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Prevalence and Associations of *tcpC*, a Gene Encoding a Toll/Interleukin-1 Receptor Domain-Containing Protein, among *Escherichia coli* Urinary Tract Infection, Skin and Soft Tissue Infection, and Commensal Isolates▽

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TcpC, a new Toll/interleukin-1 receptor domain-containing protein of uropathogenic *Escherichia coli* involved in the suppression of innate immunity, was found in 2008. The aim of the present study was to determine the prevalence of *tcpC* and its association with virulence factors and phylogenetic groups among strains from a collection of 212 *E. coli* isolates from urinary tract and skin and soft tissue infections and 90 commensal *E. coli* strains.

Pathogenic microbes avoid host defenses using a wide array of virulence factors. *Escherichia coli* strains, even though they are common bacteria of the gut microbiota, can be important pathogens due to the possession of virulence factors (5). Recently, Cirl et al. (1) reported that they found TcpC, a new Toll/interleukin-1 receptor (TIR) domain-containing protein of uropathogenic *E. coli* that inhibits Toll-like receptor (TLR) and MyD88-specific signaling, thus impairing the innate immune response. They further reported that *tcpC* homologous sequences were present in about 40% of *E. coli* isolates from individuals with pyelonephritis, 21% of isolates from individuals with cystitis, 16% of isolates from individuals with asymptomatic bacteriuria, and only 8% of commensal isolates. Their results suggested that TcpC increases the severity of urinary tract infections (UTIs) in humans and provided the first unambiguous evidence that bacterial pathogens interfere with TLR signaling to survive and spread in the human host.

The aim of our study was to determine the prevalence of *tcpC* among 212 extraintestinal *E. coli* isolates: 100 *E. coli* isolates from individuals with symptomatic UTIs, 10 *E. coli* isolates from individuals with asymptomatic UTIs, 102 *E. coli* isolates from isolates from individuals with skin and soft tissue infections (SSTIs), and 90 *E. coli* commensal isolates. In addition, we investigated the association of *tcpC* with the phylogenetic group (groups A, B1, B2, and D; *E. coli* strains causing extraintestinal infections are known to mainly belong to group B2 and, to a lesser extent, group D, while commensal *E. coli* strains belong to groups A and B1), as well as with other well-known virulence factors of extraintestinal pathogenic *E. coli* (ExPEC) strains (cytotoxic necrotizing factor 1 [*cnf1*], hemolysin [*hlyA*], P-fimbrial adhesins [*papGIII* and *papGII*], S fimbriae [*sfaDE*], Afa/Dr adhesins [*afa*/*draBC*], aerobactin [*iucD*], and uropathogenic strain-specific protein [*usp*]). To our knowledge, this is the first investigation of the prevalence of *tcpC* among *E. coli* strains causing SSTIs and of the association of *tcpC* with phylogenetic group as well as virulence factor genes among UTI, SSTI, and commensal *E. coli* isolates.

The extraintestinal *E. coli* isolates examined in this study were from our previous studies of UTIs (10, 12–14) and SSTIs (9), while the 90 *E. coli* commensal isolates were isolated for the purposes of this study. The commensal *E. coli* isolates were isolated as lactose-positive colonies on MacConkey agar plates from the feces of healthy individuals. Indole, methyl red, Voges-Proskauer, and citrate tests were performed to ascertain that the species detected were *E. coli*. The strains investigated were cultivated in Luria-Bertani medium or agar. Cell lysates of all 302 *E. coli* isolates were prepared (7) and used in the PCRs. Amplifications were performed in an automated thermal cycler (UNOII; Biometra, Göttingen, Germany) in a 25-μl reaction mixture containing template DNA (5 μl of boiled lysate), 10 pmol of forward and reverse primers (Table 1), 0.2 mM deoxynucleoside triphosphate mixture, 0.625 U *Taq* DNA polymerase, and 2.5 mM $MgCl_2$ in 1× PCR buffer (Fermentas, Vilnius, Lithuania). The amplification schemes were based on previous amplification protocols (Table 1). For amplification of the *tcpC* sequence, the following amplification scheme was employed: 1 cycle of denaturation at 94°C for 4.5 min, followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 1 min. The amplification was concluded with an extension program of one cycle at 72°C for 5 min. Fisher's exact test (two-tailed; http://www.langsrud.com/fisher.htm) and the Bonferroni correction were used to analyze the data. The threshold for statistical significance after the Bonferroni correction was set at a P value of <0.05. The PCR revealed that 49 (23%) of the pathogenic strains studied harbored the *tcpC* sequence: 23 (21%) of our UTI *E. coli* isolates (21 isolates [21%] from individuals with symptomatic UTIs and 2 isolates [20%] from individuals with asymptomatic UTIs) and 26 (25%) of our SSTI *E. coli* isolates. The prevalence of *tcpC* was much lower among commensal *E. coli* isolates, only 7 (8%), as was found in a recent study by Cirl et al. (1). Comparison of the prevalence of *tcpC* among the UTI

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TABLE 1. Sequences of primers used in this study

Functional category	Primer	Primer sequence (5′ to 3′)	Reference
Phylogenetic group	ChuA.1	GACGAACCAACGGTCAGGAT	2
	ChuA.2	TGCCGCCAGTACCAAAGACA	
	YjaA.1	TGAAGTGTCAGGAGACGCTG	
	YjaA.2	ATGGAGAATGCGTTCCTCAAC	
	TspE4C2.1	GAGTAATGTCGGGGCATTCA	
	TspE4C2.2	CGCGCCAACAAAGTATTACG	
Toxins			
Cytotoxic necrotizing factor (*cnf1*)	CNF1-1	CTGACTTGCCGTGGTTTAGTCGG	6
	CNF1-2	TACACTATTGACATGCTGCCCGGA	
Hemolysin A (*hlyA*)	hlyA.1	AACAAGGATAAGCACTGTTCTGGCT	
	hlyA.2	ACCATATAAGCGGTCATTCCCGTCA	
Fimbriae and/or adhesins			
P-fimbrial adhesin II (*papGII*)	papG_II f	GGGATGAGCGGGCCTTTGAT	4
	papG_II r	CGGGCCCCCAAGTAACTCG	
P-fimbrial adhesin III (*papGIII*)	papG_III f	CCACCAAATGACCATGCCAGAC	15
	papG_III r	GGCCTGCAATGGATTTACCTGG	
S fimbriae (*sfaDE*)	SFA-1	CTCCGGAGAACTGGGTGCATCTTAC	7
		CGGAGGAGTAATTACAAACCTGGCA	
Afa/Dr adhesins (*afa*/*draBC*)	afa/draBC-f	GGCAGAGGGCCGGCAACAGGC	3
	afa/draBC-r	CCCGTAACGCGCCAGCATCTC	
Iron uptake			
Aerobactin synthesis (*iucD*)	Aer1	TACCGGATTGTCATATGCAGACCGT	15
	Aer2	AATATCTTCCTCCAGTCCGGAGAAG	
Other			
Uropathogenic strain-specific protein (*usp*)	N6	ATGCTACTGTTTCCGGGTAGTGTGT	8
	N7	CATCATGTAGTCGGGGCGTAACAAT	
TIR domain-containing protein (*tcpC*)	tcpC for	GGCAACAATATGTATAATATCCT	
	tcpC rev	GCCCAGTCTATTTCTGCTAAAGA	1

isolates of the two studies was not possible, as we could not obtain data on the type of symptomatic UTI (cystitis, pyelonephritis), and furthermore, the number of asymptomatic UTI isolates was too small ($n = 10$) to be statistically relevant. As seen from Table 2, strong statistical correlations were found between the presence of *tcpC* and the B2 phylogenetic group, as well as between the presence of *tcpC* and the presence of *cnf1*, *hlyA*, *papGIII*, *sfaDE*, and *usp* among UTI isolates, as well as commensal strains. Among the SSTI isolates, statistically significant associations were found only between the presence

TABLE 2. Distribution of phylogenetic groups and virulence factors in relation to the presence of *tcpC*

Phylogenetic group or virulence factor	Prevalence (no. [%] of strains)[a]							
	UTI + SSTI isolates		UTI isolates		SSTI isolates		Commensal isolates	
	tcpC positive (49 [23])	*tcpC* negative (163 [77])	*tcpC* positive (23 [21])	*tcpC* negative (87 [79])	*tcpC* positive (26 [25])	*tcpC* negative (76 [75])	*tcpC* positive (7[8])	*tcpC* negative (83 [92])
Phylogenetic group								
A	5 (10)	35 (21)	0 (0)	28 (32)**	5 (19)	7 (9)	0 (0)	20 (24)
B1	3 (6)	13 (8)	0 (0)	6 (7)	3 (12)	7 (9)	0 (0)	13 (16)
B2	40 (82)	81 (50)***	23 (100)	32 (37)***	17 (65)	49 (64)	7 (100)	23 (28)**
D	1 (2)	34 (21)**	0 (0)	21 (24)*	1 (4)	13 (17)	0 (0)	27 (33)
Virulence factor								
cnf1	33 (67)	25 (15)***	16 (70)	9 (10)***	17 (65)	16 (21)**	4 (57)	1 (1)**
hlyA	33 (67)	26 (16)***	18 (78)	10 (11)***	15 (58)	16 (21)*	5 (71)	2 (2)***
papGIII	19 (39)	10 (6)***	11 (48)	3 (3)***	8 (31)	7 (9)	3 (43)	0 (0)**
papGII	13 (27)	34 (21)	11 (48)	26 (30)	2 (8)	8 (11)	0 (0)	7 (8)
sfaDE	33 (67)	30 (18)***	19 (83)	7 (8)***	14 (54)	23 (30)	7 (100)	8 (10)***
afa/*draBC*	0 (0)	3 (2)	0 (0)	2 (2)	0 (0)	1 (1)	0 (0)	4 (5)
iucD	24 (49)	70 (43)	13 (57)	33 (38)	11 (42)	37 (49)	2 (29)	33 (40)
usp	44 (90)	49 (30)***	22 (96)	26 (30)***	22 22 (85)	23 (30)*	6 (86)	1 (1)***
Average virulence score	4.06	1.52		4.78, 1.33		3.19, 1.72		3.86, 0.67

[a] The *P* values obtained following Bonferroni correction are indicated by asterisks when *P* is <0.05, as follows: *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$.

of *tcpC* and the presence of *cnf1*, *hlyA*, and *usp*. As ExPEC strains mainly belong to the B2 phylogenetic group, these correlations and the higher virulence scores of the *tcpC*-encoding strains are not surprising. Interestingly, when the UTI and SSTI isolates were compared, major differences were observed. While the prevalence rates of *tcpC* sequences were similar in both groups, 21% among UTI isolates and 25% among SSTI isolates, suggesting an important role of TcpC in UTIs as well as in SSTIs, *P* values establishing significant correlations were higher among UTI isolates than among SSTI isolates. The differences between the UTI and SSTI *E. coli* strains observed are most likely due to differences in pathogenic mechanisms; nevertheless, the possession of TcpC seems to be an important factor in establishing UTIs and SSTIs. As the bowel flora is a reservoir of ExPEC, it is not surprising that *tcpC* was also found to be significantly associated with the B2 phylogenetic group among commensal strains. Our results suggest that even though *E. coli* strains able to induce disease outside the gastrointestinal tract are collectively designated ExPEC (11), it could be worthwhile to consider strains from different sites or syndrome-specific pathotypes separately.

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