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Extra-intestinal pathogenic *Escherichia coli* from human and avian origin: Detection of the most common virulence-encoding genes

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Abstract

Pathogenic *Escherichia coli* strains cause a wide range of extra intestinal infections including urinary tract infection in humans and colibacillosis in poultry. They are classified into uropathogenic *E. coli* (UPEC) and avian pathogenic *E. coli* (APEC) with genetic similarities and variations. Their pathogenicity is related to the virulence-encoding genes like *sfa*, *papG II*, *ompT*, *iutA,* and *iss* with zoonotic potentials. One hundred isolated *E. coli* from patients with urinary tract infection and 100 *E. coli* from chickens with colibacillosis were evaluated for the presence of the most common virulence-encoding genes including *sfa, papG II, ompT, iutA,* and *iss* by multiplex polymerase chain reaction. While the frequency of *sfa, papG II, ompT, iutA* and *iss* encoding genes in APEC isolates were respectively 0.00%, 67.00%, 63.00%, 89.00% and 89.00%, the frequency of these encoding genes in UPEC isolates were 18.00%, 40.00%, 40.00%, 74.00% and 48.00%, respectively. Except for *sfa*, the frequencies of other encoding genes in APEC were more than those in UPEC isolates. The *iutA* as the most common UPEC encoding gene and *iss* as the most common APEC encoding gene were the most prevalent virulence factors in the examined *E. coli* isolates. Finding out the distribution of virulence-associated genes could be helpful to identify similarities and differences between APEC and UPEC isolates in order to provide more substantial evidence of their common virulence traits and potential zoonotic threats.

اشریشیا کولای خارج روده ای بیماریزا با منشاء انسان و پرندگان: شناسایی معمولترین ژن‌های کدکننده حدت

چکیده

سویه های بیماریزای *اشریشیا کولای*، در ایجاد طیف وسیعی از عفونت های خارج روده ای از جمله عفونت دستگاه ادراری در انسان و کلی باسیلوز در طیور نقش دارد. این سویه ها به *اشریشیا کولای* بیماری زای ادراری (UPEC) و *اشریشیا کولای* بیماری زای پرندگان (APEC) براساس تشابهات و تنوع ژنتیکی تقسیم می شوند. بیماریزایی آن ها به ژن های کدکننده حدت نظیر *sfa*، *papG II*، *ompT* و *iutA* و *iss* با قابلیت های بیماری زایی مشترک بستگی دارد. تعداد ۱۰۰ جدایه *اشریشیا کولای* جدا شده از بیماران واجد عفونت دستگاه ادراری و ۱۰۰ جدایه از جوجه های مبتلا به کلی باسیلوز با استفاده از واکنش زنجیره ای پلیمراز چندگانه از نظر وجود معمولترین ژنهای کدکننده حدت شامل *sfa*، *papG II*، *ompT*، *iutA* و *iss* مورد ارزیابی قرار گرفتند. در حالی که فراوانی ژن های *sfa*، *pap GII*، *ompT*، *iutA* و *iss* در جدایه های APEC به ترتیب ۰/۰۰، ۱۷/۰۰، ۶۳/۰۰، ۸۹/۰۰ و ۸۹/۰۰ درصد بود، فراوانی این ژن‌های کدکننده در جدایه های UPEC به ترتیب ۱۸/۰۰، ۴۰/۰۰، ۴۰/۰۰، ۷۴/۰۰ و ۴۸/۰۰ درصد بود. به جز ژن *sfa* فراوانی های سایر ژن های کدکننده در APEC بیشتر از جدایه های UPEC بود. ژن کد کننده *iutA* به عنوان معمولترین ژن کننده UPEC و ژن کد کننده *iss* به عنوان معمولترین ژن کننده APEC شایع ترین عوامل حدت در جدایه های *اشریشیا کولای* مورد آزمایش بودند. پی بردن به توزیع ژنهای مرتبط با حدت می تواند به شناخت تشابهات و اختلاف بین جدایه های APEC و UPEC در راستای فراهم شدن شواهد محکم تری از ویژگی های حدت و تهدید های بیماری زایی مشترک بالقوه معمول آنها کمک کند.

واژه های کلیدی: اشریشیا کولای، ژنهای کدکننده حدت، عفونت دستگاه ادراری، کلی باسیلوز پرندگان

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Introduction

Escherichia coli is the most significant Gram-negative facultative anaerobic bacterium with a wide range of pathogenicity and genotypic diversity.[1] It is a commensal bacterium in the gut of humans and other vertebrate and is responsible for a wide range of intestinal and extra-intestinal infections.[2] Accordingly, it is divided into two pathotypes of intestinal pathogenic *E. coli* and extra-intestinal pathogenic *E. coli* (ExPEC). Each pathotype is divided into sub-pathotypes based on certain common traits of host specificity, pathogenesis mechanism, and infected organ.[3] For example, serogroups named O18 with large virulence plasmids and K1 capsular antigen cause meningitis.[4-6] It is originated from human neonates and named as neonatal-meningitis *E. coli*. Human uro-pathogenic *E. coli* (UPEC) lacking virulence plasmids tends to be of the O2 and O6 serogroups.[6] Similarly, avian pathogenic *E. coli* (APEC), responsible for poultry coli-septicemia, possesses large virulence plasmids and great diversity in serogroups like O1, O2, and O78.[7,8]

Urinary tract infections (UTIs) are among one of the highest medical costs bacterial infections affecting healthcare, with 150 million cases occurring annually worldwide.[9] The virulence-associated factors involved in the establishment of UTIs enable them to survive and invade noxiously to the host tissues, induce the inflammatory response via disruption of the defense mechanisms and finally cause a variety of diseases even out of the urinary tract.[10] Another virulence trait of this organism is due to the expression of a wide spectrum of antimicrobial resistance genes.[11]

Avian pathogenic *E. coli* induces multi-target infection in broilers called colibacillosis with large losses to the poultry industry. Its disseminated infection induces fibrinous lesions in internal organs from air sacs and pericardium to peritonea associated with septicemia.[12,13]

According to the relationship between UPEC and APEC genes for virulence and pathogenicity, the zoonotic risk of isolated avian *E. coli* should be considered.[14-16] Despite the variable virulence gene profile engaged in the pathogenicity of APEC and human ExPEC, the similarities between these two strains may also share a common ancestor.[17] A lot of studies were done to find serogroup similarity and link between genotype of virulence factors between APEC and UPEC.[18] Common reported virulence-associated genes between APEC and UPEC include iron-limited urinary tract (*iutA*) related to iron acquisition systems, increased serum survival (*iss)*, S-fimbrial adhesion (*sfa*), pyelonephritis-associated pili (*papG II*) and outer-membrane protein T (*ompT*) genes showing their common traits.[10] Additionally, the prevalence of wide serological diversity was revealed in several studies with particular combinations of virulence-associated genes among APEC strains.[6,8,19] Different virulence assays were mentioned in APEC with various similarities to the UPEC. The most prevalent genes in *E. coli* isolated from poultry colibacillosis in Iran were *hly F*, *ompT*, *iss*, *iutA* and *pap G II*.[20] While *tsh*, *iss*, *astA*, *iucD*, *vat,* and *papC* were the most frequent virulence genes isolated from APEC, other genes including *fim*, *aaadA1* and *qnr* have been reported as the most virulence genes isolated from UPEC.[21,22] Likewise, the clonal relations between some APEC and human ExPEC strains have been demonstrated.[23-25] Also, serotype O18:K1:H7 isolated from avian has been reported to have pathogenicity for human.[26]

Epidemiological studies have showed genetic similarity and variation between *E. coli* isolated from poultry and human.[27,28] In order to get distribution of virulence-associated genes in UPEC and APEC strains, we gathered a relatively large collection of them and made a systemic comparison between the prevalence of their virulence-encoding genes. The aim of this study was to find out the distribution of virulence-associated genes to identify the similarities and differences between these groups in order to provide more substantial evidence of their common virulence traits and potential zoonotic threats.

Materials and Methods

Sampling and bacterial culture. A total of 200 *E. coli* isolates were studied in this survey. They were isolated from the pericardium of broiler chickens suffering or died of colibacillosis (n = 100) and from the urine samples of the patients suffering from urinary infection (n = 100). The urine isolates were collected from the patients having at least 10^5 colonies forming unit of a bacterium per milliliter of urine. The isolates were routinely grown in eosin methylene blue agar (Merck, Darmstadt, Germany). After confirming the bacteria like *E. coli* by biochemical tests, each one was incubated in trypticase soy broth (TSB; Merck) for 18 hr at 37 ˚C. Then, each culture was centrifuged at 3000 *g* for 15 min. After discarding the supernatant, 1.00 µL of the bacterial pellet with 1.00 µL of glycerol and 9.00 µL of TSB were cast in a microtube and stored at – 20 ˚C for the following steps.

DNA extraction. The bacterial samples were mixed with lysis buffer (Bio-Rad, Watford, UK) and incubated for 10 min in a water bath at 55 ˚C. Proteinase K (Sigma-Aldrich Co. Ltd., Dorset, UK) was added into each micro tube, vortexed carefully and incubated at 55 ˚C for 20 min. In the next step, binding buffer was added and incubation was done again at 70 ˚C for 10 min. After adding pure ethanol (Merck) and vortexing, the microtubes were then centrifuged at 8000 *g* for 1 min. Briefly, by using washing buffer and then centrifuging them, the ethanol was cleaned up. Finally, the elution buffer was used followed by multistage centrifuge to purify the extracted DNA.

Gene amplification. The *E. coli* specific gene *uidA* and virulence-associated genes including *sfa*, *papG II*, *ompT*,

iutA, and *iss* were detected by multiplex polymerase chain reaction (PCR) assay. The oligonucleotide sequences of primers used for amplifications of the target genes are shown in Table 1. In PCR run, two virulence genes were amplified in a 30.00 µL reaction mixture. The mixture was included Taq DNA Polymerase Master Mix (12.50 µL), Ampliqon (Odense, Denmark), template DNA (8.00 µL) and each 2.00 µL of primes (Metabion international AG, Planegg, Germany) supplemented with 5.50 µL distilled water. The pure *E. coli* culture (ATCC 10536) harboring target genes was obtained from Tehran University, Tehran, Iran and used as a positive control and the mixture of Taq DNA Polymerase Master Mix and distilled water was used as a negative control. Each tube reaction mixture was subjected to denaturation (94 ˚C for 30 sec), annealing (58 ˚C for 30 sec) and extension (72 ˚C for 90 sec), followed by one cycle consisting of 5 min at 72 ˚C in a thermal cycler (ABI 2720; Applied Biosystems, Vilnius, Lithuania). After gene amplification, every PCR product was run on horizontal gel electrophoresis with 2.00% agarose gel, stained with SYBER safe (SinaClon, Tehran, Iran) and illuminated by ultraviolet exposure.

Statistical analysis. In order to compare the frequency of virulence genes, Chi-square test and to investigate the relationship between the frequency of genes, Chi-square test, and Spearman correlation index were used. All analyses were performed with SPSS (version 18.0; SPSS Inc., Chicago, USA) and p value < 0.05 was the threshold for significance.

Table 1. The sequence of primers used for amplifications of *uidA, sfa, papG II, ompT, iutA,* and *iss* encoding genes.

Genes	Oligonucleotide sequence	Expected size (bp)	Reference
uidA	F: tggtaattaccgacgaaaacggc R: acgcgtggttacagtcttgcg	147	29
sfa	F: ctccggagaactgggtgcatcttac R: cggaggagtaattacaaacctggca	410	10
papG II	F: gggatgagcgggcctttgat R: cgggcccccaagtaactcg	190	10
ompT	F: atctagccgaagaaggaggc R: cccgggtcatagtgttcatc	559	10
iutA	F: ggctggacatcatgggaactgg R: cgtcgggaacgggtagaatcg	302	10
iss	F: cagcaacccgaaccacttgatg R: agcattgccagagcggcagaa	323	10

Results

Figure 1 shows the electrophoresis of the PCR products. While the isolates from poultry colibacillosis (P1-P7) and human UTIs (H1-H7) were *uidA* positive, the samples of P2-P7, H1, H2, H6, and H7 were also *ompT* positive (Fig. 1A). The P1-P6, P8, P10-P14, H1-H4, H7, H8, and H11-H13 isolates were positive for the *iutA* gene (Fig. 1B). While H1 and H8 contained *sfa*, none of the isolated strains from poultry colibacillosis contained this virulence gene.

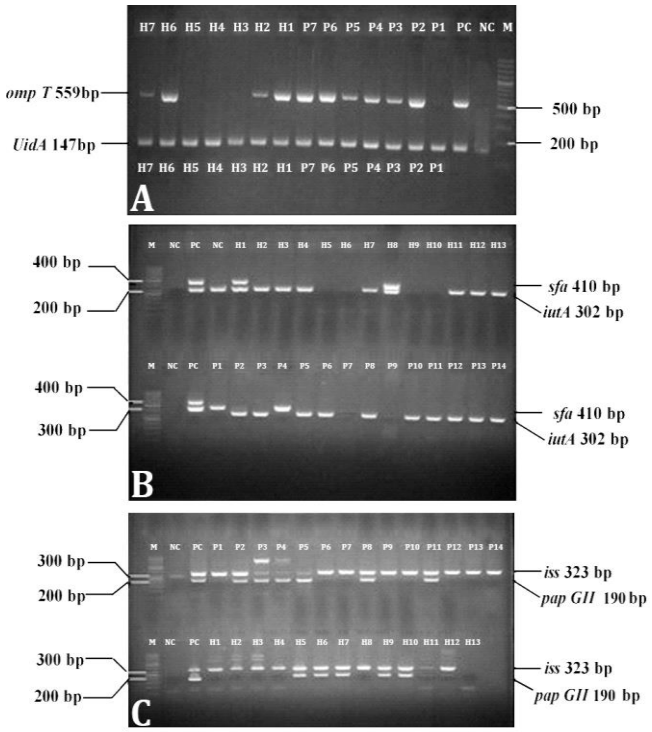

Fig. 1. Gene-specific polymerase chain reaction product electrophoresis to evaluate *uidA* and *ompT* (A), *sfa* and *iutA* (B) and *iss* and *papG II* (C) genes. M: Marker; PC: Positive control; NC: Negative control; P: *E. coli* strains isolated from poultry colibacillosis; H: *E. coli* strains obtained from human urinary infection.

The electrophoresis of genes products of *iss* and *papG II* from colibacillosis and human UTIs is shown in Figure 1C. The isolates P1, P2, P6-P14, H1-H10, and H12 contained *iss* gene. The *papG II* was present in P2-P5, P8, H5-H7, H9, and H10 isolates.

The frequency of the *sfa*, *pap GII*, *ompT*, *iutA* and *iss* genes in *E. coli* strains isolated from APEC and UPEC is shown in Table 2. Except for *sfa* gene, the frequency of other genes from APEC was significantly higher than that of UPEC (p < 0.05). According to Table 3 summarizing the virulence- encoding genes in APEC and UPEC, most isolates of APEC and UPEC had three (40.00%) and two (44.00%) virulence-encoding genes, respectively. None of them had all five virulence-encoding genes.

Table 2. The frequency (%) of virulence encoding genes in avian pathogenic *E. coli* (n = 100) and human uropathogenic *E. coli* (n = 100).

Genes	APEC[1] isolates	UPEC[2] isolates	*p value*
sfa	0	18	0.000*
papG II	67	40	0.000*
ompT	63	40	0.001*
iutA	89	74	0.006*
iss	89	48	0.000*

* indicates significant differences between APEC and UPEC isolates at p < 0.001.

[1]Avian pathogenic *Escherichia coli;* [2] Human uropathogenic *E. coli*.

Table 3. The frequency of avian pathogenic *E. coli* (n = 100) and human uropathogenic *E. coli* (n = 100) in terms of the number of virulence encoding genes.

Isolates	Number of genes					
	0	1	2	3	4	5
	Frequency (%)					
APEC[1]	1	4	18	40	37	0
UPEC[2]	2	36	44	12	6	0

[1]Avian pathogenic *Escherichia coli;* [2] Human uropathogenic *E. coli.*

Table 4 shows the simultaneous presence of two virulence-encoding genes in APEC and UPEC isolates. The correlation between the frequency of virulence- encoding genes between APEC and UPEC isolates is also listed. While in UPEC isolates, between *sfa*, *iss*, *iutA*, *iss* and *papG II* a significant negative and between *sfa* and *papaG II* a significant positive correlation was observed, no correlation was seen between any of these genes in APEC isolates.

In Table 5, the results of the simultaneous presence of three and four genes in APEC and UPEC isolates are shown. Fifty-three percent of APEC isolates had *iss*, *iutA,* and *papG II* and 53.00% percent of APEC isolates had also *iss*, *ompT* and *iutA* simultaneously. While 39.00% of APEC isolates had *iutA*, *papG II* and *ompT* together and 41.00% had *iss*, *ompT,* and *papG II* simultaneously, in UPEC isolates only one or two percent of strains had these genes together. Thirty-seven percent of UPEC isolates had four genes of *papG II*, *iutA*, *ompT* and *iss* in common.

Table 5. The frequency (%) of avian pathogenic *E. coli* (n = 100) and human uropathogenic *E. coli* (n = 100) in terms of carrying three and four virulence encoding genes.

Virulence encoding genes	APEC[1]	UPEC[2]
sfa, iss, ompT	0	0
sfa, papG II, iss	0	2
iutA, iss, sfa	0	0
iss, ompT, papG II	41	2
iss, ompT, iutA	53	1
iutA, papG II, ompT	39	2
sfa, ompT, iutA	0	1
iss, iutA, papG II	53	1
ompT, papG II, iss	0	1
papG II, iutA, sfa	0	1
sfa, iss, ompT, papG II	0	1
papG II, iutA, ompT, iss	37	1
iutA, ompT, iss, sfa	0	0
iutA, ompT, papG II, sfa	0	3
iss, papG II, iutA, sfa	0	0

[1]Avian pathogenic *Escherichia coli;* [2] Human uropathogenic *E. coli.*

Discussion

Avian pathologic *E. coli* is one of the most bacterial cause of infectious diseases in poultry and the main cause of poultry colibacillosis. This infection is responsible for great losses in the poultry industry.[1-3,30] Recently, the zoonotic potential of APEC strains was considered by some researchers.[19,31] In several studies, the phylogenetic, genotypic and serotype relation between APEC strains and extra-intestinal *E. coli* strain in human, like UPEC, has been demonstrated. Similar virulence factors with the same mechanism between APEC and UPEC strains have been concluded.[10,26,32]

In the present study, we evaluated the frequency of *sfa*, *papG II*, *ompT*, *iutA* and *iss* as virulence-encoding genes of *E. coli* and identified four genes including *papG II*, *ompT*, *iutA*, and *iss* in both APEC and UPEC. The similar result was reported previously.[10]

These results evidently have concluded the relation between APEC and UPEC and confirmed the previous knowledge of the role of certain genes specified to a particular host (human versus avian and/or urinary tract versus respiratory tract).[33,34] The frequency of virulence-related genes in APEC including *sfa*, *papG II*, *ompT*, *iutA* and *iss* in our study was respectively 0.00%, 67.00%, 63.00%, 89.00% and 89.00% versus 2.00%, 43.00%, 60.00%, 90.00% and 81.00% in the report of Zhao *et al*.[10] In another study, *iutA*, *iss* and *papG II* genes had the frequencies of 50.00%, 40.00% and 15.20%, respectively.[35] It was also reported that more than 97.00% of APEC had *sfa* and *fim* virulence-encoding genes.[26] In our study, none of the APEC isolates had *sfa*, which is in agreement with Zhao *et al*.[10] and is in contrast with Moulin-Schouleur *et al*.[26] Among the genes examined for this study, *iutA,* and *iss* as virulence factors encoding genes were detected in the

Table 4. The frequency (%) of avian pathogenic *E. coli* (n = 100) and human uropathogenic *E. coli* (n = 100) in terms of carrying two virulence encoding genes.

Genes	APEC[1]	Spearman correlation index	*p value*	UPEC[2]	Spearman correlation index	*p value*
ompT, iss	57	0.03	0.70	17	-0.09	0.30
papG II, iss	60	0.06	0.50	11	-0.27†	0.007
iutA, iss	80	0.06	0.50	12	-0.31†	0.002
iss, sfa	0	-	-	5	-0.26*	0.01
sfa, ompT	0	-	-	6	-0.26	0.40
sfa, papG II	0	-	-	12	0.74†	0.007
sfa, iutA	0	-	-	8	0.27	0.10
ompT, papG II	23	0.10	0.10	16	0.04	0.60
ompT, iutA	62	0.03	0.70	14	-0.85	0.40
papG II, iutA	60	0.00	1.00	14	-0.85	0.40

*† indicate significant differences between APEC and UPEC isolates at $p < 0.05$, $p < 0.001$, respectively

[1] Avian pathogenic *Escherichia coli;* [2] Human uropathogenic *E. coli*.

majority of APEC isolates which was similar to the other reports.[10,26,35] These results were in contrast with other study reporting *papG*, the virulence genes coding adhesion, had the highest frequency among the other virulence genes.[27]

In the present study, the presence of virulence-encoding genes from UPEC isolates was *sfa* (18.00%), *papG II* (40.00%), *ompT* (40.00%), *iutA* (74.00%) and *iss* (48.00%). Among these, the gene encoding *iutA* followed by *iss* were the most frequent (74.00% and 48.00%, respectively) which is almost similar to previous reports including *sfa* (49.00%), *pap G II* (38.00%), *ompT* (63.00%), *iutA* (83.00%) and *iss* (53.00%).[10] According to these results, the genes encoding *iutA* and *iss* were more frequent in both APEC and UPEC and *iutA* was occurred in the majority of UPEC isolates. So, it could be considered as important virulence factors in avian colibacillosis and human UTIs caused by UPEC in Iran. For example, the high prevalence of *iss* in both APEC and UPEC has shown its resistance efficacy against the complement and their survival promotion in serum.[36] On the other hand, because of having plasmid-linked genes,[10] it can be demonstrated that most *E. coli* strains inducing poultry colibacillosis and human UTIs have considerable antibiotic resistance in Iran. Moreover, we showed the simultaneous presence of virulence-encoding genes *iutA* and *iss* in 80.00% of APEC isolates indicating high resistance of this strain against various antibiotics. These results were almost the same as the previous study reporting that *iss*, *iucC*, *iutA,* and *iroN* occur in almost 75.00% of APEC and UPEC isolates.[6]

There were variations among the frequency of APEC virulence-encoding genes in our study and the others of Zhu *et al*. in China and Moon *et al*. in Thailand and these variations were also seen in the frequency of UPEC virulence genes between our report in Iran and Zhu *et al*. in China.[28,35] The differences between the frequencies of virulence-encoding genes in our study and previous reports could be related to the capability of *E. coli* strains in a high rate of genetic exchange and mutual transfer of genes related to virulence and resistance. On the other hand, the use of different antibiotics in different parts of the world would eliminate different sensitive strains in these areas. Therefore, these antibiotics could impact the frequency of *E. coli* virulence genes dramatically in different regions of the world.

According to the abundance of genes encoding virulence factors, *papG II*, *ompT* and *iss* in APEC and UPEC, it was found that the frequency of genes in APEC was significantly more than UPEC isolates which is in agreement with Zhao *et al*.[10] who have shared the similar virulence gene profile. By contrast, UPEC contained more *iutA* compared to APEC in their studies which is in contrast with our results.

In the present study, most of the strains isolated from poultry had three of the five virulence-encoding genes and more strains isolated from human UTIs had two virulence-encoding genes. Thus, none of the isolates of avian pathogenic and human uropathogenic *E. coli* had all five genes of virulence factors at the same time. Accordingly, they might have another gene (s) responsible for their virulence traits.

In UPEC, the negative correlation was seen between *papG II* and *iss*, between *iutA* and *iss* and between *iss* and *sfa*, unlike the positive correlation seen between *sfa* and *papG II*. The positive correlation indicates that by increasing the frequency of gene encoding virulence adhesion factors, *sfa*, the frequency of the gene encoding adhesion factor *papG II* was also increased. But, none of the virulence genes in APEC showed a significant correlation, which means that change in the frequency of none of the mentioned genes affects each other.

Generally, the results of the present study indicated the relation of genes encoding virulence factors between human and poultry and showed that both APEC and UPEC isolates encounter similar challenges in establishing infection. These results indicate the zoonotic risk of APEC. Therefore, further studies have to be done in poultry products such as chicken meat and eggs. However, due to the limitation of the present study in terms of lack of solid phylogenetic linkage between APEC and UPEC strains, the number of virulence-associated genes and investigating the ability of APEC to get passed the body's defenses to cause UTIs in humans can be considered in future studies.

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Conflict of interest

The authors declare that there is no conflict of interest.

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