iss* gene while the frequency of *iss* in AFEC was 37.2% ( $p < 0.05$ ). In *E. coli* isolated from the liver of poultry with colibacillosis, *irp2* was present in a relatively high frequency (60%) compared with the frequency observed in kidney-origin *E. coli* isolates (58.7%). It is also not a statistical difference ( $p > 0.05$ ). Based on the presence of *irp2* in *E. coli* isolates, 59.3% of positive isolates were appertained to diseased birds and 27.9% of positive isolates were coming from healthy birds ( $p < 0.05$ ). With no significant differences, 50% and 54.3% isolates isolated from the liver and kidney of poultry (respectively) with colibacillosis harbored *iss* and *irp2* simultaneously. But, 52.17% of isolates from colibacillosis and 19.62% of isolates from healthy chicken feces were positive for both genes ( $p < 0.05$ ).

## Discussion

This study, regarding the studied genes, was carried out as a first work in one of border region of Iran to identify some of major traits known as factors that may play an important role in virulence of APEC and apply the results in future epidemiologic studies and further genotypical researches and also will help to

establish the proper preventive and treatment strategies.

In Iran, according to our literature review, the prevalence of *iss* in the current study (86.9%) was different from those of Derakhshandeh et al. (2009: 53%), Nateghi et al. (2010: 68.2%), Haghghi Khoshkhoo and Mokhayeri (2012: 38%) and Arabi et al. (2013: 96.43%), while it was higher than those obtained in China (58.5%), Hungary (63%), Korea (41.5%), Brazil (38.5%) (Delicato et al. 2003, Jin et al. 2008, Dziva and Steven 2008, Won et al. 2009) and other studies conducted in different parts of the world (Jin et al. 2008: 72-82%). It may be related to the complement effect of serum and the pathogenicity of *E. coli*. It is reported that *in vitro* investigation about the resistance of microorganisms to serum activity has been considered as a suitable method for pathogenicity evaluation (Lee et al. 1991, Nolan et al. 2002). Although frequencies of virulence genes in APECs vary according to location and host, studies have shown that the frequency of *iss* gene in avian pathogenic *E. coli* was higher compared to *E. coli* isolated from healthy poultry (Derakhshandeh et al. 2009) what is consistent with our results and could be considered useful to differentiate APEC from the commensal strains or target gene for vaccine production in study area.

In the present study, for the first time, the frequency of *irp2* in *E. coli* isolated from colibacillosis cases was compared with AFEC strain in Iran. The findings of the present study, like previous research, demonstrated a strong correlation between the production of *irp2* and virulence of poultry *E. coli*. Nateghi and colleagues (2010) investigated the prevalence of *irp2* in APEC (53.57%) and UPEC (33%) strains in West region of Tehran, Iran. The frequency of *irp2* in APEC strains was reported 53.5%, by Arabi et al. (2013), among *E. coli* strains isolated from farms of Mazandaran province, Iran. It seems that the prevalence of *irp2* gene in our study is higher than in other studies in different areas of Iran (Nateghi et al. 2010, Arabi et al. 2013). Iron uptake systems is found in invasive strains of *E. coli* that compete with host transferrin for available iron molecules (Williams 1979, Lafont et al. 1987). Iron-repressible protein 2 (*irp2*), which is involved in iron acquisition in *Yersinia*, are found in human *E. coli* and APEC strains (Schubert et al. 1998, Gophna et al. 2001). High frequency of *irp2* in the study area demonstrated that poultry fecal *E. coli* could play a role as a source to transfer *irp2* to competent cells and may be considered for a further study. The high prevalence of this gene may be due to the dietary source which may be iron-rich and

therefore the bacterium in host shows more demand to express iron binding proteins to uptake the element from the environment, although, this hypothesis needs more investigation.

In our study, 5.9 percent of *E. coli* isolated from colibacillosis cases, were evaluated negative for both genes. There are several reports showing that *E. coli* isolated from colibacillosis lacks some of the most important virulence genes (Delicato et al. 2003). These *E. coli* strains, also could be remarked as opportunistic agents that could affect the occurrence and severity of disease due to apt factors. Probably these isolates could cause disease due to suitable disease-associated factors or adverse environmental and cultural conditions and it is possible even they are involved in the transfer cycle of *irp2* gene to other EXPEC strains, like UPEC. On this basis, it is suggested that the role of risk factors and environmental conditions in the intensity of colibacillosis and frequency of virulence genes in APEC be investigated. On the other hand, virulence factors are multifactorial phenomenon with interaction mechanisms. For example, resistance of *E. coli* to serum complement was mediated via several structural factors such as K1 capsule, LPS and outer membrane proteins, including ompT, ISS and ompA (Haghghi Khoshkhoo and Mokhayeri 2012). Thus, a virulence factor like serum resistance may be controlled by more than one gene. It means that the absence of a virulence gene does not lead to the absence of associated phenotype. It implies that different strains of APEC could apply alternative approaches to infect the host (Nakazato et al. 2009). It is recommended that overlay effects and alternatives of these factors and associated genes be investigated regarding to region (Dias da Silveira et al. 2002).

The frequency of the simultaneous presence of both genes in isolated *E. coli* were significantly different ( $p < 0.05$ ). Detection, definition and determination of distribution of molecular pathotypes of APEC in poultry farms of every province of Iran, in different hosts, could be considered as a potential approach to control colibacillosis in the country (Johnson et al. 2008). Our preliminary work used this objective in our study area to find the prevalent molecular pathotype in border region of Iran.

In conclusion, *irp2* and *iss* genes could be considered as main factors for pathogenicity of *E. coli* in the Sistan region and could be used for diagnosis of APEC and act as virulence factors. This research can also be an effort to define the future of these genes as targets for vaccine designation and disease control.

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