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LETTER-TO-THE-EDITOR

Enhanced Isolation of *Shigella* Species by Extended Incubation of Primary Isolation Plates

Sir,

Shigellosis, a disease associated with significant morbidity and mortality among pre-school children (1), is caused by any one of the four species of *Shigella,* namely *Shigella dysenteriae*, *S. flexneri, S. boydii,* and *S. sonnei*. Specific diagnosis of *Shigella* infection depends on isolation of the organism from microbiological cultures of stool or rectal swab samples.

Stool or rectal swab samples were obtained from patients who attended the Dhaka Hospital of ICDDR,B: Centre for Health and Population Research and from patients who were referred here from private hospitals in Dhaka for stool culture during February 2000–April 2002. All samples were transported either in Cary-Blair (CB) medium or in Buffered Glycerol Saline (BGS) and were processed within an hour.

The specimens were processed in MacConkey agar (MCA) and *Salmonella-Shigella* agar (SSA) and incubated for 16-24 hours at 35-37 °C. All non-lactose-fermenting colonies were tested biochemically and identified serologically following the standard methods (2), using commercial antisera (Denka Seiken Co. Ltd., Tokyo, Japan; BBL/Difco Laboratories, Detroit, Michigan, USA). All primary plates with no visible non-lactose-fermenting colonies were placed again in the incubator at 35-37 °C for another 16-18 hours. The primary plates showing non-lactose-fermenting colonies were examined and identified following the standard procedures after the extended incubation.

Of 38,243 stool specimens processed, 11,577 non-lactose-fermenting suspicious colonies were picked. Of positive isolates, 3,809 (10.0%) were identified as *Shigella* spp., 545 (1.4%) as *Salmonella* spp., and 162 (0.42%) as *Hafnia alvei* from colonies after 16-24 hours of incubation (Table). The remaining 26,666 samples were reincubated for another 16-18 hours. An additional 194 *Shigella* spp. (4.85%) and 6 *Salmonella* spp. (1.1%) were isolated from 587 non-lactose-fermenting suspicious plates. These additional 194 *Shigella* spp. were isolated mostly from *Salmonella-Shigella* agar (SSA) plate and very few from MacConkey agar plate. Of the *Shigella* isolates, *S. flexneri* (53.6%) was more frequently isolated, followed by *S. boydii* (24.2%), *S. dysenteriae* (15.0%), and *S. sonnei* (7.2%).

For the isolation of *Shigella* spp., the absence of non-lactose-fermenting colonies on the primary plates after

Table. Number of *Shigella, Salmonella,* and *Hafnia alvei* isolates obtained by extended incubation of primary plates up to 48 hours in Clinical Microbiology Laboratory of ICDDR,B during February 2000–April 2002

Pathogen	Incubation period				Total	
	24 hours		48 hours			
	No.	%	No.	%	No.	%
Shigella	3,809	95.15	194	4.85	4,003	100
S. dysenteriae	552	95.0	29	5.0	581	100
S. flexneri	2,313	95.7	104	4.3	2,417	100
S. boydii	614	92.9	47	7.1	661	100
S. sonnei	330	95.9	14	4.1	344	100
Salmonella	545	98.9	06	1.1	551	100
S. Typhi	74	97.4	02	2.6	76	100
S. Paratyphi	23	95.8	01	4.2	24	100
Salmonella species	448	99.3	03	0.7	451	100
Hafnia alvei	162	98.8	02	1.2	164	100

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initial incubation means that they are usually discarded without further testing. Slow-growing members of *Shigella* species are largely unknown, except for *S. sonnei* (2). The primary isolation plates lacking non-lactose-fermenting colonies were kept in the incubator at 35-37 °C for further 24 hours (extended up to 48 hours). The extended incubation yielded additional isolation of *Shigella* and *Salmonella* spp. (3). The system of extended incubation is now a routine practice in our clinical microbiology laboratory. Since the adoption of the extended incubation period from February 2000 to April 2002, we were able to isolate an increased number of all *Shigella* species and also some *Salmonella* Typhi and *S.* Paratyphi.

Extended incubation of conventional stool culture media to enhance isolation of *Shigella* has not been evaluated. As this extended period of incubation yielded a good number of additional *Shigella* isolates, the technique is useful for enhancing isolation of *Shigella* spp., with no additional costs, except some man-hours. Based on the findings of this study, we recommend that incubation of primary plates for isolation of *Shigella* species be extended to improve isolation rates of *Shigella* species in clinical microbiology laboratories.

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