Vaginal *Escherichia coli* share common virulence factor profiles, serotypes and phylogeny with other extraintestinal *E. coli*

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Characteristics of *Escherichia coli* residing in the vagina and their role in extraintestinal infections are largely unknown. In this study, 88 vaginal *E. coli* (VEC) isolates from Japanese women were characterized by extraintestinal virulence factor (VF) profiling, O:H serotyping and phylogenetic analysis. The prevalence of *papC*, *hlyA*, *cnfl*, *PAI*, *ibeA* and K1 antigen among the VEC strains were 45, 22, 19, 78, 32 and 44%, respectively. Phylogenetic analysis identified 76, 16 and 8% of the VEC strains in groups B2, D and A, respectively. The VEC strains were distributed into 31 serotypes, including 8 common serotypes (O1:K1:H1, O1:K1:H7, O2:K1:H7, O4:H5, O6:H1, O18ac:K1:H7, O25:H1 and O75:HNM) that were identified in three or more isolates. Comparative analysis with 61 stool isolates from healthy Japanese men and women, and with data from previous studies, revealed that, although some geographical specificities do exist, the VEC strains shared common VF profiles, O:K:H serotypes and phylogeny with uropathogenic *E. coli* and *E. coli* of neonatal septicaemia and meningitis. This study provides additional evidence for a link among extraintestinal *E. coli*, supporting the concept that the VEC are a reservoir along the 'faecal-vaginal-urinary/neonatal' course of transmission in the extraintestinal *E. coli* infections.

Keywords: vagina, *Escherichia coli*, extraintestinal virulence factors, phylogenetic grouping, O:K:H antigens

INTRODUCTION

The vaginal microflora is normally made up of 5–15 different bacterial species, including aerobes and anaerobes, and has the potential to inflict different clinical syndromes (e.g. bacterial vaginosis) and diseases (e.g. pelvic inflammatory disease). Gram-negative facultative anaerobic *Escherichia coli* is one of the common organisms in the microflora of pregnant as well as non-pregnant women. *E. coli* have been reportedly identified in 9–28% of non-pregnant women (Bartlett *et al.*, 1977; Chow *et al.*, 1986; Tashjian *et al.*, 1976) and 24–31% of pregnant women (Hillier *et al.*, 1993; Tashjian *et al.*, 1976).

Vaginal colonization with *E. coli* is associated with

various genitourinary, obstetric and neonatal complications, such as the severe form of pelvic inflammatory disease (Heinonen & Miettinen, 1994; Larsen & Galask, 1980), urinary tract infections (O'Grady *et al.*, 1970; Stamey & Sexton, 1975), very-low-birth-weight infants (Krohn *et al.*, 1997) and early-onset neonatal septicaemia and meningitis (Sarff *et al.*, 1975; Schiffer *et al.*, 1976). Vaginal *E. coli* has also been reported to be sexually transmissible to a male partner (Hebelka *et al.*, 1993).

The *E. coli* implicated in various human infections can be broadly classified as intestinal and extraintestinal. Among the extraintestinal *E. coli*, uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC) have been characterized in some detail by diverse approaches including phylogenetic analysis, serotyping and molecular typing such as virulence factor (VF) profiling, multilocus enzyme electrophoresis (MLEE), outer-membrane protein profiling and plasmid profiling. Vagina-colonizing *E. coli*, however, remains largely uncharac-

Abbreviations: CSF, cerebrospinal fluid; HNM, H non-motile; MLEE, multilocus enzyme electrophoresis; NMEC, neonatal meningitis *E. coli*; PAI, pathogenicity-associated island marker; UPEC, uropathogenic *E. coli*; VEC, vaginal *E. coli*; VF, virulence factor.

terized with regard to its genotypes, serotypes, clonality and phylogenetic linkage with other *E. coli*.

In this paper we report on the characteristics of *E. coli* isolates from vaginal swab samples of both pregnant and non-pregnant women as determined by extraintestinal *E. coli* VF profiling, PCR-based phylogenetic grouping and O:H serotyping. We also report on the results of comparative analyses with *E. coli* isolates from normal stool samples and with previous data from stool, urine, blood and cerebrospinal fluid (CSF) isolates (Bingen *et al.*, 1997, 1998; Duriez *et al.*, 2001; Johnson & Stell, 2000; Johnson *et al.*, 2002; Miyazaki *et al.*, 2002; Sarff *et al.*, 1975; Siitonen, 1992; Tsukamoto, 1997; Yamamoto *et al.*, 1995b).

METHODS

Study populations. A total of 2575 vaginal swabs (one swab per patient) were collected from patients who visited the Obstetrics and Gynaecology Department in the Mito Saiseikai General Hospital, Ibaraki Prefecture, East Japan between May 1999 and February 2001. Normal stool samples from 106 healthy adult men and women working at a food court in the Tokyo University Hospital, Tokyo, East Japan were kindly provided by Dr Shigemi Hitomi (Department of Infectious Diseases, Institute of Clinical Medicine, University of Tsukuba, Japan).

For comparative analyses with respect to the characteristics of VEC, previously published data on the following were included: (i) VF profiles of 2245 *E. coli* strains of various sources from East Japan (Ibaraki), West Japan (Kyoto, Osaka, Shiga) and other countries, including strains isolated from adult stool [$n=80$ (Yamamoto *et al.*, 1995a), $n=287$ (Siitonen, 1992)], neonatal stool [$n=1169$ (Sarff *et al.*, 1975)], neonatal CSF (bacterial meningitis) [$n=67$ (Bingen *et al.*, 1997), $n=70$ (Johnson *et al.*, 2002)], neonatal blood (septicaemia) [$n=47$ (Bingen *et al.*, 1997)], adult urine (acute simple cystitis) [$n=194$ (Yamamoto *et al.*, 1995b), $n=256$ (Miyazaki *et al.*, 2002)] and adult blood (urosepsis) [$n=75$ (Johnson & Stell, 2000)]; (ii) phylogenetic analysis of 307 *E. coli* strains, including strains from normal stool [$n=168$ (Duriez *et al.*, 2001)], neonatal CSF (bacterial meningitis) [$n=69$ (Bingen *et al.*, 1998), $n=70$ (Johnson *et al.*, 2002)]; (iii) profiles of O:H serotypes and K1 antigen of 320 extraintestinal *E. coli* strains from neonatal CSF (bacterial meningitis) ($n=52$), neonatal blood (septicaemia) ($n=74$) and adult urine (acute simple cystitis) ($n=194$) from our previous study (Tsukamoto, 1997).

Sample collection and processing. After obtaining approval from the Ethical Committees of the hospitals concerned and informed consent from the patients, we collected clinical samples. The vaginal swabs were taken from the posterior vaginal fornix by experienced interns and were immediately streaked onto Trypticase soy/5% sheep blood agar and Drigalsky agar for aerobic cultures. The same swabs were used for microbial identification by Gram staining. Isolates that had been preliminarily identified as *E. coli* by colony morphology and Gram-stain reactions were confirmed using an API 20E (bioMérieux) kit. The normal stool *E. coli* strains isolated from Drigalsky agar were also confirmed using an API 20E (bioMérieux) kit. Boiled lysates from at least two batches of overnight-grown bacterial cultures were used as PCR templates for the determination of VFs and phylogenetic groups.

VF profiling. The prevalence of extraintestinal VFs among *E. coli* isolates was examined by a multiplex PCR-based screen-

ing method that uses the primer pairs described elsewhere: *papC* (pilus associated with pyelonephritis) [pap1/2: gacggctgtactgcagggtgtggcg/atactcttctgcaggatgcaata (Le Bouguenec *et al.*, 1992)]; *sfaDE* (S fimbriae) [sfa1/2: ctccggagaactgggtgca-tcttac/cggaggagtaattacaaacctggca (Johnson & Brown, 1996)]; *afa/draBC* (afimbrial adhesins) [afa1/2: gctgggcagcaactgata-actctc/catcaagctgtttgtctcgccg (Le Bouguenec *et al.*, 1992)]; *iucD* (aerobactin synthesis) [aer1/2: taccggattgtcatatgcagaccgt/aatatcttctccagtcggagaag (Yamamoto *et al.*, 1995a)]; *cnf1* (cytotoxic necrotizing factor) [cnf1/2: aagatggagtttctatgcag-gag/cattcagagtctctgcctcattatt (Yamamoto *et al.*, 1995a)]; *hlyA* (haemolysin) [hly1/2: aacaaggataagcactgttctggct/accatataag-cggctattcccgta (Yamamoto *et al.*, 1995a)]; PAI (pathogenicity-associated island marker) [RPAi f/r: ggacatctgttacagcgca/ca/tcgccaccaatcacagccgaac (Johnson & Stell, 2000)]; *fimH* (mannose-specific adhesin subunit of type 1 fimbriae) [fimH f/r: tgcagaacggataagccgtgg/gcagtcacgtccctccggta (Johnson & Stell, 2000)]; and *ibeA* (invasion brain endothelium) [ibe10 f/r: aggaggtgtgcccgcgtac (Huang *et al.*, 1995) and tgggtctcc-ggcaaacatgc (Johnson & Stell, 2000)].

A PCR mixture (50 μ l) containing 5 μ l bacterial lysate, 62.5 μ M dNTPs, 5 μ l 10 $\times$ PCR buffer (100 mM Tris/HCl, pH 8.8, 500 mM KCl, 15 mM MgCl₂, 1% Triton X-100), Ex Taq DNA polymerase (0.03 U μ l⁻¹) (TaKaRa Shuzo) and primer set A (20 pmol each of pap1/2, sfa1/2, afa1/2, aer1/2 and cnf1/2 primers; 30 pmol each of hly1/2 primers) or primer set B (10 pmol each of RPAi f/r, fimH f/r and ibe10 f/r primers) was amplified in a thermal cycler (PTC-100; MJ Research) for 30 cycles with a profile of 94 °C for 1 min, 63 °C for 30 s and 72 °C for 3 min, followed by a final extension at 72 °C for 3 min. Amplified products were separated in 2% agarose gels, and ethidium bromide-stained gels were visualized with an ultraviolet transilluminator for photographic imaging.

Three methods were used for K1 antigen determination: (i) PCR-based detection of *neuS* (K1-specific gene) (Tsukamoto, 1997), (ii) antiserum and (iii) bacteriophage-based detection using five K1-specific bacteriophages (Gross *et al.*, 1977). Five bacteriophages specific to the K1 polysaccharide antigen of *E. coli* were kindly supplied by Dr B. Rowe (Central Public Health Laboratory, London, UK) (Gross *et al.*, 1977).

Phylogenetic analysis. Phylogenetic classification of *E. coli* isolates was determined using triplex PCR-based phylotyping described by Clermont *et al.* (2000), which is a simple method comparable to MLEE-based typing. Briefly, genomic DNA of bacterial strains was amplified by triplex PCR using primers targeted to three markers, *chuA*, *yjaA* and TspE4.C2. The phylogenetic grouping was made on the basis of the presence of specific PCR-amplified fragments as follows: group B2 (*chuA*+, *yjaA*+, TspE.C2 $\pm$), group D (*chuA*+, *yjaA*-, TspE.C2 $\pm$), group B1 (*chuA*-, *yjaA* $\pm$, TspE.C2+) and group A (*chuA*-, *yjaA* $\pm$, TspE.C2-).

O:H serotyping. The *E. coli* isolates were serotyped in the Osaka Prefectural Institute of Public Health, Japan, following the method described by Orskov & Orskov (1975). The O-antigen confirmation for some questionable isolates was performed by the Statens Serum Institute, Denmark. PCR-based H7 typing (Tsukamoto & Kanki, 1999) was used to assign H7 to some non-motile (HNM) strains.

Statistical analysis. Significance of differences between the variables was tested with the χ^2 test and the Fisher's exact test.

RESULTS

Eighty-eight VEC isolates from 2575 vaginal swabs were grouped into four subsets according to the clinical background of patients (Table 1) and examined for

Table 1. Clinical backgrounds of patients in this study

	Non-pregnant women		Pregnant women	
	Asymptomatic (<i>n</i> = 5)	Symptomatic (<i>n</i> = 43)	Asymptomatic (<i>n</i> = 20)	Symptomatic (<i>n</i> = 20)
Age (years, mean)	59.6	44.9	29.2	29.9
Gravidity (mean)	2.6	2.19	1.2	1.4
Parity (mean)	2.0	1.35	0.7	0.8
Gestational weeks (mean)			14.0	30.5
Clinical history*				
None	5†		20‡	
Lower abdominal pain		5		
Atypical bleeding		3		
Discharge		23		
Itchiness		10		
Signs and symptoms of cystitis		1		
External genital pain		1		
Preterm labour				11
Preterm PROM§				6
Triplet pregnancy				2
Intrauterine fetal death				1

* Only chief complaints are listed.

† Patients visited for screening of cancer and sexually transmitted diseases.

‡ Patients visited for prenatal care.

§ PROM, premature rupture of membranes.

Table 2. Prevalence of virulence factors among vaginal *E. coli* isolates

Data are shown as no. (%).

Virulence factor	Non-pregnant women		Pregnant women		Total (<i>n</i> = 88)
	Asymptomatic (<i>n</i> = 5)	Symptomatic (<i>n</i> = 43)	Asymptomatic (<i>n</i> = 20)	Symptomatic (<i>n</i> = 20)	
Adhesins					
<i>papC</i>	3 (60)	20 (47)	7 (35)	10 (50)	40 (45)
<i>sfaDE</i>	1 (20)	8 (19)	3 (15)	6 (30)	18 (20)
<i>afa/draBC</i>	0	3 (7)	1 (5)	1 (5)	5 (6)
<i>fimH</i>	5 (100)	43 (100)	20 (100)	20 (100)	88 (100)
Toxins					
<i>blyA</i>	1 (20)	10 (23)	2 (10)	6 (30)	19 (22)
<i>cnf1</i>	1 (20)	8 (19)	2 (10)	6 (30)	17 (19)
Siderophores					
<i>iucD</i>	3 (60)	16 (37)	4 (20)	8 (40)	31 (35)
Invasin					
<i>ibeA</i>	1 (20)	15 (35)	7 (35)	5 (25)	28 (32)
PAI*	4 (80)	34 (79)	17 (85)	14 (70)	69 (78)
K1	2 (40)	21 (49)	11 (55)	5 (25)	39 (44)

* PAI, *Escherichia coli* CFT073 antiterminator homologue and MalX homologue genes in pathogenicity-associated island.

extraintestinal VF profiles, phylogenetic groups and O:H serotypes. Sixty-one additional *E. coli* isolates from the stool samples of 106 healthy people were also

included for extraintestinal VF profiling and phylogenetic analysis. The VF profiles of 2245 *E. coli* strains, together with the phylogenetic grouping of 307 *E. coli*

Table 3. Prevalence of virulence factors among *E. coli* from various sources

Data are shown as no. (%).

Virulence factor	Present study		Previous studies						
	Vagina* (n = 88)	Stool† (n = 61)	Stool‡ (n = 80)	Urine‡ (n = 194)	Urine§ (n = 256)	Blood (n = 75)	Blood¶ (n = 47)	CSF¶ (n = 67)	CSF# (n = 70)
Adhesins									
<i>papC</i>	40 (45)	11 (18)	22 (28)	125 (64)	107 (42)	58 (77)	24 (51)	33 (50)	
<i>sfaDE</i>	18 (20)	7 (11)	11 (14)	71 (37)	44 (17)	19 (25)	14 (30)	29 (44)	43 (61)
<i>afa/draBC</i>	5 (6)	4 (7)	0 (0)	17 (9)	13 (5)	7 (9)	3 (6)	2 (3)	18 (26)
<i>fimH</i>	88 (100)	60 (98)				75 (100)			
Toxins									
<i>blyA</i>	19 (22)	3 (5)	14 (18)	80 (41)	48 (19)	31 (41)			6 (9)
<i>cnf1</i>	17 (19)	4 (7)	10 (13)	61 (31)	44 (17)	12 (16)			6 (9)
Siderophore									
<i>iucD</i>	31 (35)	18 (30)	17 (21)	93 (48)	104 (41)				
Invasin									
<i>ibeA</i>	28 (32)	16 (26)				4 (5)	8 (17)	22 (32)	23 (33)
PAI**	69 (78)	28 (46)				53 (71)			55 (79)
K1	39 (44)	13 (21)		93 (48)			33 (70)	55 (83)	57 (81)

* Isolates from East Japan (Ibaraki).

† Isolates from East Japan (Tokyo).

‡ Strains from West Japan (Kyoto, Osaka, Shiga), as detected by K1 antigen-specific phages and probe hybridization for other virulence factors (Yamamoto *et al.*, 1995b; Tsukamoto *et al.*, 1997).§ Strains from East Japan (Ibaraki), as detected by PCR (Miyazaki *et al.*, 2002).

|| As detected by PCR (Johnson & Stell, 2000).

¶ Strains from neonates, as detected by antisera for K1 antigen and PCR for other virulence factors (Bingen *et al.*, 1997).# Strains from neonates, as detected by probe hybridization and PCR (Johnson *et al.*, 2002).** PAI, *Escherichia coli* CFT073 antiterminator homologue and MalX homologue genes in pathogenicity-associated island.

strains and serotype data of 320 extraintestinal *E. coli* strains were used for comparison with that of the VEC isolates.

VF profile

The prevalence of VFs among the VEC isolates of four clinical subsets is shown in Table 2. There were no significant differences between the non-pregnant women and the pregnant women or between the asymptomatic women and the symptomatic women.

Table 3 shows the comparison of the prevalence of VFs among the *E. coli* isolates from the present study and previous studies. The prevalence of *papC* (45%), *blyA* (22%), *cnf1* (19%) and PAI (78%) in the VEC isolates was significantly higher than that in the normal stool *E. coli* isolates of this study ($P < 0.05$). The prevalence of *papC*, *sfaDE*, *afa/draBC*, *blyA*, *cnf1* and *iucD* of our VEC isolates in Ibaraki prefecture were very similar to those of *E. coli* strains from cystitis in the same prefecture (Miyazaki *et al.*, 2002), whereas, compared with those in *E. coli* isolates of cystitis in West Japan and of urosepsis origins, the prevalence of *papC*, *blyA* and

cnf1 in the VEC isolates appeared lower ($P < 0.05$). The prevalence of *ibeA* in the VEC isolates (32%) matched that in the NMEC strains (Table 3) (Bingen *et al.*, 1997; Johnson *et al.*, 2002) and was significantly higher than that in the uroseptic strains (5%) (Table 3) (Johnson & Stell, 2000) ($P < 0.0001$).

The prevalence of K1 in the VEC isolates (44%) was significantly higher than that in the normal stool *E. coli* isolates (21%) of this study (Table 3) ($P < 0.005$) and that in the normal stool *E. coli* strains from adults (30%) and neonates (19%) of previous studies (Sarff *et al.*, 1975; Siitonen, 1992) ($P < 0.05$), but was similar to that in the UPEC strains (48%) (Table 3) (Tsukamoto, 1997; Yamamoto *et al.*, 1995b). About 92% (48/52) of NMEC strains and 61% (45/74) of neonatal septicaemia *E. coli* strains were K1 positive in our previous study (supplementary data available at <http://mic.sgmjournals.org>. See also Tsukamoto, 1997). The mean prevalence of K1 among the NMEC strains (48 + 55 + 57/52 + 67 + 70; 85%) (Bingen *et al.*, 1997; Johnson *et al.*, 2002; Tsukamoto, 1997) was significantly higher than that among the neonatal septicaemia strains (45 + 33/74 + 47; 64%) (Bingen *et al.*, 1997;

Table 4. Distribution of phylogenetic groups among vaginal *E. coli* isolates

Data are shown as no. (%).

Phylogenetic group	Non-pregnant women		Pregnant women		Total (n=88)
	Asymptomatic (n=5)	Symptomatic (n=43)	Asymptomatic (n=20)	Symptomatic (n=20)	
A	1 (20)	2 (5)	1 (5)	3 (15)	7 (8)
B1	0	0	0	0	0
B2	3 (60)	33 (77)	17 (85)	14 (70)	67 (76)
D	1 (20)	8 (19)	2 (10)	3 (15)	14 (16)

Table 5. Distribution of phylogenetic groups among *E. coli* from various sources

Data are shown as no. (%).

Phylogenetic group	Present study		Previous studies		
	Vagina (n=88)	Stool (n=61)	Stool* (n=168)	CSF† (n=69)	CSF‡ (n=70)
A	7 (8)	17 (28)	67 (40)	4 (6)	1 (1)
B1	0	0	57 (40)	0	7 (10)
B2	67 (76)	27 (44)	19 (11)	47 (68)	57 (81)
D	14 (16)	17 (28)	25 (15)	18 (26)	5 (7)

* As detected by multiplex PCR (Duriez *et al.*, 2001).† As detected by RFLP (Bingen *et al.*, 1998).‡ As detected by RAPD (Johnson *et al.*, 2002).

Tsukamoto, 1997) and the prevalence of K1 among *E. coli* from any source (Table 3) ($P < 0.0001$).

Phylogenetic groups

There were no significant differences in the distribution of phylogenetic groups between the VEC subsets (non-pregnant vs pregnant or asymptomatic vs symptomatic) (Table 4).

The prevalence of groups B2 and D in the VEC isolates (92%) was significantly higher than that in the normal stool isolates from this study (72%) (Table 5) ($P < 0.0005$). The VEC isolates shared a very similar distribution of phylogenetic groups with the CSF isolates (Table 5) (Bingen *et al.*, 1997; Johnson *et al.*, 2002). The prevalence of groups B2 and D in the normal stool isolates from this study was far higher than that in the normal stool isolates from the previous study (26%; Table 5, $P < 0.0001$) (Duriez *et al.*, 2001).

Serotypes

A supplementary table is available at <http://mic.sgmjournals.org>. See also Tsukamoto (1997). Among the VEC isolates, 71% (62/88) were typable for O antigen, whereas 25% (22/88) were O non-typable and

5% (4/88) were O rough. The 62 O-typable VEC strains were found to be distributed into 20 serogroups or 31 different serotypes including HNT (H non-typable) and HNM (H non-motile). Identified in three or more VEC isolates were 8 common serotypes, including O1:K1:H1 ($n=5$), O1:K1:H7 ($n=5$), O2:K1:H7 ($n=6$), O4:H5 ($n=3$), O6:H1 ($n=3$), O18ac:K1:H7 ($n=4$), O25:H1 ($n=3$) and O75:HNM ($n=6$). These serotypes accounted for 56% (35/62) of serotypable isolates.

Among the 31 VEC serotypes, 10 serotypes (O1:K1:H7, O2:K1:H7, O4:H5, O6:H1, O6:NM, O16:K1:H6, O16:K1:NM, O18ac:K1:H7, O75:HNM and O77:HNM) were also identified in the strains from urine and neonates. Seven ONT:K1:H4 strains were observed among the VEC isolates. All of them belonged to phylogenetic group B2 and had identical VF profiles; they all were positive for *ibeA*, PAI and *fimH*, and negative for other VFs.

DISCUSSION

The relatively low presentation of *papC*, *blyA* and *cnf1* of VEC and UPEC strains in East Japan (Ibaraki prefecture) compared to that of UPEC strains in West Japan indicates the geographical distribution of VFs. Similarly, the lower presentation of *papC*, *blyA* and *cnf1*

was also seen in the stool strains of East Japan (18%, 5% and 7%) compared to that of West Japan (28%, 18% and 13%). The high occurrence of *ibeA* among VEC strains appears to reflect the population under scrutiny because the normal stool strains in this study also exhibited a higher prevalence of *ibeA* (26%) than that in the other stool isolates (2%) (Duriez *et al.*, 2001). The increasing occurrence of K1 antigen among the *E. coli* from the stool, the vagina and the neonatal blood to neonatal CSF indicates a progressive accumulation of K1-positive *E. coli* strains along the faecal–vaginal–neonatal course of infection. Since the K1 capsular antigen is known for serum resistance, anti-phagocytosis and crossing the blood–brain barrier (Kim, 2001), the selection of faecal strains carrying such a pathogenic trait by the vaginal ecosystem could play an important role in extraintestinal pathogenesis.

Taken together, the VEC strains are very similar to other extraintestinal *E. coli* with respect to the overall distribution of VFs. With their common VF profiles, all extraintestinal *E. coli* can be considered ‘virulent clones’ (Finlay & Falkow, 1997; Ochman *et al.*, 2000). This in turn supports the concept of the recently proposed term, ‘extraintestinal pathogenic *E. coli*’ (ExPEC) (Russo & Johnson, 2000).

Among four recognized phylogenetic groups (A, B1, B2 and D) (Herzer *et al.*, 1990; Selander *et al.*, 1996), the virulent extraintestinal *E. coli* strains belong mainly to group B2 and, to a lesser extent, to group D, whereas most commensal strains belong to group A (Bingen *et al.*, 1998; Johnson & Stell, 2000; Picard *et al.*, 1999). The fact that most of the VEC strains (92%) were grouped into B2 or D suggests that most of the VEC strains are potentially virulent. It should be noted, however, that this high occurrence of group B2 (76%) in the VEC strains might have originated from the higher occurrence of group B2 (44%) in the commensal faecal *E. coli* strains of Japanese populations. Duriez *et al.* (2001) has suggested that the commensal strains are phylogenetically distributed among geographically distinct human populations. This hypothesis could explain the difference between the distribution of phylogenetic groups of Japanese faecal strains (this study) and that of European and African faecal strains (Duriez *et al.*, 2001). Therefore, it is worth examining more VEC isolates from distinct geographical populations to better understand the role of VEC in extraintestinal infections.

The serogroups O1, O2, O4, O6, O18, O25 and O75 found in three or more VEC strains have also been reported as the common serogroups in UPEC (Czirok *et al.*, 1986; Korhonen *et al.*, 1985; Mulder *et al.*, 1984; Orskov *et al.*, 1982; Sandberg *et al.*, 1988; Stenqvist *et al.*, 1987; Vaisanen-Rhen *et al.*, 1984) and *E. coli* of neonatal meningitis or septicaemia (Cross *et al.*, 1984; Korhonen *et al.*, 1985; McCabe *et al.*, 1978; McCracken *et al.*, 1974; Robbins *et al.*, 1974). Among 10 serotypes that were distributed elsewhere in the VEC and other extraintestinal strains of our study, O18ac:K1:H7 strains of cystitis, neonatal bacterial meningitis

and faecal origin have already been shown to be clonally derived (Johnson *et al.*, 2001). The strains belonging to the remaining nine serotypes could also be clonal, though further examination is needed for confirmation.

Inasmuch as 39% (12/31) of serotypes in the VEC strains were restricted to the vagina and the remaining 61% (19/31) can be found elsewhere, it is likely that a subset of the VEC, the former group, is confined locally as the vagina-specific residents whilst the latter group can spread to and exist in other extraintestinal sites.

In conclusion, our data demonstrate that the VEC share common VF profiles, phylogenetic groups and serotypes with *E. coli* strains from urinary and neonatal (blood and CSF) origins. This study provides additional evidence for a link among extraintestinal *E. coli*, which in turn supports the concept that VEC are a reservoir along the ‘faecal–vaginal–urinary/neonatal’ course of transmission in extraintestinal *E. coli* infections.

ACKNOWLEDGEMENTS

We thank Dr Kozo Fujita (Sapporo City Institute of Public Health, Japan), Dr Hiroyuki Kitajima (Osaka Medical Center and Research Institute for Maternal and Child Health, Japan), Dr Shingo Yamamoto (Department of Urology, Faculty of Medicine, Kyoto University, Japan) and Dr Shigemi Hitomi (Department of Infectious Diseases, Institute of Clinical Medicine, University of Tsukuba, Japan) for providing bacterial strains. This work was supported by Grant No. 12877045 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Received 20 March 2002; revised 29 May 2002; accepted 30 May 2002.