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Correlation of *Escherichia coli* Strains Isolated from Wild Bird Feces and Human Urinary Tract Infections from Phylogenetic Point of View

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Abstract

Background: *Escherichia coli* is a typical occupant of the enteric system of vertebrates. Some *E. coli* strains are related to urinary tract infections (UTIs) in human. *E. coli* strains are divided namely to the four phylogenetic groups, A, B_1, B_2, and D. Some investigations have indicated the relationship between phylogenetic characteristics and pathogenicity of E. coli. Thus, determining the phylogeny of unknown *E. coli* strains may be useful in predicting the pathogenesis.
Objectives: In the present study, we aimed to compare the distribution of *E. coli* phylogroups in human UTIs and wild bird feces as a possible source of infection for human in a cross-sectional survey.
Methods: A total of 264 *E. coli* isolates were obtained from human UTIs and feces of wild birds around and phylogenetic determination was carried out using the Clermont Triplex-PCR technique.
Results: Our results showed that phylogenetic group B_2 strains were the most prevalent in UTI cases (47.2%) followed by group D (30.2%). Group B_1 contained 32.5% of the isolates in feces of wild birds, followed by group A (27.5%). There was a significant difference in *E. coli* phylogeny between hosts so that groups B_2 and D were more prevalent in human UTIs and groups B_1 and A in wild birds. Also, when comparing the phylogroups within a host, group B_1 showed a higher rate in wild birds than in human UTIs.
Conclusions: Although the majority of isolates from wild birds belonged to nonpathogenic phylogenetic groups B_1 and A, further research seems to necessary to assess the exact relation of wild birds as pathogen sources for human by genotyping *E. coli* strains via high throughput genotyping assays.

Keywords: Phylogeny, Human UTI, Wild Bird Feces, *Escherichia coli*

1. Background

Few microorganisms act as *Escherichia coli*, an important representative of the enteric microbiota of humans and other animals, because it can be a highly all-around pathogen from beneficial to lethal forms (1). Pathogenic *E. coli* can cause a wide spectrum of human diseases that extent from the enteric sites to extraintestinal areas such as the urinary tract, bloodstream, and central nervous system (2). The prompt source of *E. coli* that is implicated in extraintestinal infections such as urinary tract infections (UTIs) is the enteric lumen of the person and characterization of such strains suggests that environmental sources, perhaps contaminated foods especially with animal origin, can play a role in the local spread of intently associated *E. coli* strains (3, 4). Through the ages, wildlife has been a significant source of infectious diseases transmissible to humans (5). Currently, zoonosis with a wildlife source constitute can act as a remarkable public health problem all over the world. *E. coli* strains exist commonly in the gastrointestinal tract of wild birds and such animals can achieve the mentioned pathogens from infected surroundings and expand it directly to humans or indirectly by contaminating livestock farms (5, 6). *E. coli* strains based on the presence of three genetic markers (chuA, yjaA, and TspE4.C2) are divided in four phylogroups, namely A, B_1, B_2, and D, as follows: subgroup A0 (group A), lacking chuA, yjaA, and TspE4.C2; subgroup A1 (group A), lacking chuA and TspE4.C2 and having yjaA; subgroup B22 (group B_2), having chuA and yjaA and lacking TspE4.C2; subgroup B23 (group B_2), having chuA, yjaA, and TspE4.C2; subgroup D1 (group D), having chuA and lacking yjaA and TspE4.C2; and subgroup D2 (group D), having chuA and TspE4.C2 and lacking yjaA. Group B_2 and, to a lesser degree, group D, harbor the most of virulent extraintestinal *E. coli*, while groups A and B_1 mainly perform commensal, low-pathogenic *E. coli*

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or animal enteric pathogen *E. coli* (7). Based on these observations, we determine the phylogenetic groups of *E. coli* strains isolated from the feces of wild birds and human UTI cases to assess the correlation of such strains from phylogenetic point of view and evaluate whether wild birds can be involved in human urinary tract infections as pathogen sources or not.

2. Methods

Bacterial isolation and identification: 159 *E. coli* isolates from patients (both sexes) aged 20-55 years with UTIs in Semnan, Iran, during 2015 were isolated according to the protocol described by Manges et al. (8) and UTI was defined via the presence of more than two specific clinical symptoms (9). Also 105 *E. coli* strains from wild birds were isolated through cloacal swabs and feces of the birds in Semnan suburbs (40 strains from captured wild birds and 65 strains from free living wild birds, mainly wild Mallard ducks) and bacterial isolation was carried out according to the protocol of Carrillo-Del Valle et al. (10). Isolated strains with biochemically *E. coli* characteristics were stocked in nutrient broth with 15% glycerol at -20°C until genotypic steps.

DNA Extraction and Multiplex PCR experiments: Before DNA extraction, isolated *E. coli* strains were grown up in Luria Bertani agar (LB) plates and incubated at 37°C for 24 hours. Next, genomic DNA was extracted from the isolated strains with alkaline lysis method using NaOH and Tris-Hcl reagents, as described by Staji et al. (11). Then, *E. coli* isolates were phylogrouped to A, B_1, B_2, and D, using an established Triplex-PCR based method by detection of the chuA, yjaA, and tspE4.C2 genetic markers as previously described by Gordon et al. (12).

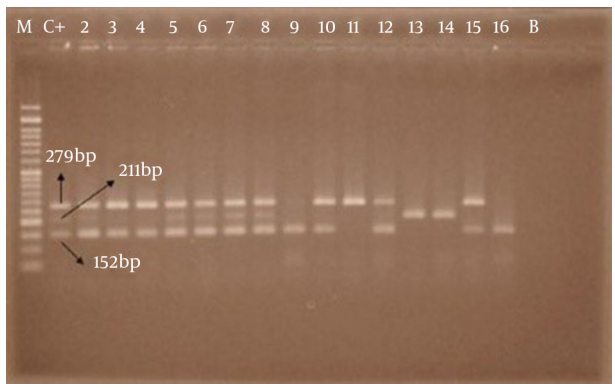
2.1. Statistical Analysis

The present research is a Cross-Sectional study. Phylogenetic distribution within *E. coli* strains of a host and comparison of phylogroups prevalence between the hosts (human and wild birds) were carried out by SPSS 20 using the Chi square test at significance level of $P < 0.05$.

3. Results

The results of detection of phylogenetic markers are shown in Figure 1. Distribution of *E. coli* phylogroups are presented in Table 1. Based on the detection of phylogenetic markers via the mentioned Triplex-PCR, group B_2 was the most prevalent phylogroup within isolates from both human with UTIs and wild birds. In total, 75 isolates (46.9%) from human UTIs belonged to phylogenetic group B_2, followed by group D (48 isolates, 30%), group A (25 isolates, 15.6%), and group B_1 (12 isolates, 7.5%). 34 isolates (32.5%) from wild birds belonged to group B_1, followed by group A (29 isolates, 27.5%), group B_2 (23 isolates, 22%), and group D (19 isolates, 18%). In the case of *E. coli* isolates from human UTIs, the distribution of phylogroup B_2 was significantly higher than that of others groups ($P = 0.00$) within human strains. In addition, the sum of isolates belonging to groups B_2 and D was the most prevalent phylogroups ($P < 0.05$) in overall human isolates, significantly. There was no significant difference between distributions of *E. coli* phylogroups from feces of wild birds. Statistically, the binary correlation of phylogroup B_1 showed higher distribution of group B_1 among isolates of wild birds than among human UTI strains, significantly ($P = 0.00$).

Figure 1. Gel Electrophoresis of Multiplex-PCR Results for the Detection of *E. coli* Phylogroups



M, marker (50 bp); C+, positive control for three genes (ECOR62); 2 -16, *E. coli* isolates harboring different phylogenetic markers (test strains); B, blank as negative control (reactions lacking DNA template).

4. Discussion

This study was planned to find the distribution of phylogroups in *E. coli* strains from human urinary tract infections in Semnan, Iran, and feces of captive and free-living wild birds in this region zoo and suburbs and compare the phylogroups distribution between these hosts. There is much evidence showing that Extraintestinal pathogenic *E. coli* (ExPEC) strains usually exist in groups B_2 and D, and the enteric pathogenic strains are related to groups A, B_1, and D, while the commensal strains exist in groups A and B_1 (11, 13). In the present study, we analyzed phylogenetic group distribution in 264 *E. coli* isolates (159 from human UTIs and 105 from feces of wild birds) and our results showed that in human strains, B_2 is the most preva-

lent group (47.2%) followed by group D (30.2%), and these two phylogroups covered 77.4% of human isolates (Table 1). Clermont et al. (2000) suggested that strains belonging to B_2 and to a lesser extent to D, mainly are related to ExPEC and according to the fact that uropathogenic *E. coli* strains are a subgroup of ExPEC, results of our work are in parallel with the results of these researchers (14). About *E. coli* strains from feces of wild birds, the A (27.5%) and B_1 (32.5%) phylogroups were significantly the most prevalent groups and B_2 (22%) and D (18%) phylogroups presented at lower prevalence rates. It has been concluded that A and B_1 phylogroups are commensal and low pathogenic *E. coli* strains in general; but in some non-human species especially in mammals, these strains can cause enteric infections (7, 14). Although the prevalence of B_2 and D groups in the gastrointestinal tract of wild birds of this area was not dominant, these strains covered 40% of the isolates, implying that these birds can play an important role as a source of these two phylogroups in the infection of other hosts like human (6), especially considering this fact that strains belonging to B_2 and D groups harbor the majority of virulence factors that are related to invasion of *E. coli* to extraintestinal organs and infections while commensal *E. coli* strains usually are derived from A and B_1 phylogroups, lacking specific virulence genes related to B_2 and D phylogenetic groups (13, 15, 16). Within the *E. coli* strains from human UTIs, we found that about 22.6% of these isolates belonged to groups A and B_1, in accordance with the studies of Bingen et al. and Pupo et al., demonstrating that the cause of infection can be intestinal pathogenic *E. coli* and commensal strains (17, 18) and one of the most important causes of infection with commensal agents is poor observation of preventative criteria in people. On the other hand, some investigations show that members of B_1 phylogroup can persist in environment (12) and sometimes these strains carry virulence factors such as shiga-like toxins (stx1 and stx2), which these toxins are implicated in hemolytic uremic syndrome (HUS) caused by some uropathogenic *E. coli* in human. Thus, the strains belonging to B_1 group cannot be ignored and presence and distribution of such bacteria in feces of wild birds can be a potential hazard for humans that are in contact with them (19).

There is much documented evidence showing that extension of *E. coli* phylogroups and virulence genes are not accidental in different hosts, and frequency of phylogenetic groups in hosts is influenced by some factors including host characteristics and food regimen, the antibiotic pressure in each geographic region, ecological differences, body volume, and climate conditions (20, 21). Escobar-Paramo et al. (2006) analyzed *E. coli* strains from fecal samples of birds, human, and other mammals and observed

Table 1. Phylogenetic Analysis of 164 *E. coli* Strains Isolated from Human UTI Cases and Wild Bird Feces[a]

Phylogenetic Group	Frequency of *E. coli* Isolates		
	Human UTIs	Wild bird feces	Both hosts
A	24 (15)	29 (27.5)	53 (20)
B1	12 (7.6)	34 (32.5)	46 (17.5)
B2	75 (47.2)	23 (22)	98 (37.2)
D	48 (30.2)	19 (18)	67 (25.3)
Total	159	105	264

[a]Values are expressed as No. (%).

the significant distribution of phylogroups D and B_1 in birds droplets and A and B_2 in human strains (22). These authors found out that domestication could act as a major force to shape the genetic arrangement of *E. coli* populations in a host. Therefore, observation of some differences in the percentage of phylogroups distribution in *E. coli* strains from wild birds in comparison with the mentioned studies may be related to geographical, domestication, or climate variations or host species between wild birds in the present study and the birds that had been monitored in other investigations. In our study, identification of phylogenetic groups showed that the distribution of B_1 phylogroup in wild bird isolates was significantly higher compared to human isolates ($P = 0.00$) and analysis of data by Phi test showed a negative significant correlation in the distribution of this phylogroup between human UTIs and wild birds isolates.

There are no data available on the distribution of *E. coli* phylogroups and distribution of virulence genes and antimicrobial resistance patterns within these phylogroups in different hosts and regions of Iran. As *E. coli* is one of the major pathogenic agents for human and animals and due to the influence of various environmental factors and host characteristics in shaping the genetic characteristics of *E. coli* population circulating in various hosts of an area, we decided to evaluate the phylogroups similarity in *E. coli* isolates from human UTIs and wild birds of Semnan suburb in Iran as the first step. We found out that distribution of phylogroups in human UTIs resembles to majority of other investigations but phylogroups circulating in wild bird populations are a little bit different from ones in human UTIs especially in the case of B_1 group. Moreover, considering the potential role of *E. coli* in the transmission from wild life to human via various routes, it is quite possible that this less frequent *E. coli* phylogroups (B_2 and D in wild birds of area) may be related to human infections. In conclusion, considering that most of *E. coli* isolates in wild birds of our

study were commensal strains and due to their ability to transmit virulence factors vertically and horizontally, further research to comprehensively diagnose *E. coli* genetic pools in both hosts and their virulence factors in detail as epidemiological data by some high throughput molecular techniques like DNA microarrays seems to necessary to assess exact relationships of isolates from these hosts, and phylogrouping may not be a very good technique to evaluate the relationships of different isolates of *E. coli*.

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