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Short Communication

Pathogenicity of *Shigella* in Healthy Carriers: a Study in Vientiane, Lao People's Democratic Republic

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SUMMARY: *Shigella* spp. isolated from diarrheal patients and non-diarrheal carriers were examined by PCR for the presence of two pathogenic genes, chromosomal *ipaH* and invasive plasmid encoded *ial*. *Shigella* spp. were detected in 7 of 72 diarrheal cases examined (9.7%), and 9 of 145 non-diarrheal cases (6.2%). All isolates from diarrheal cases harbored both *ipaH* and *ial*, while all isolates from non-diarrheal cases were positive for *ipaH* but not *ial*. These results suggested that *Shigella* spp. in healthy carriers were basically non-pathogenic.

Intestinal infection due to *Shigella* spp. results in a severe inflammation of the colon accompanied by muco-purulent-sanguineous diarrhea. This infection, shigellosis, is endemic throughout the world, but occurs mainly in developing countries. It is estimated that there are about 165 million cases of *Shigella* infection per year, causing death in 1.1 million people (1). The morbidity and mortality of shigellosis are as serious as those of malaria. The problems remaining to be solved are as follows: i) there are many false negative cases arising from the routine culture method to detect *Shigella* from the stools; ii) the drug resistance patterns of the organisms vary; and iii) it is expected that there are many healthy carriers. A comparative study to find shigellosis patients using real-time PCR and a routine culture method demonstrated that the detection rate of the *ipaH* gene from stools using real-time PCR was 3 times higher than that using the routine culture method (2). The drug susceptibility of *Shigella* varied from district to district or even from case to case in the same district (3); therefore, a drug susceptibility test for the isolate from each patient must always be performed. Although *Shigella* is a potently pathogenic organism, there are also many healthy carriers of *Shigella*. Unfortunately, there have been only a few studies on the prevalence of such carriers. In Japan, it has been reported that the carrier-rate of *Shigella* in healthy food handlers was 0.28% in 1961, 0.01% in 1969, and almost 0% between 1976 and 1997 (4). In Lao People's Democratic Republic (Laos), 8 of 185 children (4.3%) in a kindergarten and 1 of 26 healthy adults (3.8%) were positive for *Shigella* on examination in 1997 (5). However, in the present study, none of the carriers in the Lao kindergarten developed any sign of diarrhea within 2 weeks after the date of *Shigella* isolation. Therefore, it is unclear whether *Shigella* spp. in healthy carriers are really pathogenic.

We directed our attention to the pathogenicity of *Shigella* isolates from diarrheal and non-diarrheal stools. The study was carried out at the Center for Laboratory and Epidemiology, Vientiane, Lao People's Democratic Republic. All diarrheal stools submitted from the hospitals in Vientiane to this laboratory were examined. The stool samples submitted to this laboratory for parasitological examination included both diarrheal and non-diarrheal stools. The non-diarrheal (formed) stools were used in order to compare their *Shigella* isolates with those from diarrheal stools.

Stool specimens were collected in a plastic container or by swab with Cary-Blair transport medium. There were 72 diarrheal stool specimens, of which 14 were collected in 2002, 27 in 2003, and 31 in 2004. There were 147 non-diarrheal stool specimens, of which 54 were collected in 2002, 37 in 2003, and 56 in 2004. The stool samples or swab contents were directly inoculated on SS agar plates and incubated at 37°C overnight. Lactose non-fermentative colonies were subcultured on nutrient agar plates to proliferate and confirm the purity of the original colonies. Identification of *Shigella* was made by examining a colony on the nutrient agar using Kligler iron agar (BBL, Cockeysville, Md., USA), SIM semisolid agar, Simond Citrate medium, Voges Proskauer medium, and lysin decaboxylase medium (Eiken Co., LTD., Tokyo, Japan).

Shigella isolates were classified into four subgroups (species) and further serotyping was performed using commercialized anti-sera (Denka Seiken Co., LTD., Tokyo, Japan). The presence of pathogenic genes, *ipaH* on the chromosome (also present on the plasmid) and the invasion associated locus (*ial*) on the plasmid, was examined by polymerase chain reaction (PCR). DNAs were extracted from the organisms as described by Yokoyama (6). The *ipaH* gene was detected by PCR using the primer 5′-GTTCCTTGACCGCCTTTCCGATACCGTC-3′ (ipaIII) and 5′-GCCGGTCAGCCACCCTCTGAGAGTAC-3′ (ipaIV) and produced a 619 bp amplicon (7). The PCR conditions consisted of 30 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min. The primer set for the *ial* consisted of 5′-CTGGTAGGTATGGTGAGG-3′ (ial-I) and 5′-CCAGGCCAACAATTATTT-3′ (ial-II) and produced a 320 bp amplicon (7). The PCR conditions were 30 cycles at 95°C for 1 min, 52°C for 1 min, and 72°C for 1 min.

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Table 1. Information of the *Shigella* isolates

Strains	Age	Sex	Stools*	Date	Serogroup	Type	*ipaH*	*ial*
02SHL1	42	M	N	7/2/02	D	II	+	−
02SHL2	3	M	N	7/2/02	B	Y	+	−
02SHL3	27	F	N	7/3/02	B	Y	+	−
02SHL4	14	M	N	15/2/02	D	II	+	−
02SHL5	2	M	D (bld)	19/6/02	B	3a	+	+
02SHL6	7	F	D	20/6/02	B	3a	+	+
02SHL7	3	F	N	28/6/02	B	2b	+	−
02SHL8	16	M	N	12/7/02	B	2b	+	−
03SHL14	9	F	D	27/7/03	B	2b	+	+
03SHL15	4	M	N	27/7/03	B	2a	+	−
03SHL16	1.7	M	N	21/7/03	D	II	+	−
04SHL17	25	M	N	17/1/04	B	2a	+	−
04SHL19	2.4	M	D	11/3/04	B	2a	+	+
04SHL22	7	F	D	28/7/04	B	2b	+	+
04SHL23	3.2	F	D	12/8/04	B	2a	+	+
04SHL25	9	F	D(bld)	14/9/04	B	2a	+	+

*N, non-diarrheal stool; D, diarrheal stool; (bld), bloody.

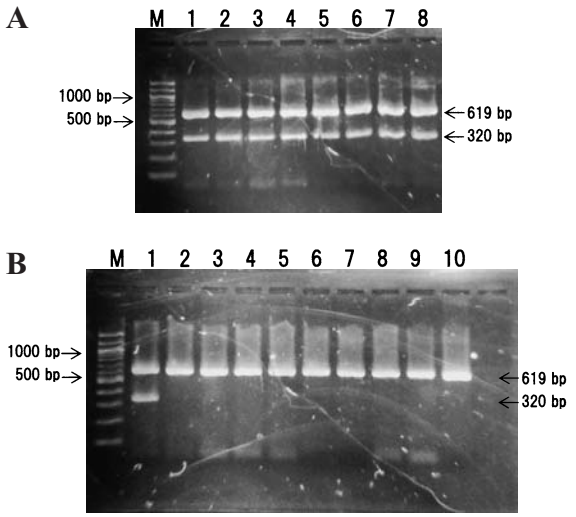

Fig. 1. A: lane 1: *ipaH*/*ial* positive control strain (*S. dysenteriae* 93SHL), lane 2: 02SHL5, lane 3: 02SHL6, lane 4: 03SHL14, lane 5: 04SHL19, lane 6: 04SHL22, lane 7: 04SHL23, lane 8: 02SHL25, lane M: molecular marker (100 bp ladder). B: lane 1: *ipaH*/*ial* positive control, lane 2: 02SHL1, lane 3: 02SHL2, lane 4: 02SHL3, lane 5: 02SHL4, lane 6: 02SHL7, lane 7: 03SHL8, lane 8: 03SHL15, lane 9: 03SHL16, lane 10: 04SHL17, lane M: molecular marker (100 bp ladder).

Shigellae were isolated from 7 of 72 diarrheal stools (9.7%), and all isolates belonged to subgroup B (*S. flexneri*). In non-diarrheal samples, *Shigellae* were isolated from 9 of 145 stools (6.2%). Among these, 6 belonged to subgroup B and 3 belonged to subgroup D (*S. sonnei*) (Table 1). All isolates from diarrheal stools were positive for both *ipaH* and *ial*. All isolates from non-diarrheal stools were positive for the *ipaH* gene but negative for the *ial* (Table 1, Fig. 1).

These results suggested that there are many healthy carriers of *Shigella* in the developing world, and that the distribution of *ipaH* and *ial* was clearly different between isolates from diarrheal stools and isolates from non-diarrheal stools. However, there is a limitation of the method used in this study, since spontaneous loss of the invasive plasmid or selective deletion of the plasmid-encoded genes may occur during the storage period of the isolates. The *ial*-negative strain is regarded as free from the *Shigella* invasive plasmid, and therefore it must not be pathogenic. If the *Shigellae* in healthy carriers are not pathogenic, this is significant information for the administration of public health. Although, none of the healthy carriers in the Lao kindergarten developed diarrhea (data not published), there has been no other follow-up study on healthy carriers. It is not clear whether the data in this study would reflect the situation in other developing countries. However, if so, there would be no need to worry about healthy carriers as infection foci. A large scale study on healthy carriers is now ongoing in our group. Since shigellosis is a typical communicable disease, we must consider how to treat healthy carriers.

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