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Original Article

Association between *Escherichia coli* with NotI-restriction resistance and urinary tract infections

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KEYWORDS
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Abstract *Background: Escherichia coli* is the most common cause of urinary tract infections (UTIs). It is widely accepted that uropathogenic *E. coli* (UPEC) mainly emerge from the distal gut microbiota. Identification of bacterial characteristics that are able to differentiate UPEC

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Uropathogenic *E. coli*;
NotI;
PFGE;
RFLP;
Restriction enzyme resistance;
Phylogenetic group B2

from fecal commensal strains will facilitate the development of novel strategies to detect and monitor the spread of UPEC.

Methods: Fifty fecal commensal, 83 UTI-associated and 40 biliary tract infection (BTI)-associated *E. coli* isolates were analyzed. The NotI restriction patterns of chromosomal DNA in the isolates were determined by pulse-field gel electrophoresis. The phylogenetic types and the presence of 9 known virulence genes of each isolate were determined by PCR analyses. Additionally, the susceptibilities of the isolates to antibiotics were revealed. Then the associations of NotI resistance with UTI-associated isolates, phylotypes, and antibiotic resistance were assessed.

Results: NotI resistance was correlated with UTI-associated isolates, compared to the fecal isolates. Consistently, NotI-resistant isolates harbored a greater number of virulence factors and mainly belonged to phylotype B2. Additionally NotI resistance was correlated with chloramphenicol resistance among the bacteria. Among the fecal, UTI-associated and BTI-associated groups, the distribution of NotI-resistant group B2 isolates was correlated with UTI-associated bacteria.

Conclusion: NotI resistance alone is a potential marker for distinguishing fecal strains and UPEC, while the combination of NotI resistance and B2 phylogeny is a candidate marker to differentiate UPEC from fecal and other extraintestinal pathogenic *E. coli*. Additionally, NotI resistance may be valuable for assessing the potential of chloramphenicol resistance of *E. coli*.

Introduction

Urinary tract infections (UTIs) are the most common bacterial infections, which result in substantial economic and public health burden and significantly affect the life quality of patients.[1,2] *Escherichia coli* is the most common causative agent of UTIs.[3,4] It is widely accepted that uropathogenic *E. coli* (UPEC) mainly emerge from the distal gut microbiota.[3,5] Thus, compared to the fecal commensal strains of *E. coli*, UPEC have additional virulence factors (VFs) that allow them to successfully transit from the intestinal tract to the urinary tract.[3] The presence of VFs reflects the virulence potential of an *E. coli* strain to cause the infection. However, current understanding of the critical VFs that differentiate UPEC from commensal strains is limited because the pathogens require a combination of multiple VFs to cause infections and the composition of VFs is very diverse among UPEC.[3,6,7] In addition, *E. coli* strains are classified mainly into 4 phylogenetic groups, A, B1, B2, and D. The strains responsible for extra-intestinal infections, including UTIs, are more likely belongs to groups B2 or D.[3,6,8,9] Since *E. coli* characteristics that associate with UTIs are potential markers of UPEC, in the present study we attempted to identify UTI-associated characteristics of *E. coli*.

In bacteria, chromosomal DNA restriction patterns are commonly utilized for subtyping bacterial pathogens. Restriction fragment length polymorphism (RFLP) analyses depend on the susceptibility of the bacterial chromosome to restriction enzyme digestion. However, resistance of bacterial chromosome to digestion by some restriction enzymes has been epidemiologically associated with infections. It has been shown that Sau3A1 resistance is prevalent in epidemic-associated *Listeria monocytogenes* strains.[10,11] Similarly, Chiou et al. have shown that 6 out of 10 *E. coli* O157:H7 strains (the *E. coli* serotype associated with hemorrhagic colitis and hemorrhagic uremic syndrome) exhibit NotI resistance,[12] suggesting that the restriction enzyme resistance may associate with intestinal infection of *E. coli*. NotI is a rare-cutting restriction enzyme (restriction site: GCGGCCGC) and is commonly utilized in RFLP analyses. We speculate that NotI-resistant *E. coli* strains may have a greater potential to cause disease. In this study, we determined that *E. coli* strains with NotI resistance are significantly associated with UTIs.

Materials and methods

E. coli isolates

The *E. coli* isolates used in this study were collected from National Cheng Kung University Hospital at Tainan city, Taiwan and have been described previously.[7,13] The UTI-associated strains were isolates from urine samples obtained from patients with UTIs. The biliary tract infection (BTI) associated bacteremia *E. coli* isolates were obtained from the blood specimens of BTI patients with bacteremia. The fecal isolates were collected from healthy human feces. Bacteria were grown in Luria Bertani (LB) broth at 37 °C for 16 h unless otherwise indicated and were stored in LB with a final concentration of 15% glycerol at −80 °C.[4,14,15]

PCR-based genotyping, phylogenetic typing, and *z2389* detection

The frequencies of the 9 known virulence genes among the *E. coli* isolates were determined by PCR-based assays using primers and PCR conditions as described previously.[13,16e18] The phylogenetic group of each *E. coli* isolate was determined by the PCR-based method described by Clermont

et al.[19] This typing technique, which classifies *E. coli* into A, B1, B2, and D groups, is based on triplex PCR by detecting the presence *chuA* and *yjaA* genes, and the DNA fragment TspE4.C2. *chuA* is present in the strains belonged to groups B2 and D, but not in the strains belonged to group A and B1. The presence of *yjaA* was able to discriminate groups B2 and D, while the presence of TspE4.C2 was utilized to differentiate group A from group B1. Strains in groups B2 and D are *yjaA* positive and negative, respectively. Strains in groups B1 and A are TspE4.C2 positive and negative, respectively.[20] To detect *z2389*, two sets of primers Z2389-F1 and R1 (5^0-TTGCACGTCCAAGAAGATGT and 5^0-CCAATCAGGGAAGCCTTGTA) and Z2389-F2 and Z2389-R2 (5^0-ATGAATGTAATAGATTTGTTTTC and 5^0-CTTTGGAAGTTAGGGTATAA) were used. The PCR reactions for *z2389* detection were heated to 95 °C in an automated thermal cycler for 5 min, followed by 30 cycles of denaturation (95 °C, 45 s), annealing (59 °C, 45 s), and extension (72 °C, 50 s). All PCR amplifications were performed in a 25 ml reaction mixture and carried out in an Eppendorf Mastercycler® gradient thermal cycler (Eppendorf, Hamburg, Germany). Taq polymerase was used in the reactions. All PCR tests were performed 2 times with independently prepared boiled bacterial lysates. Additional assays were conducted if discrepancies between the independent assays occurred.

For the PCR-based analysis of the known virulence genes and *z2398*, the *E. coli* strains that served as positive controls included CFT073 (*papGII*, *sat*, *iha*, *usp*, *ireA*, *iroN*, and *hlyA*), UTI89 (*sfaS*), J96 (*papGIII*), and EDL933 (*z2398*). MG1655 was used as a negative control for all the genes.[7,21e23]

Antimicrobial susceptibility testing

Susceptibility of the *E. coli* isolates to each of five antimicrobial agents (nalidixic acid, ciprofloxacin, levofloxacin, chloramphenicol, and tetracycline) was determined by the disk diffusion method described by the Clinical and Laboratory Standards Institute (CLSI).

Pulsed-field gel electrophoresis

PFGE was performed to analyze *E. coli* chromosomal DNA digested with XbaI or NotI, following the protocol described previously.[24,25] The resulting DNA profiles were recorded using a digital camera system (Kodak Electrophoresis Documentation and Analysis System 290; Kodak, Rochester, New York, USA). PFGE fingerprints were analyzed using BioNumerics software version 4.6 (Applied Maths; Kortrijk, Belgium). The dendrograms for the NotI- and XbaI-PFGE patterns were constructed using the Dice similarity coefficient and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm, with settings for pattern optimization of 1.5% and band tolerance of 0.75%.

Statistical analysis

All of the comparisons between different bacterial groups, except those for the virulence scores, were measured using the two-tailed Fisher's exact test.[20] The comparisons of the virulence scores between the NotI-resistant and NotI-susceptible *E. coli* groups were measured using the ManneWhitney U test. A P value of <0.05 was set as the threshold for statistical significance.

Results

E. coli with NotI resistance is associated with UTIs

To investigate whether UPEC and fecal commensal *E. coli* isolates can be discriminated by the PFGE subtyping analysis with NotI. Fifty *E. coli* fecal isolates from healthy human subjects and 83 *E. coli* isolates from patients with UTIs were analyzed. Among the 133 *E. coli* isolates 20 were shown to be NotI-resistant and thus clustered together in the dendrogram based on the PFGE pattern (Fig. 1A; Supplement Fig. 1). The NotI-sensitive isolates exhibited distinct restriction patterns except for 2 isolates, one UTI-associated and one fecal isolates (Fig. 1A). Based on the dendrogram, three clusters of NotI-sensitive bacteria (Clusters I, II, and III) exhibited high frequencies of UTI-associated isolates (91%, 83%, and 100%, respectively), suggesting the NotI restriction patterns in these clusters may be correlated with UTIs. Since the NotI-resistant strain showed an identical pattern of NotI PFGE (Supplement Fig. 1), they were possibly originated from a bacterial clone and thus likely derived from an epidemiological outbreak. If the NotI-resistant isolates were derived from an epidemiological outbreak, it may cause a bias inference with the epidemiological distribution of NotI resistance among the *E. coli* isolates. To exclude this possibility, the 20 NotI-resistant isolates were further analyzed using XbaI PFGE. These isolates exhibited distinct XbaI restriction patterns, suggesting that they were not derived from an epidemiological outbreak (Fig. 1B). Among the NotI-resistant strains, 19 isolates were UTI-associated isolates, whereas only one was a fecal isolate. The frequency of NotI resistance in UTI-associated isolates (23%; 19/83) was significantly higher than that in the fecal isolate (2%; 1/50), indicating that NotI resistance of *E. coli* is statistically associated with UTIs (Fig. 2).

NotI resistance is associated with phylogenetic group B2 *E. coli*

We further investigate whether the distribution of NotI resistance is associated with phylogenetic groups. As shown in Table 1, a majority of the NotI-resistant isolates (17 of 20 isolates) belonged to phylogenetic group B2. Therefore, the distributions of NotI resistance were significantly associated with phylogenetic group B2 among all the isolates (both commensal and UTI-associated) and among the UTI-associated isolates. In contrast, NotI resistance among all the *E. coli* isolates was negatively associated with phylogenetic group A, whose member strains are often devoid of extraintestinal VFs (Table 1).[26]

Association of NotI resistance with virulence genes

Because *E. coli* isolates with NotI resistance were significantly associated with UTIs, we further investigated

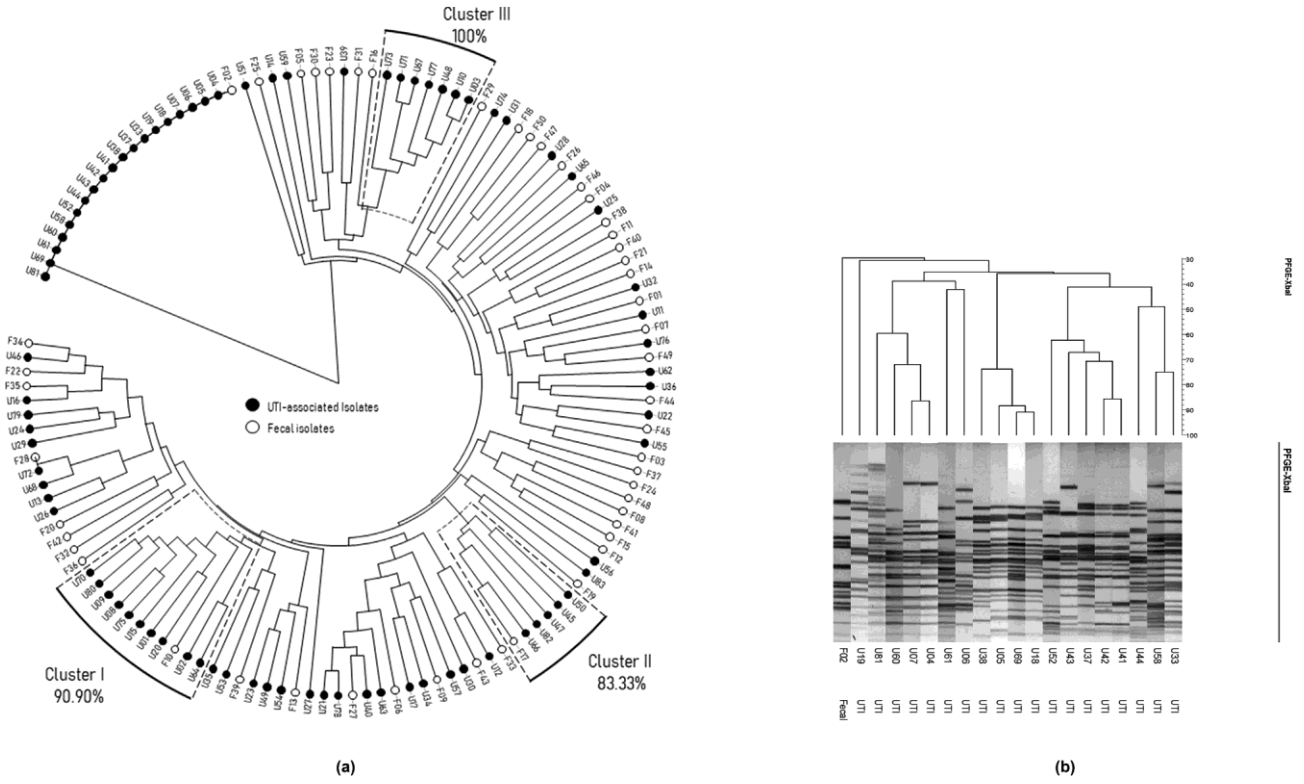

Figure 1. Dendrograms of the PFGE patterns of the fecal and UTI-associated *E. coli* isolates. (A) The dendrogram of the NotI PFGE patterns of the 113 *E. coli* isolates. Twenty isolates exhibited NotI-resistant pattern. All the NotI-sensitive isolates showed distinct NotI restriction patterns except for two isolate, F28 and U72 which showed an identical pattern. Among the NotI-sensitive isolates, three clusters of isolates contained high frequencies of UTI-associated strains (Cluster I, II, and III). The isolates in each of the clusters showed higher than 62% of similarity (Supplementary Fig. 1) (B) The dendrogram of the XbaI PFGE patterns of the 20 NotI-resistant isolates. The isolates with names beginning with "U" and "F" are UTI-associated and fecal isolates, respectively.

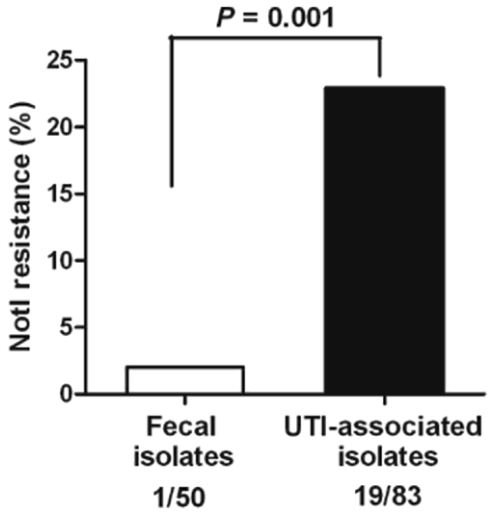

Figure 2. The distribution of NotI resistance among the fecal isolates and UTI-associated isolates.

whether the distribution of NotI resistance is associated with virulence. Therefore, the distribution of NotI resistance was compared to the distributions of 9 known VFs (*iha*, *papGII*, *papGIII*, *cnfI*, *hlyA*, *sat*, *ireA*, *iroN*, and *usp*) among the *E. coli* isolates.[7,21,27] Among all of the *E. coli* strains, the NotI-resistant isolates exhibited significantly higher frequencies of *papGII*, *sat*, *ireA*, and *usp* than the NotI-sensitive isolates (Table 1). Among the UTI-associated *E. coli* strains, the NotI-resistant isolates exhibited significantly higher frequencies of *papGIII* and *usp* than the NotI-sensitive isolates. In addition, the NotI-resistant isolates exhibited significantly higher average virulence scores than the NotI-sensitive *E. coli* among all the isolates and the UTI-associated isolates, suggesting that NotI-resistant *E. coli* strains are associated with a higher virulence potential.

Association of NotI resistance with antibiotic resistance

In addition, the correlation of NotI resistance with antibiotic resistance among the *E. coli* isolates was investigated (Table 1). The NotI-resistant isolates showed a significantly higher frequency of chloramphenicol resistance than the NotI-susceptible isolates among all the isolates and among the UTI-associated isolates, suggesting the distribution of NotI resistance is associated with chloramphenicol resistance among the *E. coli* isolates (Table 1). On the other hand, NotI resistance show no significant association with the resistance to nalidixic acid, ciprofloxacin, levofloxacin, and tetracycline (Table 1).

Table 1 Distribution of NotI resistance in relation to phylogenetic groups, VFs, and antibiotic resistance among the *E. coli* isolates used in this study.

	All strains (n Z 133)[a]			UTI-associated strains (n Z 83)		
	NotI resistant (n Z 20)	NotI susceptible (n Z 113)	P-value[b]	NotI resistant (n Z 19)	NotI susceptible (n Z 64)	P-value[b]
Phylogenetic group						
A	0 (0%)	30 (27%)	0.007	0 (0%)	6 (9%)	NS
B1	1 (5%)	10 (9%)	NS	1 (5%)	7 (11%)	NS
B2	17 (85%)	57 (50%)	0.006	17 (89%)	39 (61%)	0.025
D	2 (10%)	16 (14%)	NS	1 (5%)	12 (19%)	NS
Virulence factor						
iha	10 (50%)	33 (29%)	NS	9 (47%)	24 (38%)	NS
papGII	12 (60%)	28 (24%)	0.003	12 (63%)	22 (34%)	0.034
papGIII	0 (0%)	7 (6%)	NS	0 (0%)	5 (8%)	NS
cnf1	2 (10%)	18 (16%)	NS	2 (11%)	15 (23%)	NS
hlyA	6 (30%)	25 (22%)	NS	6 (32%)	17 (26%)	NS
sat	10 (50%)	27 (24%)	0.028	9 (47%)	22 (34%)	NS
ireA	8 (40%)	20 (18%)	0.036	8 (42%)	15 (23%)	NS
iroN	8 (40%)	37 (33%)	NS	8 (42%)	24 (37%)	NS
usp	16 (80%)	49 (43%)	0.003	16 (84%)	36 (56%)	0.032
Average score[c]	3.6	2.2	0.001	3.7	2.8	0.033
Antibiotic resistance						
Nalidixic acid	11 (55%)	42 (37%)	NS	10 (53%)	26 (41%)	NS
Ciprofloxacin	1 (5%)	13 (12%)	NS	1 (5%)	11 (17%)	NS
Levofloxacin	1 (5%)	12 (11%)	NS	1 (5%)	9 (14%)	NS
Chloramphenicol	16 (80%)	35 (31%)	<0.001	15 (79%)	28 (44%)	0.009
Tetracycline	15 (75%)	71 (63%)	NS	14 (74%)	43 (67%)	NS

[a] All strains include the 50 fecal isolates and the 83 UTI-associated isolates.
[b] Only P values < 0.05 are shown; NS, not significant.
[c] The average scores of the indicated bacterial groups represent the means of virulence scores, which were calculated for each isolates as the sum of all the 9 known virulence genes detected (*iha*, *papGII*, *papGIII*, *cnfI*, *hlyA*, *sat*, *ireA*, *iroN*, and *usp*).

The combination of NotI resistance and phylogeny B2 may be a marker specific to UPEC

To investigate whether NotI resistance is specifically associated with UPEC, but not with other extraintestinal pathogenic *E. coli*, we further analyzed 40 bacteremia *E. coli* isolates associated with biliary tract infections (BTIs). The frequency of NotI resistance in the BTI-associated *E. coli* (10%; 4/40) was lower than that in the UTI-associated isolates (23%; 19/83), although the difference was not statistically significant (Table 2). These findings suggested that NotI resistance may be correlated with UTIs (or UPEC), when UTI-associated isolates was compared with BTI-associated isolates. However, a further study with larger sample sizes is required to draw a conclusive conclusion.

NotI resistance was shown to be associated with phylogenetic group B2 *E. coli* (Table 1). Since UPEC are mainly belonged phylogenetic groups B2 and D,[7] we further assessed whether the association of NotI resistance with UTIs (or UPEC) is due to the association of the restriction resistance with the group B2 *E. coli*. Among the group B2 *E. coli*, the frequency of the NotI resistance in the UTI-associated isolates (30%; 17/56) was still higher than those in the fecal isolates (0%; 0/18) and BTI-associated isolates (10%; 1/10) (Table 2). These findings suggest that the association of NotI resistance with UTIs is not due to the association of the restriction resistance with the group B2 bacteria (i. e. NotI resistance specifically associates with UTIs).

We further stratified all the *E. coli* isolates into two groups: the group of isolates with both NotI resistance and phylogeny B2 and the group of the other isolates (Table 3). The frequency of the NotI-resistant B2 bacteria in the UTI-associated isolates (20%; 17/83) was significantly higher then those in the fecal isolates (0%; 0/50) and BTI-associated isolates (3%; 1/40), while the distribution of the NotI-resistant B2 bacteria in the fecal and BTI-associated *E. coli* showed no significant difference (Table 3). These findings suggest that NotI resistance plus phylogeny B2 is specifically associated with UTIs, when UPEC are compared to fecal and other extraintestinal pathogenic *E. coli* strains.

Association of NotI resistance with *z2389*

Chiu et al. have shown that the gene *z2389*, which encodes a DNA cytosine methyltransferase, is responsible for methylation of the first cytosine residue in the NotI site (GCGGCCGC), thus causing NotI resistance in *E. coli* O157:H7 strains.[12] To investigate whether *z2398* is responsible for the NotI resistance in our *E. coli* isolates, 2 sets of primers specific to *z2398* were used to detect this gene in

Table 2 The frequency of NotI resistance in Fecal, UTI-associated, and BTI-associated *E coli* isolates.

	Fecal isolates (N Z 50)			UTI-associated isolate (N Z 83)			BTI-associated strains (N Z 40)			P value[a,b]		
	Total isolate N (%)	Not-1 resistant N (%)	Frequency of NotI resistance	Total strains N (%)	Not-1 resistant N (%)	Frequency of NotI resistance	Total strains N (%)	Not-1 resistant N (%)	Frequency of NotI resistance	Fecal vs. UTI	BTI vs UTI	Fecal vs BTI
Total strains	50 (100%)	1 (100%)	2%	83 (100%)	19 (100%)	23%	40 (100%)	4 (100%)	10%	0.0008	NS	NS
B2 strains	18 (36%)	0 (0%)	0%	56 (67%)	17 (89%)	30%	10 (25%)	1 (25%)	10%	0.0078	NS	NS
Non-B2 strains	32 (64)	1 (100%)	3%	27 (33%)	2 (11%)	7%	30 (75%)	3 (75%)	10%	NS	NS	NS

[a] The statistical analysis of the frequency of NotI resistance in the indicated *E. coli* groups.
[b] Only P values < 0.05 are shown; NS, no significant.

the NotI-resistant isolates. However, *z2398* was not detected, suggesting that *z2398* is not responsible for the NotI resistance (data not shown).

Discussion

This study is the first to demonstrate that UPEC is statistically associated with NotI-resistant chromosomal DNA when compared with fecal commensal *E. coli*, suggesting that NotI resistance is a bacterial characteristic able to discriminate UPEC and commensal *E. coli*. Consistent with this finding, the NotI resistance was significantly associated with higher virulence scores and the phylogenetic group B2. We have also firstly demonstrated that the distribution of NotI-resistant group B2 *E. coli* was significantly correlated with UTI-associated bacteria, when the UTI-associated, BTI-associated, and fecal isolates were compared. This finding suggest that NotI resistance plus the B2 phylotype is a potential marker to distinguish UPEC from fecal and other extraintestinal pathogenic *E. coli*. In addition, NotI resistance was shown to be associated with chloramphenicol resistance, which may contribute to evaluating the potential of *E. coli* strains in resisting the antibiotic treatment.

Resistance of chromosomal DNA to restriction enzyme digestion has been a valuable marker for epidemiological investigations of pathogenic bacteria. One example is the food-borne bacterial pathogen *L. monocytogenes*. Food contamination by the bacteria is involved in numerous outbreak and sporadic human listeriosis. However, it is believed that only a fraction of *Listeria monocytogeness* strains identified in foods are able to cause illness.[28] Since an epidemic-associated clone of *L. monocytogeness* is associated Sau3AI resistance, Sau3AI restriction analyses can be a tool to assess the hazard posed by *Listeria*-contaminated food.[10,11] In addition, SmaI resistance among methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with MRSA strains originating in animals.[29] Therefore, SmaI resistance has been utilized to investigate the entry of animal-originated MRSA strains into human populations.[30,31] Accordingly, the association of the NotI resistance of *E. coli* chromosomal DNA with UTIs and chloramphenicol resistance may benefit future epidemiological investigations of UPEC.

The association of the NotI-resistant *E. coli* isolates with UTIs may be explained by the possibility that NotI resistance contributes to the urovirulence of *E. coli*. Restriction resistance of bacterial chromosomal DNA is mainly acquired by epigenetic modification, most commonly by DNA methylation of restriction sites.[32] This epigenetic modification has been shown to affect transcriptional regulation of bacterial virulence genes, and therefore, it may regulate the virulence of pathogenic bacteria.[32] For example, such epigenetic modification is required for the full virulence of *Salmonella* in mice and is involved in regulating the expression of *E. coli*'s pyelonephritis-associated (P) pili that contribute to kidney colonization.[32,33] Therefore, the epigenetic modification of the NotI cutting sites may be involved in the transcriptional regulation of urovirulence genes. Another nonexclusive explanation for the association is that the

Table 3 The distribution of NotI-resistant B2 *E. coli* in fecal, UTI-associated, and BTI-associated *E. coli* isolates.

Strains	Fecal isolate (N Z 50)	UTI-associated isolates (N Z 83)	BTI-associated isolates (N Z 40)	P value[a]		
				Fecal vs. UTI	BTI vs UTI	Fecal vs BTI
B2 plus NotI resistant	0 (0%)	17 (20%)	1 (3%)	0.0002	0.0065	NS
The others	50 (100%)	66 (80%)	39 (98%)			

[a] Only P values < 0.05 are shown; NS, no significant.

gene(s) responsible for NotI resistance may be genetically linked to genes encoding urovirulence. Many virulence genes of pathogenic *E. coli* are acquired through horizontal transfer of mobile genetic elements.[3,34] The NotI resistance-encoding genes may be carried by mobile elements that also harbor virulence genes and thus are usually co-transferred with the VFs.[9] The association of NotI resistance with higher virulence scores may support this hypothesis.

When the fecal and UTI-associated isolates (113 isolates in total) were included in the analysis of virulence gene distributions, the frequencies of the virulence genes, *papGII*, *sat*, *ireA*, and *usp* in the NotI-resistant isolates were significantly higher than those in the NotI-sensitive isolate (Table 1). These findings may simply reflect the fact that the frequency of UTI-associated isolates in the NotI-resistant group (19/20; 95%) was higher than that in the NotI-sensitive group (64/93; 69%) (i. e. NotI resistance is associated with UTI), because virulence genes are associated with UPEC in comparison with fecal commensal strains.[7] When only the UTI-associated strains (83 isolates) were analyzed, the distributions of *papGII* and *usp* in the NotI-resistant group were still significantly higher than those in the NotI-resistant group (Table 1). These findings suggest that NotI-resistant UPEC strains have a higher potential to harbor *papGII* and *usp* than NotI-sensitive UPEC strains. In addition, in the UTI-associate isolates, the NotI-resistant group exhibited a significant higher virulence score than the NotI-sensitive group (Table 1). It is likely that NotI-resistant UPEC strains are correlated with a higher virulence potential than NotI-sensitive UPEC strains.

The correlations of NotI resistance to the *E. coli* phylogenetic groups were consistent with the associations of NotI resistance with UTIs and greater virulence potential. Most *E. coli* strains fall into four main phylogenetic groups, A, B1, B2, and D.[19] It is known that extraintestinal pathogenic *E. coli* strains, including UPEC, mainly belong to phylogenetic group B2 and, to a lesser extent, group D.[3] It is also known that group A and B1 strains are most often devoid of extraintestinal VFs.[26,35] In agreement with these previous studies, NotI resistance was positively associated with group B2 strains but negatively associated with group A strains among the *E. coli* isolates in the present study.

NotI resistance was associated with chloramphenicol resistance among the *E. coli* isolates (Table 1). Chloramphenicol is an old broad-spectrum antibiotic with potent therapeutic effect against bacterial infections, including UTIs. Although chloramphenicol is no longer a first-line antibiotic for treating infections in developed countries due to its bone marrow toxicity,[36] this agent has regained popularity in light of the rising incidence of multidrug-resistant (MDR) bacteria and for managing patients with beta-lactam allergy.[36] In view of the fact that chloramphenicol has become an important alternative antimicrobial agent, understanding the resistance potential of pathogens to chloramphenicol treatment will greatly facilitate the decision-making of antibiotic selection. The chloramphenicol resistance rates in the UPEC ranged from 16% to 55% around the world (51.8% in the present study),[37e39] suggesting that the distribution of chloramphenicol resistance may significantly differ in distinct geographic areas. NotI restriction resistance may serve as a predictor to assess the potential of chloramphenicol resistance in pathogenic *E. coli*. Similarly, the association between restriction resistance and antimicrobial resistance has been shown in other bacteria. MobI resistance is associated with erythromycin resistance among swine isolates of *Campylobacter coli*, which is a pathogen capable of causing human and animal diarrhea.[40]

Chiou et al. have identified that the gene *z2398*, which encodes a DNA cytosine methyltransferase, is responsible for the NotI resistance of the *E. coli* O157:H7 strains by methylating the first cytosine residue of the NotI site (GCGGCCGC).[12] *z2398* is located within the prophage CP-933 R in the sequenced *E. coli* O157:H7 strain EDL933,[12] suggesting that *E. coli* O157:H7 strains horizontally acquired the NotI resistance phenotype through phage infection. However, we failed to detect *z2398* in our NotI-resistant strains using two pairs of primers; this finding suggests that a gene other than *z2398* is responsible for modification of the NotI sites in these NotI-resistant UTI-associated strains and that the mechanism by which the NotI-resistant phenotype was acquired is different than that of the *E. coli* O157:H7 strains.

In conclusion, NotI resistance may be valuable for the differentiation of uropathogenic and non-pathogenic *E. coli* strains and for the assessment of chloramphenicol resistance among *E. coli* strains. In addition, the combination NotI resistance and B2 phylogeny may be a potential marker for distinguishing UPEC from fecal and other extraintestinal pathogenic *E. coli*. Studies are in progress to further identify the genes responsible for NotI resistance in UTI-associated bacteria and to determine whether NotI resistance contributes to the pathogenesis and antibiotic resistance of UPEC.

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Author contributions

MH WCH and BHM carried out the experiments in this study. CSC and CHT contributed to the study conception, planning experiments, data analysis, and interpretation. JJW and SLJ participated in conceptualization and interpretation of the data. IBNPD, MCW, WHL, and CCT contributed materials and technical support. CHT, MH, and BHM wrote the manuscript. All authors contributed to the article and approved the submitted version.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or other association that might pose a conflict of interest.

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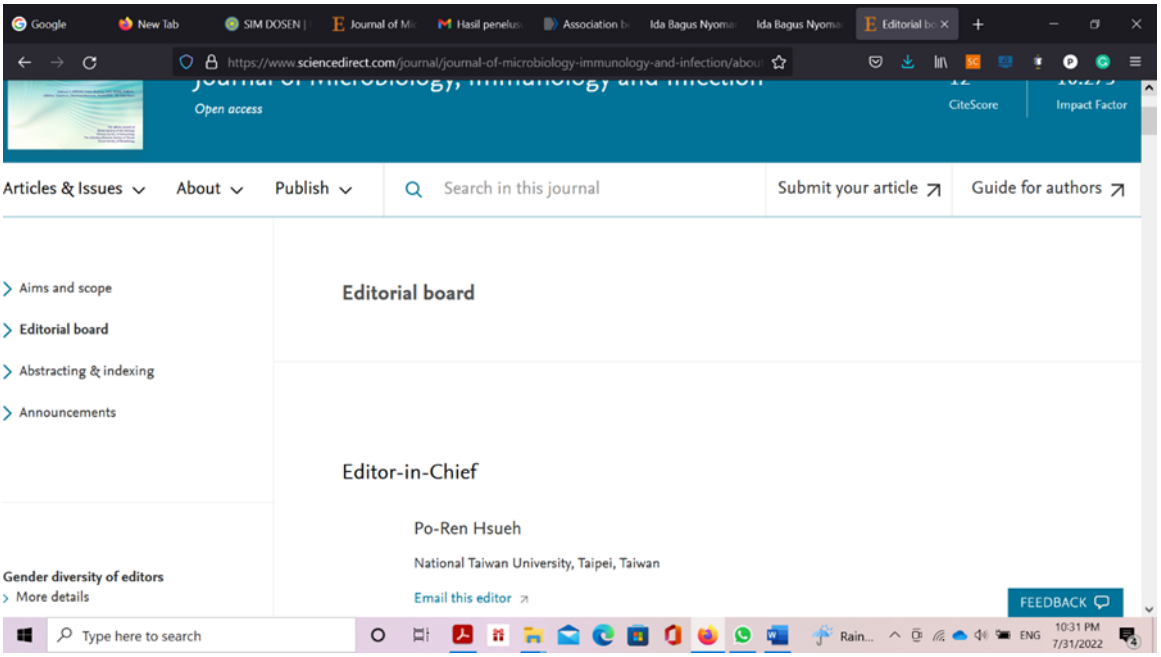
References

1. Medina M, Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. *Ther Adv Urol* 2019;11. 1756287219832172.
2. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol* 2015;13:269e84.
3. Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. *Int J Med Microbiol* 2005;295:383e404.
4. Huang WC, Lin CY, Hashimoto M, Wu JJ, Wang MC, Lin WH, et al. The role of the bacterial protease Prc in the uropathogenesis of extraintestinal pathogenic *Escherichia coli*. *J Biomed Sci* 2020;27:14.
5. Gruneberg RN. Relationship of infecting urinary organism to the faecal flora in patients with symptomatic urinary infection. *Lancet* 1969;2:766e8.
6. Marrs CF, Zhang L, Foxman B. *Escherichia coli* mediated urinary tract infections: are there distinct uropathogenic *E. coli* (UPEC) pathotypes? *FEMS Microbiol Lett* 2005;252:183e90.
7. Mao BH, Chang YF, Scaria J, Chang CC, Chou LW, Tien N, et al. Identification of *Escherichia coli* genes associated with urinary tract infections. *J Clin Microbiol* 2012;50:449e56.
8. Marrs CF, Zhang L, Tallman P, Manning SD, Somsel P, Raz P, et al. Variations in 10 putative uropathogen virulence genes among urinary, faecal and peri-urethral *Escherichia coli*. *J Med Microbiol* 2002;51:138e42.
9. Lloyd AL, Rasko DA, Mobley HL. Defining genomic islands and uropathogen-specific genes in uropathogenic *Escherichia coli*. *J Bacteriol* 2007;189:3532e46.
10. Zheng W, Kathariou S. Host-mediated modification of Sau3AI restriction in *Listeria monocytogenes*: prevalence in epidemic-associated strains. *Appl Environ Microbiol* 1997;63:3085e9.
11. Yildirim S, Lin W, Hitchins AD, Jaykus LA, Altermann E, Klaenhammer TR, et al. Epidemic clone I-specific genetic markers in strains of *Listeria monocytogenes* serotype 4b from foods. *Appl Environ Microbiol* 2004;70:4158e64.
12. Chiou CS, Li HY, Tung SK, Chen CY, Teng CH, Shu JC, et al. Identification of prophage gene z2389 in *Escherichia coli* EDL933 encoding a DNA cytosine methyltransferase for full protection of NotI sites. *Int J Med Microbiol* 2010;300:296e303.
13. Huang WC, Liao YJ, Hashimoto M, Chen KF, Chu C, Hsu PC, et al. cjrABC-senB hinders survival of extraintestinal pathogenic *E. coli* in the bloodstream through triggering complement-mediated killing. *J Biomed Sci* 2020;27:86.
14. Huang WC, Hashimoto M, Shih YL, Wu CC, Lee MF, Chen YL, et al. Peptidoglycan endopeptidase Spr of uropathogenic *Escherichia coli* contributes to kidney infections and competitive fitness during bladder colonization. *Front Microbiol* 2020;11:586214.
15. Hsu PC, Chen CS, Wang S, Hashimoto M, Huang WC, Teng CH. Identification of MltG as a Prc protease substrate whose dysregulation contributes to the conditional growth defect of Prc-deficient *Escherichia coli*. *Front Microbiol* 2020;11:2000.
16. Chapman TA, Wu XY, Barchia I, Bettelheim KA, Driesen S, Trott D, et al. Comparison of virulence gene profiles of *Escherichia coli* strains isolated from healthy and diarrheic swine. *Appl Environ Microbiol* 2006;72:4782e95.
17. Johnson JR, Gajewski A, Lesse AJ, Russo TA. Extraintestinal pathogenic *Escherichia coli* as a cause of invasive nonurinary infections. *J Clin Microbiol* 2003;41:5798e802.
18. Hashimoto M, Ma YF, Wang ST, Chen CS, Teng CH. Iron acquisition of urinary tract infection *Escherichia coli* involves pathogenicity in *Caenorhabditis elegans*. *Microorganisms* 2021;9.
19. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000;66:4555e8.
20. Huang WC, Wong MY, Wang SH, Hashimoto M, Lin MH, Lee MF, et al. The ferric citrate uptake system encoded in a novel bla CTX-M-3- and bla TEM-1-harboring conjugative plasmid contributes to the virulence of *Escherichia coli*. *Front Microbiol* 2021;12:667782.
21. Lin WH, Wang MC, Liu PY, Chen PS, Wen LL, Teng CH, et al. *Escherichia coli* urinary tract infections: host age-related differences in bacterial virulence factors and antimicrobial susceptibility. *J Microbiol Immunol Infect* 2021. S1684-1182(21)00091-8.
22. Xie Y, Parthasarathy G, Di Cello F, Teng CH, Paul-Satyaseela M, Kim KS. Transcriptome of *Escherichia coli* K1 bound to human brain microvascular endothelial cells. *Biochem Biophys Res Commun* 2008;365:201e6.
23. Scaria J, Warnick LD, Kaneene JB, May K, Teng CH, Chang YF. Comparison of phenotypic and genotypic antimicrobial profiles in *Escherichia coli* and *Salmonella enterica* from the same dairy cattle farms. *Mol Cell Probes* 2010;24:325e45.
24. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, et al. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, Salmonella, and *Shigella* for PulseNet. *Foodb Pathog Dis* 2006;3:59e67.
25. Janvilisri T, Scaria J, Teng CH, McDonough SP, Gleed RD, Fubini SL, et al. Temporal differential proteomes of *Clostridium difficile* in the pig ileal-ligated loop model. *PLoS One* 2012;7:e45608.
26. Bingen E, Picard B, Brahimi N, Mathy S, Desjardins P, Elion J, et al. Phylogenetic analysis of *Escherichia coli* strains causing neonatal meningitis suggests horizontal gene transfer from a predominant pool of highly virulent B2 group strains. *J Infect Dis* 1998;177:642e50.
27. Tseng CC, Lin WH, Wu AB, Wang MC, Teng CH, Wu JJ. *Escherichia coli* FimH adhesins act synergistically with PapGII adhesins for enhancing establishment and maintenance of kidney infection. *J Microbiol Immunol Infect* 2020. S1684-1182(20)30218-8.

28. Hof H, Rocourt J. Is any strain of *Listeria monocytogenes* detected in food a health risk? *Int J Food Microbiol* 1992;16:173e82.
29. Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuvel MG, Heck ME, Pluister GN, et al. Community-acquired MRSA and pig-farming. *Ann Clin Microbiol Antimicrob* 2006;5:26.
30. van Loo I, Huijsdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N, Beaujean D, et al. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. *Emerg Infect Dis* 2007;13:1834e9.
31. Huijsdens XW, Bosch T, van Santen-Verheuvel MG, Spalburg E, Pluister GN, van Luit M, et al. Molecular characterisation of PFGE non-typable methicillin-resistant *Staphylococcus aureus* in The Netherlands, 2007. *Euro Surveill* 2009:14.
32. Casadesus J, Low D. Epigenetic gene regulation in the bacterial world. *Microbiol Mol Biol Rev* 2006;70:830e56.
33. Garcia-Del Portillo F, Pucciarelli MG, Casadesus J. DNA adenine methylase mutants of *Salmonella typhimurium* show defects in protein secretion, cell invasion, and M cell cytotoxicity. *Proc Natl Acad Sci USA* 1999;96:11578e83.
34. Kuo CJ, Chen JW, Chiu HC, Teng CH, Hsu TI, Lu PJ, et al. Mutation of the enterohemorrhagic *Escherichia coli* core LPS biosynthesis enzyme RfaD confers hypersusceptibility to host intestinal innate immunity *in vivo*. *Front Cell Infect Microbiol* 2016;6:82.
35. Boyd EF, Hartl DL. Chromosomal regions specific to pathogenic isolates of *Escherichia coli* have a phylogenetically clustered distribution. *J Bacteriol* 1998;180:1159e65.
Sood S. Chloramphenicol - a potent armament against multi- drug resistant (MDR) gram negative bacilli? *J Clin Diagn Res* 2016;10:DC01e3.
36. Starcic Erjavec M, Rijavec M, Krizan-Hergouth V, Fruth A, Zgur-Bertok D. Chloramphenicol- and tetracycline-resistant uropathogenic *Escherichia coli* (UPEC) exhibit reduced virulence potential. *Int J Antimicrob Agents* 2007;30:436e42.
37. Hozzari A, Behzadi P, Kerishchi Khiabani P, Sholeh M, Sabokroo N. Clinical cases, drug resistance, and virulence genes profiling in Uropathogenic *Escherichia coli*. *J Appl Genet* 2020;61:265e73.
38. Paniagua-Contreras GL, Monroy-Perez E, Rodriguez-Moctezuma JR, Dominguez-Trejo P, Vaca-Paniagua F, Vaca S. Virulence factors, antibiotic resistance phenotypes and O-serogroups of *Escherichia coli* strains isolated from community-acquired urinary tract infection patients in Mexico. *J Microbiol Immunol Infect* 2017;50:478e85.
39. Wright S, Wilson S, Miller WG, Mandrell RE, Siletzky RM, Kathariou S. Differences in methylation at GATC sites in genomic DNA of *Campylobacter coli* from turkeys and swine. *Appl Environ Microbiol* 2010;76:7314e7.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2021.11.010.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