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Molecular Epidemiological Characterization of Uropathogenic *Escherichia coli* from an Outpatient Urology Clinic in Rural Japan

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In the remote Japanese community of Saku, a rural town in the Nagano Prefecture, a large proportion of outpatient urinary tract infections was caused by well-recognized globally dispersed clonal lineages of uropathogenic *Escherichia coli* (UPEC). However, most of these strains were drug susceptible, suggesting that factors other than selection pressure account for the clonal spread of drug-susceptible UPEC.

In Japan, most characterizations of uropathogenic *Escherichia coli* (UPEC) strains have been limited to hospital settings where the proportion of drug-resistant strains may be higher than that in community-associated UPEC strains (1–4). Therefore, to assess the prevalence of antimicrobial resistance and the clonal composition of UPEC in a community setting, we studied patients at an outpatient clinic in Saku, a remote rural town in the Nagano Prefecture of Japan.

Patients presenting to the clinic between October 2008 and September 2009 with clinically suspected urinary tract infection (UTI) and the presence of pyuria were recruited into the study. All men and women reporting any urinary tract abnormality, history of urinary catheter use, cancer, diabetes, or other medical condition requiring hospitalization were classified as having a complicated UTI. Women without these features were classified as having an uncomplicated UTI. Patients also completed a questionnaire which included various medical, exposure, and demographic questions. Each patient provided written informed consent to participate. Approval was obtained from the institutional review board of the National Institute of Infectious Diseases, Japan. Antimicrobial susceptibility testing was performed for all *E. coli* isolates with the Vitek 2 compact system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. The antimicrobial agents tested were cefazolin, cefotiam, ceftriaxone, piperacillin-tazobactam, levofloxacin, and trimethoprim-sulfamethoxazole (SXT). An isolate was considered to be multidrug resistant if it exhibited nonsusceptibility to at least one agent in three or more antimicrobial categories (5). Each *E. coli* isolate was typed by multilocus sequence typing (MLST). MLST was performed according to the protocol described on the University of Warwick MLST database website (http://mlst.warwick.ac.uk/mlst/). Allelic profiles and sequence types (ST) were assigned according to the aforementioned website's scheme. Clonal complexes (CCs) were defined as groups of isolates with an identical ST or one or two allele differences from another ST within the CC. The risk factors were compared by assessing odds ratios (OR) with 95% Cornfield approximation confidence intervals (CI). Statistical significance was defined as a P value of <0.05.

Among 199 participants, 129 completed the questionnaire. Of these, 114 (88%) were women and 15 (12%) were men; 99 (77%) had an uncomplicated UTI, whereas 30 (23%) had a complicated UTI. Patients who had diarrhea during the 6-month period prior to the current UTI and patients who lived in a household of more than three people were more likely to have uncomplicated UTI (OR, 2.44 [95% CI, 1.06 to 5.61] [$P < 0.05$] and 2.39 [95% CI, 1.03 to 5.57] [$P < 0.05$], respectively). Conversely, patients who were older than 65 years old and patients who were hospitalized during the 1 year period prior to the current UTI case had increased odds of having a complicated UTI (OR, 6.34 [95% CI, 2.48 to 16.19] [$P < 0.001$] and 14.76 [95% CI, 2.88 to 75.78] [$P < 0.001$], respectively).

Of the 199 urine samples, 159 distinct bacterial isolates were identified, of which 80 (50%) were *E. coli*. The highest prevalence of resistance was observed for SXT at 8%, followed by levofloxacin at 6%. Four isolates were multidrug resistant. Of 80 *E. coli* isolates, 66 (82.5%) grouped into 15 CCs (Table 1). Each of the remaining 14 (17.5%) isolates belonged to a distinct ST. The most frequent CC was CC95, which constituted 20 (25%) of the 80 isolates; 17 were ST95, 2 were ST979, and 1 was ST3664. CC131 comprised 7 (8.8%) isolates, of which 6 were ST131 and 1 was ST2279. Of the six ST131, four were serotyped as O25:H4.

The association of CC95 isolates with various patient characteristics was examined by univariate analyses (Table 2). Patients who lived with three or more people had higher odds of CC95 isolation than those who lived with fewer than two people (OR,

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TABLE 1 Characteristics of *E. coli* MLST clonal complexes

Clonal complex	No. (%) of isolates	Serogroup(s)	ECOR[b] phylogenetic group
95	20 (25)	O1, O18, O125	B2
131	7 (8.8)	O25, OUT[a]	B2
357	6 (7.5)	OUT	
73	6 (7.5)	O6, O25, OUT	B2
69	4 (5)	O25, O15, OUT	D
23	3 (3.8)	O78, OUT	
405	3 (3.8)	OUT	
568	3 (3.8)	OUT	
86	2 (2.5)	OUT	
14	2 (2.5)	O18, OUT	
144	2 (2.5)	OUT	
127	2 (2.5)	O6	
141	2 (2.5)	OUT	
676	2 (2.5)	OUT	
420	2 (2.5)	O125, O8	

[a] OUT, O antigen untypeable.
[b] ECOR, *Escherichia coli* reference.

TABLE 2 Univariate analysis of risk factors for CC95 and non-CC95 infections[a]

Risk factor	Questionnaire ($n = 62$)				
	CC95 ($n = 16$)	Non-CC95 ($n = 46$)	OR	95% CI	*P* value[b]
Mean age (yrs)	42	46			0.38
Age ≥ 65 yrs	1	10	0.24	(0.01, 2.00)	0.16
Gender					
Female	16	45			
Male	0	1			
UTI type					
Complicated	2	9	0.59	(0.06, 3.41)	0.52
Uncomplicated	14	37			
Diarrhea in past 6 mo	11	23	2.20	(0.67, 7.05)	0.19
Treated with antimicrobial drugs	0	0			
Recurrent UTI					
Past 3 mo	1	2	1.47	(0.02, 29.9)	0.76
Past yr	11	31	1.06	(0.28, 4.63)	0.92
Treated with antimicrobial drugs	11	25	1.85	(0.49, 7.84)	0.31
Antimicrobial drug use in past 1 mo	0	4			
Hospitalization					
Past 1 mo	0	2			
Past 1 yr	1	6	0.44	(0.01, 4.21)	0.46
No. in household					
≥3	15	29	8.79	(1.12, 391.4)	<0.05
≤2	1	17			
Travel					
Outside prefecture in past 3 mo	7	21	0.93	(0.25, 3.36)	0.89
Foreign country in past 1 yr	0	3			
Sick during travel	0	0			

[a] CC, clonal complex; OR, odds ratio; CI, cornfield approximation confidence interval.
[b] Two-tailed Fisher exact test results were reported when ≤5 patients were in a category.

8.79 [95% CI, 1.12 to 391.4] [$P < 0.05$]). Adjusting for age group (≥65 or <65 years), a significant association was still found between the number of people in the household and CC95 isolation (Mantel Haenszel odds ratio, 7.00 [95% CI, 0.73 to 66.7] [$P < 0.05$]).

In this study, a high proportion of the *E. coli* isolates grouped into 15 CCs. These findings suggest that a distinct and limited number of strains cause UTI in this community. The most common lineage was CC95. Interestingly, we observed that patients who share a household with three or more members had higher odds of being infected with CC95 than those who lived with two or fewer members. A previous study reported that within-household sharing of fecal *E. coli* clones, including those isolated from patients with acute UTI, varied linearly with the number of individuals per household (6). If the within-household spread of *E. coli* clones is a common occurrence, CC95 may be a clone adapted to being transmitted among humans. ST95 has also been increasingly associated with other extraintestinal diseases in human and avian hosts, particularly in poultry (7–9). In recent years, the zoonotic potential of ST95 has been reported, suggesting a threat to human and animal health (10).

Even in this relatively remote rural community, UPEC strains recognized to belong to globally dispersed clonal groups (ST131, ST69, ST95) were found to predominate. ST131, a clonal group commonly associated with CTX-M type extended-spectrum β-lactamase (ESBL) production, has been found across Canada, Europe, the Middle East, and Asia, including Japan (1, 11–15). Interestingly, none of the ST131 strains identified in this study produced ESBL. It is not clear why these ST131 strains have not yet gained the CTX-M type ESBL genes (11, 13, 16). In Japan, SXT is not indicated in guidelines for the treatment of uncomplicated UTI, and the use of SXT is highly limited (17). Despite the limited use of SXT, the rates of nonsusceptibility to SXT have been documented to be consistently over 10% among *E. coli* isolates collected from hospitals in Japan over the past decade (18, 19). Indeed, *E. coli* exhibited the highest rate of nonsusceptibility to SXT in our study. Since the clinical use of SXT is limited in Japan, the possibility of resistance resulting from selection pressure of high clinical usage of this drug is unlikely. It is possible that SXT use in agriculture and animal husbandry may not only be maintaining the pool of drug-resistant strains but also the mobile genetic elements harboring drug-resistant genes that are introduced into the human population (20, 21). This study demonstrates that even in a relatively remote community in Japan, UPECs, whether resistant or not, exhibit a clonal distribution. The clinical use of antimicrobial agents can select for drug-resistant UPEC, but additional factors may account for the clonal spread of drug-susceptible UPEC.

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