

BODY OF REPORT

SEATO Medic Study No. 60 Bacteriologic Survey of Stools from Patients
with Acute Diarrhea

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Objective: To determine the types, frequency of occurrence and distribution of *Salmonellae* and *shigellae* in stools of patients with acute diarrhea and to determine the incidence of other Enterobacteriaceae and their relationships to this disease.

Description: The 4,123 specimens included in this study were from patients of both sexes from hospitals throughout Thailand. All were hospitalized for acute diarrhea, and most specimens were taken during the acute stage of the disease. In the Bangkok area 2 rectal swabs were taken from each patient. One was placed in a screw-capped tube of selenite-F enrichment broth and the second into a tube of alkaline peptone broth. Specimens from outside of Bangkok were submitted in a holding medium designed for transport of enteric bacteria. Upon

arrival at the laboratory tubes of selenite-F and alkaline peptone broth were inoculated from the holding medium.

Each selenite-F broth was subcultured on desoxycholate-citrate and MacConkey agar plates. After overnight incubation, the selenite-F broth was again subcultured, this time to salmonella-shigella and eosin-methylene blue or MacConkey agar plates. The alkaline peptone broth was subcultured on alkaline lauryl sulfate tellurite agar plates. All incubations were at 37 c.

All plates were examined after 24 and 48 hours incubation. Lactose negative colonies were transferred to Kligler's iron agar slants and subsequently into a variety of media to determine patterns of biochemical activity. Colonies typical of vibrios on alkaline lauryl sulfate tellurite agar were processed in the same way. Those isolates showing biochemical patterns typical of salmonella, shigella or vibrio were definitively identified serologically in accordance with methods described by Edwards and Ewing.

Progress: Results in Table I show that recognized diarrheal agents were isolated from approximately 25 percent of all specimens. While the overall figure was approximately that of last year, the distribution is different in that isolations of vibrios have decreased from approximately 12 percent last year to less than one percent this year. There has been a corresponding increase in the percentages of other enteropathogens isolated.

The incidence of diarrheae associated with Salmonellae was 15.6 percent, (Table I) a percentage that is somewhat above the average of the past three years. The results in Table II show that 27 species of salmonellae were identified from the 649 positive swabs. More than half of the positive specimens were S. montevideo, a species that has dominated salmonellosis in Thai children for the past several years. S. montevideo and S. derby - the second most frequently isolated species - were involved in outbreaks at Children's Hospital during this reporting period. Fewer isolations of Salmonella anatum and Salmonella paratuberculosis B were made this year than in previous years. The number of Salmonella typhosa isolations was about the same as last year and all represented sporadic unrelated cases. In summary salmonellosis continues to be an important enteric disease and there is no indication that progress is being made in its eradication.

Only 84 shigella isolates were made during this period but these represent 17 species (Table III) None of these were associated with outbreaks and the importance of these data is that the inocula for outbreaks in present. The percentage of shigella isolates has been less than two percent of total diarrheal stools examined every year for the last four years.

The decrease of isolations of agglutinable vibrios from about 12 percent last year to 0.4 percent this year is explained by the almost total decrease in the number of cases of clinical cholera. It is of interest that non-agglutinating

vibrios were isolated from patients every month of the year.

While their role in the etiology of diarrheal diseases is unproven, Paracolobactrum sp. were listed in this report for the sake of completeness. Almost 60 percent of the patients were positive for one or more species of this genus. There was no pattern of distribution of these organisms that suggested any role as an enteric pathogen except that no other recognized enteric pathogen was isolated from most of these patients.

In July 1964 this department's assistance was requested to help control an outbreak of salmonellosis in Children's Hospital, Bangkok. After a detailed study of procedures and hospital personnel a number of changes in procedure designed to reduce hospital-acquired infections were put into effect. At the same time it was determined that the strain of S. montevideo involved was resistant to all readily available antibiotics except colistin which was made the antibiotic of choice on 15 August. As seen in Table IV the number of cases was decreased significantly during the remainder of August and all of September. The rate increased again during the last three months of the year and much of the increase was related to decreased use of colistin during this period. It was of interest that none of the isolates of S. montevideo tested developed resistance to this antibiotic during this period. It can also be seen in Table IV that many of these organisms were acquired during hospitalization. Repeated checks of hospital personnel and fomites failed to reveal salmonella except in one instance when it was isolated from a "community" hand towel in the diarrhea ward. A possible explanation for the failure to find the source(s) of hospital-acquired S. montevideo could be that this strain is rarely found in adults and, in a previous study, Gaines found that 24 percent of the stools of non-diarrhea patients in Children's Hospital harbored this organism. It is evident that both S. montevideo and S. derby are resident in the hospital and continue to serve as a reservoir of hospital-acquired salmonellosis.

Summary: Approximately 25 percent of stool specimens were positive for known enteric pathogens and approximately another 60 percent were positive for paracolons. Most of the enteric pathogens were Salmonellae with S. montevideo and S. derby predominating. Outbreaks caused by these two organisms in Children's Hospital were curtailed but not completely eradicated. Many of the cases of salmonellosis caused by these organisms are acquired in the hospital.

Conclusion: Salmonellosis and shigellosis remain serious medical problems in Thailand. There is no indication that these problems will diminish in the immediate future.

Table I
 ENTEROBACTERIACEAE ISOLATED FROM ACUTE DIARRHEA CASES IN THAILAND FROM APRIL 1964
 THROUGH MARCH 1965

Months	No. of Specimens	Enteropathogenic coli			Vibrio cholerae		
		Shigellae	Salmonellae	El Tor	NAGS	Pracolon	
April 1964	371	8	1	26	10	26	170
May	191	4	3	8	2	61	128
June	283	6	10	26	0	5	183
July	389	19	5	74	1	4	287
August	384	31	4	101	0	7	265
September	386	9	6	100	0	9	228
October	386	20	8	76	0	3	269
November	435	12	6	69	0	1	207
December	361	6	8	51	0	2	199
January 1965	326	12	10	40	0	2	229
February	282	14	3	45	4	5	169
March	320	14	7	26	0	6	108
Total	4,123	155	71	642	17	131	2,442
Percent of total specimens		3.8	1.7	15.6	0.4	3.2	59.2

Table II
 SALMONELLA SPECIES ISOLATED IN THAILAND FROM APRIL 1965
 THROUGH 26 MARCH 1965

Species	Group	Children	Adults	Unknown age	Total
<u>Salmonella derby</u>	B	111	1	6	118
<u>Salmonella paratyphi B</u>	B	13	-	1	14
<u>Salmonella sandiego</u>	B	-	-	2	2
<u>Salmonella saintpaul</u>	B	1	1	-	2
<u>Salmonella stanley</u>	B	4	1	10	15
<u>Salmonella typhimurium</u>	B	1	1	1	3
Salmonella group B species (untyped)	B	1	1	-	2
<u>Salmonella montevideo</u>	C ₁	332	-	2	334
<u>Salmonella virchow</u>	C ₁	1	-	2	3
<u>Salmonella tennessee</u>	C ₁	-	-	1	1
<u>Salmonella bovismorbificans</u>	C ₂	1	4	3	8
<u>Salmonella newport</u>	C ₂	7	-	2	9
<u>Salmonella tananarive</u>	C ₂	-	1	-	1
<u>Salmonella manhattan</u>	C ₂	1	-	-	1
Salmonella group C ₂ species (untyped)	C ₂	-	-	2	2
<u>Salmonella dublin</u>	D	34	1	-	35
<u>Salmonella panama</u>	D	3	-	-	3
<u>Salmonella typhosa</u>	D	20	2	-	22
<u>Salmonella portland</u>	D	1	-	-	1
Salmonella gr. D species (untyped)	D	4	-	2	6
<u>Salmonella anatum</u>	E ₁	10	5	1	16
<u>Salmonella lexington</u>	E ₁	19	-	1	20
<u>Salmonella meleagridis</u>	E ₁	6	2	1	9
<u>Salmonella weltevreden</u>	E ₁	-	-	3	3
Salmonella gr. E. species (untyped)	E	5	2	1	8
<u>Salmonella senftenberg var new castle</u>	E ₄	-	-	1	1
Salmonella species (not type)	-	10	1	-	11
Total	-	585	23	42	650

Table III
SHIGELLA SPECIES ISOLATED IN THAILAND FROM 1 APRIL 1964
TO 26 MARCH 1965

Species	Group	Children No.	Adults No.	Unknown age No.	Total No.
<u>Shigella dysenteriae</u> 1	A	-	1	3	4
<u>Shigella dysenteriae</u> 2	A	-	1	-	1
<u>Shigella dysenteriae</u> 3	A	-	1	2	3
<u>Shigella dysenteriae</u> 4	A	-	-	1	1
<u>Shigella flexneri</u> 2	B	2	1	-	3
<u>Shigella flexneri</u> 3	B	4	2	-	6
<u>Shigella flexneri</u> 4	B	2	1	-	3
<u>Shigella flexneri</u> 6	B	-	2	1	3
<u>Shigella boydii</u> 2	C	1	1	3	5
<u>Shigella boydii</u> 7	C	2	1	5	8
Shigella group C species (untyped)	C	-	1	-	1
<u>Shigella sonnei</u> form 1	D	5	10	6	21
<u>Shigella sonnei</u> form 2	D	6	4	2	12
Alkalescens-dispar 01	A-D group	5	1	2	8
Alkalescens-dispar 02	"	-	-	1	1
Alkalescens-dispar 04	"	1	-	1	2
Alkalescens-dispar species (untyped)	"	1	-	1	2
Total		29	27	28	84

Table IV
OCCURRENCE OF SALMONELLAE IN PATIENTS IN CHILDREN HOSPITAL
APRIL 1964 - MARCH 1965

Months	Number of Patients	Patients positive for Salmonella	Patients positive for <u>S. montevideo</u>		Patients positive for <u>S. derby</u>		Patients positive for Other salmonellae	
			Hospital		Hospital		Hospital	
			Total	Acquired	Total	Acquired	Total	Acquired
April 1964	49	14	5	0	1	0	8	0
May	28	2	2	0	0	0	0	0
June	40	7	5	0	0	0	2	0
July	119	43	42	12	1	0	0	0
August 1-15	54	34	26	1	2	1	6	3
August 16-30	54	23	7	4	6	0	10	1
September	114	31	13	10	10	7	8	2
October	110	40	21	8	14	4	5	0
November	124	38	31	12	6	2	1	0
December	115	26	22	7	1	1	3	0
January 1965	85	15	6	4	8	7	1	0
February	75	18	4	3	11	6	3	0

