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Molecular and phylogenetic characterization of Ornithobacterium rhinotracheale isolates from turkey, quail, partridge and domestic pigeon in Iran

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Abstract

Ornithobacterium rhinotracheale (ORT) is a respiratory pathogen which has been isolated throughout the world from numerous bird species. The present study was designed to investigate molecular and phylogenetic characterization of ORT isolates from turkey, quail, partridge and domestic pigeon. For this purpose, one bacterial strain from each bird species which was isolated in a previous work was compared with chicken and some other bird species isolates. Isolates were streaked on 5% sheep blood agar containing gentamicin. Genomic DNA was extracted from fresh cultures by boiling of bacteria suspension. PCR was performed by using of specific primers for partial amplification of 16S rRNA gene. PCR products of ORT isolates were sequenced in both directions by an automatic sequencer. Our ORT isolates showed high identity (98.1% to 100%) in sequence of 16S rRNA gene to related data in GenBank. Phylogenetic analysis of these sequences showed that Iranian turkey and pigeon isolates were classified into a distinctive cluster with pigeon isolates from Taiwan, while Iranian quail and partridge isolates showed more relationship with the native isolates of chicken as well as some foreign isolates from different sources in the GenBank.

Keywords: *Ornithobacterium rhinotracheale*, turkey, quail, partridge, pigeon, PCR

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Introduction

Ornithobacterium Rhinotracheale (ORT) is a gram negative, pleomorphic, rod shaped bacterium which can infect numerous bird species including chicken, turkey, partridge, pheasant, quail, duck, goose, guinea fowl, gull, ostrich and pigeon (Hafez and Sting, 1999; Soriano et al., 2002; Tsai and Huang, 2006). The ORT was first identified as *Pasteurella*- like bacterium and Taxon 28 until the present name was suggested by Vandamme in 1994 (Chin et al., 2008). Since then, it has been reported from various wild and domesticated birds in diverse countries throughout the world such as Taiwan (Tsai and Huang, 2006), Mexico (Soriano et al., 2002), Brazil (Canal et al., 2005), South Africa (Travers, 1996), Canada, and Germany (Hafez and Sting, 1999). The ORT can be a primary or secondary avian respiratory pathogen. Manifestation of clinical signs or the severity of infection are highly influenced by environmental factors such as poor management, high stocking density, poor hygiene, strain virulence of the pathogen and host factors such as immune statues and concurrent diseases. Other infectious agents of the respiratory tract such as *Escherichia coli*, *Bordetella avium* and Newcastle disease virus have also a triggering effect on the incidence of ORT infections (Schuijffel et al., 2005). Determination of the occurrence of this relatively new pathogen in a population of host species is essential for selecting and implementing appropriate control measures. Infection of some species of birds other than chickens but not about the phylogenetic characterization of ORT isolates has been reported in Iran (Mirzaie et al., 2011). Accordingly, the aim of this study was to investigate molecular and phylogenetic characterization of ORT isolates from commercially reared turkeys, quails, partridge and domestic pigeons.

Materials and Methods

ORT isolates

The ORT which were isolated from commercial turkey, quail, partridge and domestic pigeon by taking tracheal swabs as well as lung and tracheal tissue samples in a previous work of Mirzaie et al. (2011) were used to study for molecular characterization and phylogenetic relationship among ORT isolates from different host species. Isolates were streaked on 5% sheep blood agar containing 10 µg per ml gentamicin (Chin et al., 2008) and incubated for 24 to 48 hrs at 37°C in atmosphere containing 7.5% CO_2. DNA was extracted from fresh cultures by boiling of bacteria suspension at 100°C for 10 min. DNA was extracted from killed vaccine by using of genomic DNA extraction kit (Bioneer, South Korea).

Polymerase chain reaction

Polymerase chain reaction (PCR) was performed in a 25 µl final volume with 5 µl of boiled extract DNA, 2 U of *Taq* polymerase, 12.5 µM KCl, 5 µM Tris- HCl, 0.1 µM of each dNTP, 0.5 µM $MgCl_2$ and 0.2 µM of each specific primer to the 16S ribosomal RNA gene of ORT (Van Empel and Hafez, 1999) (Table 1). Initial denaturation was at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 30s, annealing at 58°C for 60s and extension at 72°C for 90s with a final extension at 72°C for 7 min. The inactivated vaccine and distilled water were used as the positive and negative controls (Hassanzadeh et al., 2010).

Table 1. PCR primers used in this study

Primer	Sequence	Target gene
OR16S- F_1	5- GAG AAT TAA TTT ACG GAT TAA G – 3	16S RNA
OR16S- R_1	5- TTCGCTTGGTCTCCGAAGAT- 3	16S RNA

DNA sequencing

PCR products of four ORT isolates from turkey, quail, partridge and pigeon were sequenced in both directions by an automatic sequencer provided by a commercial sequencing facility (Gen Fanavaran, Tehran, Iran). The nucleotide sequences editing, analysis and alignments were conducted using the BioEdit program (Hall, 1999). The nucleotide sequences had been submitted to GenBank and had been assigned accession numbers HQ696787 for turkey, HQ696786 for quail, HM246652 for partridge and HM234094 for pigeon isolates. Phylogenetic tree was constructed from the 16S rRNA nucleotide sequences, using the neighbor- joining method of MEGA version 4 (Tamura et al., 2007).

Results and Discussion

Polymerase chain reaction, DNA sequencing and analysis

An amplification product of 784 bp could be detectable in all isolates (Figure 1). The sequences of the 16S rRNA fragment showed the identity to be 98.1% to 100% with compare to all GenBank registered ORT isolates. According to the phylogenetic tree, the 16S rRNA sequence of ORT isolates can be divided into three major clusters (Figure 2). Iranian quail, partridge and chicken isolates, in addition to some foreign sequences from chicken, turkey, rook and guinea fowl fell in cluster I, while Taiwanese chicken isolates constituted cluster II. Iranian turkey and pigeon isolates with Taiwanese pigeon isolates, were in cluster III. Based on the 16S RNA nucleotide position of strain LMG 9086T

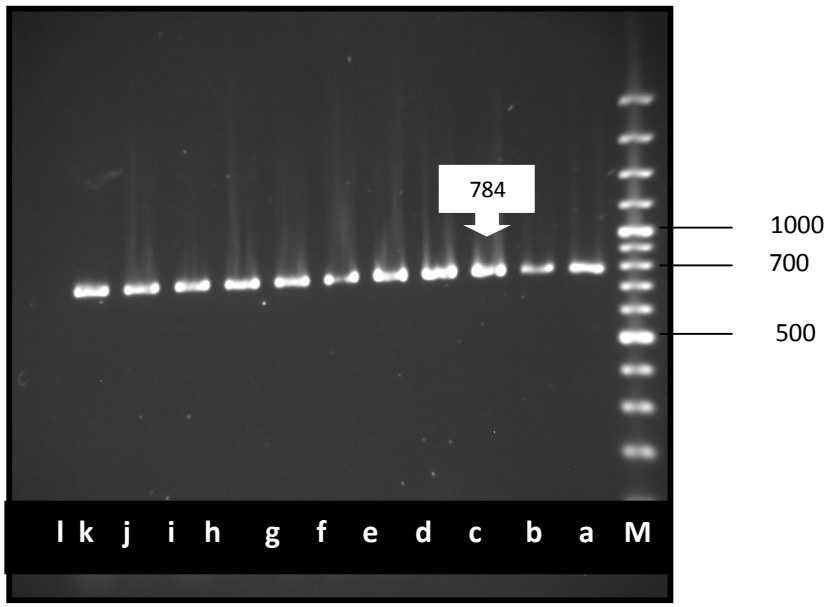

Figure 1. Electrophoresis of PCR products on 1.5% agarose gel stained with ethidium bromide: 100 bp molecular weight marker (lane M), positive control (lane a), amplification products from ORT isolates (lanes b-k) and negative control (lane l).

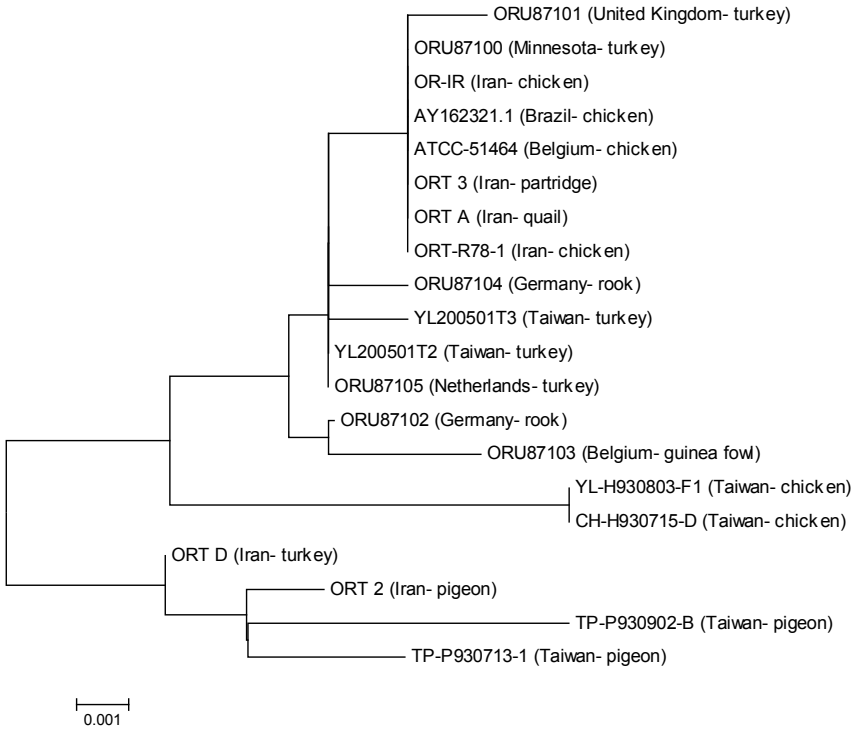

Figure 2. Phylogenic tree by neighbor- joining method constructed from the nucleotide sequences of partial 16S rRNA gene of *Ornithobacterium rhinotracheale*, showing the relationship among turkey, chicken, quail, partridge, pigeon and some other avian isolates.

(accession No. U87101), sequences of cluster I (including Iranian chicken, quail and partridge isolates), differed from sequence of clusters II and III at positions 185 and 568, where respectively G→A and A→G replacements occurred. Nucleotide sequence unique to cluster II are G at position 363 and T at 401, whereas all other sequences of cluster I and III are A and C, respectively. In cluster III (including Iranian turkey and pigeon isolates), at positions 169, 569 and 622, nucleotides are A, T and A, respectively, while at mentioned positions of clusters I and II are G, A and T instead. *Ornithobacterium rhinotracheale* has been isolated from both apparently healthy and sick birds in different studies and has been recognized as additional respiratory pathogen of different bird species, especially turkeys and chickens (Canal et al., 2005; Tsai and Huang, 2006; Roepke et al., 1998; Ozbey et al., 2004). There are several studies about ORT infection in commercial chickens in Iran (Banani et al., 2003 and 2004; Rahimi and Banani, 2007; Asadpour et al., 2008, Hassanzadeh et al., 2010), but there are a few published reports regarding the ORT infection in other susceptible bird species including turkeys, quails, partridges or pigeon in our area (Ozbey et al., 2004).

Based on the sequence analysis of 16S rRNA, the ORT isolates showed identity to the related isolates from GenBank with ranging from 98.1% to 100%. The ORT which isolated from Iranian pigeons with Taiwanese counterparts formed a distinctive cluster however Iranian turkey isolate was also fell to pigeon cluster. Quail isolate showed more genetic relationship with Iranian chicken and partridge isolates and some foreign isolates from different sources in the GenBank. The observed high sequence identity and phylogenetic relationship within the ORT isolates was in agreement with the previous reports and indicated that ORT strains originating from different bird species all over the world constitute a small group of closely related clones (Van Empel and Hafez, 1999). Other researchers by employing SDS-PAGE also confirmed high identities within ORT strains (Banani et al., 2001), However, some variations within ORT isolates were also reported by some genetic investigations especially AFLP and rep-PCR assays (Van Empel and Hafez, 1999; Amonsin et al., 1997).

Conclusion

This study showed that ORT isolates from different sources still show variations in some phylogenetic positions of conserved 16S rRNA gene.

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