Short Communication
Zoonotic Potential of Canine Uropathogenic Escherichia coli Isolated from Yangzhou, China

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ABSTRACT
Uropathogenic Escherichia coli (UPEC) is a common pathogen of urinary tract infection. To investigate its characteristics and explore the interaction between UPEC and human urinary bladder cancer T24 cells, 7 canine UPEC strains were isolated from dogs in Yangzhou, China. The adhesion-encoding genes (iha, fimH, pap4, papC, papG allele I, papG allele II, papG allele III, focA, focG, fshS), virulence-associated genes (sat, cdiI, cnf1, hlyD), iron uptake system encoding genes (iroN, iut, iroA) were investigated, and the ability of biofilm formation was examined. In addition, UPEC in invading T24 cells, cytotoxicity, immune response and synthesis of proinflammatory cytokines were discussed. The results showed that fimH was 100% detected, followed by iut, iroN, and focA, with the detection rates of 71.4, 57.1 and 57.1% respectively; while papG allele I, papG allele II, papG allele III, cdiI, sat, fshS, iha and sat were not detected. Most of the isolated UPEC strains have strong virulence on T24 cells and could induce strong immune responses. Taking these data together, canine UPEC strain may not be a canine specific pathogen, but has a certain potential for zoonosis.

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counts ≥ 10^5 colony-forming units (CFU)/mL, strains were inoculated on MacConkey agar (Hopebio, Qingdao, China) and cultured at 37°C for 24 h. Metallic green colonies were presumptively considered as indicators of E. coli. The strains were identified by bacterial 16S rDNA PCR Kit (TaKaRa, Dalian, China) (Hu et al., 2014), and were preserved as the isolated UPEC strains.

UPEC related virulence genes were detected by PCR using primers (Supplementary Table SI) (Nam, 2013), including adhesion-encoding genes (iha, fimH, papA, papC, papG allele I, papG allele I', papG allele II, papG allele III, focA, focG, sfaS), virulence-associated genes (sat, cdtI, cnfI, hlyD), iron uptake system encoding genes (iroN, iut, ire4).

For biofilm formation test, the strains were inoculated in LB broth liquid medium at 30°C in a shaking incubator overnight. The next day, the bacteria solution was adjusted to make OD_{600} = about 1.0, added in 96 well plates for 150 μL per well for inoculation in biofilm induction medium at the ratio of 1:100. After incubated at 30°C for 48-72 h, each well was rinsed for 3 times by PBS, then 200 μL 2% crystal violet was added for dyeing. After 15 min, each well was gently rinsed with distilled water for 3 times, and then 250 μL 95% ethanol solution was added. The values OD_{600} of each well were measured by a multifunctional microplate reader (Duan et al., 2013). All procedures were repeated 3 times, and 6 parallel sets for each strain at a time.

To perform the cytotoxicity assays, T24 cells were inoculated on 96 well plates at 3×10^4 CFU per well and cultured in RPMI 1640 medium (containing 10% fetal bovine serum) at 37°C with 5% CO2 for 24 h. Each isolated strain, 4 strains had strong biofilm forming ability (No. 3, 4, 5 and 7). Compared with the negative control DH5α, the biofilm forming ability was increased by 7.5, 8.6, 8.8 and 7.1 times, respectively (P<0.05).

To perform the invasion assays, the bacteria were inoculated with T24 cells at the MOI of 0.01 for 2 h, then were washed with PBS for 3 times immediately, and antibiotics (50 μg/mL gentamicin) was added to further incubate for 2 h. Then 0.5% Triton X-100 was added for 20 min to release the bacteria that had invaded the cells. After diluting the culture medium, samples were evenly spread on LB medium plate and cultured overnight at 37°C, then bacteria on plates were counted, and DH5α was used as the negative control.

The isolated bacteria were incubated with T24 cells at the MOI of 1:100 for 2 h, then the total RNA was extracted using Trizol reagent (Takara, Dalian, China) (Yang et al., 2013). The primers for pro-inflammatory factors il-8 and tnf-a were listed in Table S1. Gene gapdh was used as the housekeeper gene, and SYBR® Premix Ex Taq™II (Takara, Shiga, Japan) was employed for subsequent fluorescence quantitative test. Data were collected by ABI 7500 Real Time System (Applied Biosystems, Foster City, CA, USA), and were processed by 2^{△△Ct} method.

**Results**

Out of the 37 strains of bacteria isolated from urine samples 7 were identified as UPEC, accounting for 18.9% of the isolates. UPEC usually carries a series of virulence factors, which adhere to host cells and invade the host. The detection rate of fimH was 100%, followed by iut, iroN, and focA, with the detection rates of 71.4, 57.1 and 57.1% respectively. The following genes, including papG allele I, papG allele I’, papG allele II, papG allele III, cdtI, sat, sfaS, iha and sat, were not detected (Table 1). The ability of UPEC strains to biofilm formation was detected by crystal violet staining (Duan et al., 2013) (Fig. 1A). Among the isolated strains, 4 strains had strong biofilm forming ability (No. 3, 4, 5 and 7). Compared with the negative control DH5α, the biofilm forming ability was increased by 7.5, 8.6, 8.8 and 7.1 times, respectively (P<0.05).

The results of the invasion assays showed that compared with the control, all the isolated strains showed stronger cell invasive ability, and the number of T24 cells invaded by each UPEC strain increased by 2-13 times (Fig. 1B). The data showed that isolates 1, 2 and 7 were less invasive. At the same time, WST-1 Kit was used to test the cytotoxicity of UPEC to T24 cells. Compared with the control, the number of viable cells in each isolated group decreased by 50-70%, indicating that most of the isolated strains had strong cytotoxicity against T24 cells (Fig. 1C).

To further analyze the changes in expression level of proinflammatory cytokines after the immune response of T24 cells to UPEC strain, the transcription levels of IL-8 and TNF-α cytokines were detected by quantitative
fluenzer PCR (Fig. 1D). Compared with the control, the IL-8 transcription level of UPEC increased 49–91 times, the level of TNF-α transcription increased 69–154 times. Under the stimulation of each strain, the transcription levels of IL-8 and the cytokines TNF-α in T24 cells increased significantly (Yang et al., 2013, 2018).

Table I. Distribution of virulence genes in canine UPEC isolates.

<table>
<thead>
<tr>
<th>Virulence genes</th>
<th>Isolates</th>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>papA</td>
<td>+</td>
</tr>
<tr>
<td>papC</td>
<td>-</td>
</tr>
<tr>
<td>papG allele I</td>
<td>-</td>
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<tr>
<td>papG allele II</td>
<td>-</td>
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<tr>
<td>papG allele III</td>
<td>-</td>
</tr>
<tr>
<td>focA</td>
<td>+</td>
</tr>
<tr>
<td>focG</td>
<td>-</td>
</tr>
<tr>
<td>sfaS</td>
<td>-</td>
</tr>
<tr>
<td>iha</td>
<td>-</td>
</tr>
<tr>
<td>fimH</td>
<td>+</td>
</tr>
<tr>
<td>cdI</td>
<td>-</td>
</tr>
<tr>
<td>sat</td>
<td>-</td>
</tr>
<tr>
<td>cnf1</td>
<td>-</td>
</tr>
<tr>
<td>hlyD</td>
<td>-</td>
</tr>
<tr>
<td>ireA</td>
<td>-</td>
</tr>
<tr>
<td>iroN</td>
<td>+</td>
</tr>
<tr>
<td>iut</td>
<td>+</td>
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*, positive; -, negative

Discussion

E. coli is the main pathogenic factor of UTI in human and livestock, animal derived E. coli has zoonotic potential for human infection. This study identified the virulence genes, biofilm forming ability and the biological characteristics of the 7 isolated canine UPEC, and further discussed UPEC in invading T24 cells, cytotoxicity, immune response and synthesis of proinflammatory cytokines.

UPEC usually carries a series of virulence factors, which adhere and invade the host cells (Mulvey et al., 1998; Nagamatsu et al., 2015; Leatham-Jensen et al., 2016). In the process of infection, UPEC can resist the scouring of various body fluids, especially urine, by adhering to the host cells. Therefore, adherence is a crucial aspect of UPEC infection ability. The virulence factors related to UPEC adherence include T1F, P fimbriae and so on. T1F is usually associated with descending urethral infection, and P fimbriae are mainly related to ascending urethral infection. The acquisition of iron is the key condition for UPEC to survive in urinary iron restricted environment.

Biofilm forming ability is one of the virulence determinants of chronic and recurrent bladder infections associated with UPEC (Dhakal et al., 2008; Cai et al., 2013; Flores-Mireles et al., 2015; Spaulding and Hultgren, 2016; Sharma et al., 2016; Terlizzi et al., 2017). Biofilm is mainly composed of polysaccharides, proteins, nucleic acids and lipids, which constitute a direct environment for bacterial growth. The drug tolerance of bacterial biofilm is usually 10–1000 times that of planktonic bacteria, which is one of the main reasons for the failure of antibiotic treatment. T1F plays an important role in the formation of biofilm. Recent studies have shown that biofilm formation is an important strategy for E. coli colonization in the urinary tract (Soto et al., 2007; Mabbett et al., 2009). Among the isolated strains, 4 strains had strong biofilm forming ability, which was 7.5, 8.6, 8.8 and 7.1 times higher compared with DH5α, that can greatly improve the survival ability in different hosts and effectively improve the pathogenicity.

Epithelial cells are the first line of defense against invading pathogens, they also help to initiate the host’s innate and adaptive immune response by producing chemokines, cytokines and antimicrobial peptides (Wood, 2009; Gibson et al., 2010; Nakamura et al., 2016; Shenagari et al., 2018). The immune response to UPEC begins with bacterial invasion of bladder epithelial cells, which induces the production of pro-inflammatory cytokines and recruits neutrophils to the site of infection. A study on mouse cystitis model showed that UPEC can persist in mouse bladder cells to resist antibiotic treatment. Recent studies showed that UPEC can persist in the host epithelial cells for many days and reproduce rapidly in the cells, suggesting that the ability of UPEC to invade epithelial cells plays a key role during UTI (Xia et al., 2011). This study found that most of the isolated UPECs had strong ability to invade T24 cells, suggesting the invasive infection of UPEC does not have strict host specificity. Similarly, most of the isolated UPEC strains have strong cytotoxicity and can induce epithelial cells to form a strong host immune response, which is essential for defense against UTI. This result is consistent with previous study (Nam, 2013).

Conclusion

In conclusion, 7 canine UPEC strains were isolated from Yangzhou, the virulence factors and biofilm formation ability were identified. The results showed that the canine UPEC strains could invade T24 cells, induce cytotoxicity and activate the synthesis of proinflammatory cytokines in epithelial cells, indicating that canine UPEC has a certain potential of zoonosis.

Acknowledgement

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Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/2018.50.5.1807-1813

Statement of conflict of interests

The authors have declared no conflict of interest.

Reference


Supplementary Material

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Supplementary Table SI. The primers used in this study.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Sequence (5’-3’)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| papA  | F:ATGGCAGTGGTGTTTCTTTTGGTG  
R:CGTCCACATACGCTGCTTTTC  | (Johnson and Stell, 2000) |
| papC  | F:GTGGGCAAGATGTAATGACCGTTA  
R:ATATCCTTTTCGACAGGATGCAATA  | (Le Bouguenec and Archambaud et al., 1992) |
| papG allele I | F:TCGTGTCATCAGGCGGAAATT  
R:GCCAGAATAGCTACTAATGAACCCG  | (Mitumori and Terai et al., 1998) |
| papG allele I’ | F:CTACATATAGTTATGCTGCTGTATC  
R:CTGACATCTTCACATTATATCGA  | (Johnson and Stell, 2000) |
| papG allele II | F:GGGATGAGCGGGGCCATTGAT  
R:CGGGGCCCACAGTAGGTACTG  | (Ghanbarpour and Akhtardanesh, 2010) |
| papG allele III | F:GGCCTGCAATGGATACCTGG  
R:CCACCAATTAGCATGCACAC  | (Johnson and Stell, 2000) |
| focA  | F:ATGCCGCTCATGCTGACCGGG  
R:GGGGCTGGCTGCTGGGGAAGAC  | (Ghanbarpour and Akhtardanesh, 2010) |
| focG  | F:CGACAGAGGGCAATGGATACCA  
R:GAAATGCTGCCTGCCCAATGCTTTC  | (Johnson and Stell, 2000) |
| sfaS  | F:GTGGATACGATCATCAGCTACTGGT  
R:CCGCCAGCATTCCCTGTATTTC  | (Johnson and Stell, 2000) |
| iha   | F:CTGGCCGAGGCTGATGGAGATCA  
R:TCTTTAAGCTGCCGGCGGGCTGA  | (Ghanbarpour and Akhtardanesh, 2010) |
| fimH  | F:TGCAGAACGGATAAGCCGGTGG  
R:GCACTACCTGCCTCGCCGTA  | (Johnson and Stell, 2000) |
| cdtI  | F:GAAATATATGGAACACATGTTCG  
R:AAATCTCCTGCAATCATCCAGTTA  | (Ghanbarpour and Akhtardanesh, 2010) |

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<table>
<thead>
<tr>
<th>Genes</th>
<th>Sequence (5'→ 3')</th>
<th>Reference</th>
</tr>
</thead>
</table>
| sat    | F:GCAGCTACCGCAATAGGAGGT  
R:CATTCAGAGTACCGGGGCCTA | (Ghanbarpour and Akhtardanesh, 2010)                                      |
| cnfl   | F:ATCTTATATCTGGATGGGATCATCTTTGG  
R:GCAGAAGAGCTTTCATAAAGTAT | (Ghanbarpour and Akhtardanesh, 2010)                                      |
| hlyD   | F:CTCCGGTACGTGAAAAGGAC  
R:GCCCTGATTACTGAAGCCTG | (Johnson and Stell, 2000)                                                 |
| ireA   | F:GATGACTCAAGCCAGGGTGAA  
R:CCAGAACTCACCTACGAAT | (Ghanbarpour and Akhtardanesh, 2010)                                      |
| iroN   | F:AAATCAAGCGAGGTGTTGCCC  
R:GACGCGCAGCATTAAGACGCAG | (Ghanbarpour and Akhtardanesh, 2010)                                      |
| iut    | F:GGCTGGACATCATGGGAACTGG  
R:CGTCGGGAACGGGTAGAATCG | (Ghanbarpour and Akhtardanesh, 2010)                                      |
| il8-RT | F:TGCAAGCTGTGTAAGGTTG  
R:ACTTCTCCACAACCCCTGTC | (Yang and Zhou et al., 2014)                                             |
| tnf-α-RT | F:CCCCAGGGACCTCTCTCTAATC  
R:TGAGGTACAGGCCCTCGTAT | (Yang and Zhou et al., 2014)                                             |
| GAPDH-RT | F:GAATGGCGCTGAAGCATGAG  
R:GAGGATCTGAGCATCTTTG | (Yang and Zhou et al., 2014)                                             |

References


