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Short Communication

The Incidence of *Escherichia coli* Having Pathogenic Genes for Diarrhea: A Study in the People's Democratic Republic of Lao

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SUMMARY: The incidence of *Escherichia coli* having pathogenic genes for diarrhea was studied in Laos in 2002. A total of 525 *E.coli* strains from 278 patients (basically, two *E. coli* isolates from each patient) were examined by PCR to detect the known pathogenic genes (*stx*, *eae*, *elt*, *est*, *ipaH*, and *aggR*). These genes were detected in 23 strains from 16 patients (16/278: 5.8%). In 10 cases of the 16, one of the two isolates from each individual was negative for the gene, and in the other six cases, both isolates had the gene (same gene in four cases). *E. coli* having *eae* but no *stx* (enteropathogenic *E. coli* [EPEC]) was found in two cases out of 278 (0.7%). Nevertheless, Class I classical EPEC (serogroup-based) was found in 77 cases (28%). Enterotoxigenic *E. coli*, enteroaggregative *E. coli*, and enterohemorrhagic *E. coli* were found in 9, 4, and 1 cases, respectively. Enteroinvasive *E. coli* was not detected. This study suggested that the incidence of diarrhea due to *E. coli* is not as high as has been previously thought.

Lao People's Democratic Republic (Lao PDR, or Laos) is a landlocked country located in the center of the Indochina Peninsula. Diarrheal disease, especially in young children, is still a major public health problem in developing countries such as Laos. It has been believed that *Escherichia coli* is one of the most important etiologic agents of childhood diarrhea. Diarrheagenic *E. coli* has been classified into at least five categories (1), including enteropathogenic *E. coli* (having *eae* but not *stx*: EPEC), enteroinvasive *E. coli* (having invasive plasmid genes: EIEC), enterotoxigenic *E. coli* (having *est* and /or *elt*: ETEC), enterohemorrhagic *E. coli* (having *stx*: EHEC), and enteroaggregative *E. coli* (producing specific adhesion to HEp-2 cells, and having 60 MDa specific plasmid: EAEC).

Identification of diarrheagenic *E. coli* strains requires the differentiation of these organisms from non-pathogenic members of the normal flora. Although serogrouping proposed by Levine (2) has been carried out to define these pathogenic strains, it is now recognized that the serogroup is not well correlated with the presence of pathogenic factors (3). Recently, a practical method for detecting the EspB protein of EPEC and EHEC was reported (4), but no evaluation of this method has yet been reported. Therefore, at present, the detection of pathogenic genes by PCR may be the best way to identify the category of diarrheagenic *E. coli*. PCR assays using a single primer set for one of the pathogenic genes have been reported elsewhere (5-7). However, at least six primer sets for one strain are required to identify the five categories of diarrheagenic *E. coli*, which makes the number of tests required too large. To reduce the number of tests needed to identify the category of diarrheagenic *E. coli*, several multiplex PCR systems for one category of diarrheagenic *E. coli* have been reported (8-10). Multiplex PCR systems to determine all categories of diarrheagenic *E. coli* in one test have been reported (11,12). Although one such multiplex PCR system was proposed to detect 11 genes at a time, none of them has been used to evaluate a large panel of isolates. Recently, a new multiplex PCR system that detects six genes (*stx*, *eae*, *elt*, *est*, *ipaH*, and *aggR*) at a time was reported (13). In the present study, we used this new multiplex PCR system to determine the incidence of diarrhea due to diarrheagenic *E. coli* in Laos, and to discuss the meaning of classical serogrouping as described by Levine in 1987 (2).

A total of 525 *E. coli* strains from 278 patients (basically, two *E. coli* isolates from each patient) were collected in the City of Vientiane. The specimens were submitted to the National Center for Laboratory and Epidemiology, and were classified into three categories (121 watery, 143 muco-muddy, and 14 any bloody). The patients were mainly adult (24 were younger than 6 years; 31 from 6 to 15 years; and 223 older than 16 years). Pathogenic organisms other than *E. coli* were disregarded. The *E. coli* strains were examined by multiplex PCR to detect the genes *stx*, *eae*, *elt*, *est*, *ipaH*, and *aggR* as reported recently (13). Multiplex PCR was carried out as reported with the exception that primer set MK1-MK2 was used instead of VTcom-u-VTcom-d to detect *stx*. DNAs were extracted from the organisms as described by Yokoyama (14). The genes of positive multiplex PCR were confirmed by PCR using a single primer set. The primers used in this study are listed in Table 1. The PCR mixture used with a single primer set consisted of a total volume of 30 μl, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.75 U of *Taq* DNA polymerase (Toyobo, Osaka), 0.2 mM deoxynucleoside triphosphate, a 0.25 μM concentration of each primer, and 3 μl of the DNA template. The PCR program was 95°C for 1 min, 52°C for 1 min, and 72°C for 1 min, for 25 cycles. The O-antigen of the isolates was determined by a slide agglutination test using commercially available antisera called "diarrheagenic *E. coli* diagnostic antisera" (SEIKEN, Tokyo), which consists of 43 kinds of anti-O-antigen sera.

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Table 1. PCR primers used in this study

Designation	Sequence (5′ to 3′)	Target gene	Amplicon size (bp)	Reference
SK1	CCCGAATTCGGCACAAGCATAAGC	*eae*	881	5
SK2	CCCGGATCCGTCTCGCCAGTATTCG			
MK1	TTTACGATAGACTTCTCGAC			
MK2	CACATATAAATTATTTCGCTC	*stx*	224 or 227	21
AL65	TTAATAGCACCCGGTACAAGCAGG			
AL125	CCTGACTCTTCAAAAGAGAAAATTAC	*est*	147	22
LT_L	TCTCTATGTGCATACGGAGC			
LT_R	CCATACTGATTGCCGCAAT	*elt*	322	7
ipa III	GTTCCTTGACCGCCTTTCCGATACCGTC			
ipa IV	GCCGGTCAGCCACCCTCTGAGAGTAC	*ipaH*	619	6
aggRks1	GTATACACAAAAGAAGGAAGC			
aggRkas2	ACAGAATCGTCAGCATCAGC	*aggR*	254	23

Table 2. Detection rate of pathogenic genes

Genes	Positive No. of isolates	Positive No. of cases
eae	3	2
stx	1	1
elt	4	3
est	8	6
elt+*est*	2	2
aggR	5	4
ipaH	0	0
Total	23	16*

* In two cases , one isolate was positive for *elt* and another for *elt* / *est*.

Table 3. Serogroup of the strains (525 strains from 278 patients examined)

	Serogroups	No. of strains	No. of patients
Class I EPEC (Ref. 2)	O26	2	2
	O55	6	4
	O86a	4	4
	O111	1	1
	O119	2	2
	O125	29	22
	O126	4	3
	O127a	12	9
	O128	2	2
	O142	38	28
	Subtotal	100	77
Class II EPEC (Ref. 2)	O18ac	15	14
	O44	22	15
	O114	9	6
	Subtotal	46	35
EIEC (Ref. 2)	O28ac	2	1
	O29	5	4
	O124	2	2
	O136	1	1
	O143	2	2
	O152	1	1
	O164	4	4
	Subtotal	17	15
Others	O1	7	5
	O151	2	2
	O157	9	7
	O158	11	8
	O166	3	2
	Subtotal	32	24
	TOTAL	195	151

The pathogenic genes were detected in 23 strains from 16 patients of the 278 examined (5.8%). These *E. coli* included three strains of EPEC from two patients, 14 ETEC from nine patients, one EHEC from one patient, and five EAEC from four patients. The strains of ETEC consisted of eight *est*+, four *elt*+, and two *est*+/*elt*+ strains. In two cases with *est*+/*elt*+ ETEC, a single patient possessed two kinds of ETEC (*est*+/*elt*+ and *elt*+). One isolate of EHEC was negative for the *eae* gene. No strain of these *E. coli* carried the gene for any other category (Table 2).

In serogrouping, 195 of 525 strains examined were agglutinated with one of the 43 monovalent antisera (Table 3). Among them, 100 strains from 77 patients belonged to Class I classical EPEC (O-26, 55, 86a, 111, 119, 125, 126, 127a, 128, and 142) and 17 strains from 15 patients belonged to serogroup-based EIEC (O-28ac, 29, 124, 136, 143, 152, and 164). Among the 100 strains having the O-antigen of Class I classical EPEC, no strain had the *eae* gene. And among 17 isolates having the O-antigen of EIEC, no isolate had the *ipaH* gene.

The serogroup of the 23 *E. coli* having the genes of interest included O1 for one strain with *est*, O125 for two strains (one with *aggR* and another with *stx*), O126 for one strain with *aggR*, O151 for one strain with *aggR*, and O142 for one strain with *est*. The other 17 strains did not belong to any of the 43 serogroups included in the commercially available anti-sera (Table 4).

Diarrheagenic *E. coli* has been considered for the most frequent causative organism of diarrhea in tropical countries, and its pathogens were defined on the basis of serogrouping (15-18). However, in the present study, the isolation frequency of *E. coli* having the diarrheagenic gene was not as high as expected. Despite the fact that isolated *E. coli* having the gene was regarded as a causative pathogen for the patient, EPEC-caused diarrhea was found in only two of 278 patients (0.7%). When serogrouping was used to define EPEC in this study, Class I classical EPEC was isolated from 77 of 278 patients (28%). In the present study, the detection rate of *E. coli* having the genes for all categories of diarrheagenic *E. coli* was 5.8% (16/278) of diarrheal patients. The isolation frequency of *E. coli* having these genes may vary depending on the district, season, patient age, and so on. Notably, a high incidence of

Table 4. The relation between pathogenic genes and O-serogroups

Strains	Carrying genes	serogroups
434-1	*stx*	O125
347-2	*eae*+/*stx*-	–
473-1, 2	*eae*+/*stx*-	–
305-1*	*elt*	–
343-1**	*elt*	–
422-1, 2	*elt*	–
358-1	*est*	–
374-1, 2	*est*	–
414-1, 2	*est*	–
435-1	*est*	O142
484-1	*est*	–
529-2	*est*	O1
305-2*	*elt-est*	–
343-2**	*elt-est*	–
428-2	*aggR*	O126
501-2	*aggR*	O151
566-1, 2	*aggR*	–
569-2	*aggR*	O125

The strain 434-1 was negative for *eae*.
*, **: *E. coli* with different phenotypes were isolated from one patient.

diarrheagenic *E. coli* defined on the basis of pathogenic genes was reported in Bangladesh (19). A worldwide study would be required to determine the distribution of diarrheagenic *E. coli*.

In the 1990s, the pathogenic factors of diarrheagenic *E. coli* were intensively studied and identified, and studies on the relationship between serogrouping and pathogenic factors have been reported (2, 3, 20). Based on findings these reports, the usefulness of serogrouping to define diarrheagenic *E. coli* remains controversial. It is important to clarify this issue, given that the serogrouping method is still used in clinical laboratories to define the type of *E. coli*. According to the results of the present study, the serogroup of *E. coli* is not likely to correspond to the pathogenic factors. A large-scale study is now ongoing in our laboratory.

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