

FINAL REPORT

BODY OF REPORT

SEATO Medic Study No. 69 Study of Enteropathogenic Organisms in
Premature Baby

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S. E. Asia

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Objective: The objective of this study was to study diarrhea in premature babies. Diarrhea in babies is one of the most important medical problems because it seems to be the major cause of death. Premature babies are especially susceptible to infectious diseases, particularly diarrheal diseases. This study was done at the premature ward of Women's Hospital, Bangkok, Thailand. The outbreak of acute diarrhea on this premature ward was a serious occurrence in terms of infant mortality and the added burden placed on the hospital staff.

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Description: This study involved the determination of the cause of diarrhea, types of the organisms, frequency of occurrence and the distribution of salmonellae, shigellae, and enteropathogenic E. coli. Also included in the study were determinations of antibiotic sensitivities of representative enteropathogens and an epidemiologic study of the outbreak. Once the etiology was determined, studies were carried out on personnel and the environment to determine factors involved.

Ractal swabs were obtained daily on each baby for the first three days after birth. Additional swabs were taken at the onset of diarrhea. Swabs were inoculated into alkaline peptone broth pH 8.6 and selenite F. enrichment broth. After incubation at 37°C for 6-8 hrs, subcultures from alkaline peptone broth were plated onto tellurite agar while subcultures from selenite F broth were plated on salmonella-shigella agar and MacConkey agar respectively. After 24 hours additional incubation, the subcultures of selenite F broth were made to desoxycholate citrate agar and eosin methylene blue agar. Non-lactose fermenting colonies were identified for salmonella and shigella and lactose-fermenting colonies were checked for identification as enteropathogenic E. coli. The serologic types of these enteropathogens were determined by the slide agglutination method. Cases caused by enteropathogenic bacteria were cultured daily until three consecutive negative cultures were obtained. Some of isolates were examined for the sensitivity to tetracycline, chloramphenicol, neomycin, kanamycin, penicillin, streptomycin, erythromycin, novobiocin and colimycin. Most of the sensitivity determinations were carried out on nutrient agar plate by the disc method and a few by the tube dilution method. An attempt were made to disrupt the spread of the disease by treatment of carriers. Cultures from hospital personnel and environment were taken initially and at intervals when there was the diarrhea outbreak.

During the period covered by this report, there were two diarrheal outbreaks. The first outbreak, caused by S. paratyphi B was from 15 July 1964 to 30 July 1964 and the second caused by S. derby, began at the end of September 1964 and ended in December 1964.

Progress: Examinations of 1672 specimens from 500 premature babies were done. Results in Table I show that enteropathogenic organisms were isolated about 15 percent of all specimens. Salmonellae were found in 12 percent of the specimens while 0.25 percent yielded shigellae and 2.5 percent yielded enteropathogenic E. coli. Types of the enteric pathogens are showed in Table II. There were 196 salmonellae representing 6 species isolated. Enteropathogenic E. coli were isolated from 42 specimens representing 6 serotypes and only 4 shigellae representing 3 species were isolated.

It was noted that almost 80 percent of S. paratyphi B isolates were obtained from premature babies during their first few days of life. Epidemiologic study were performed on the personnel and the environment in the labor room. The results in Table III show that 214 specimens from 210 doctors and nurses in the labor room were examined. Seven strains of Salmonellae representing 4 species and 2 strains of enteropathogenic E. coli serotype 0119:B14 were isolated. There

Table I
 ENTERIC PATHOGENS ISOLATED FROM PREMATURE BABIES IN WOMEN'S HOSPITAL, BANGKOK, THAILAND
 FROM JULY 1964 TO MARCH 1965

Total Premature babies Sample	Total Specimens Taken	Babies pos. for Salmonella	Salmonellae isolated	Babies pos. for Shigella	Shigellae isolated	Babies pos. for Enteropathogenic E. coli	Enteropathogenic E. coli isolated
500	1672	74	196	4	4	37	42

Table II
 SEROTYPES OF ENTERIC PATHOGENS ISOLATED FROM PREMATURE
 BABIES

	No. of Patients	No. of isolates
<u>Salmonellae</u>		
S. derby	50	158
S. paratyphi B	12	26
S. montevideo	6	6
S. lexington	4	4
S. anatum	1	1
Salmonella Gr. C ₂	1	1
Total	74	196
<u>Shigellae</u>		
Sh. sonnei form II	1	1
Sh. dysenteriae 6	1	1
Alkalescens-dispar 04	2	2
Total	4	4
<u>Enteropathogenic E. coli</u>		
0119:B14	16	19
025:B19	10	11
086:B7	5	5
0126:B16	2	3
0125:B15	2	2
0124:B17	2	2
Total	37	42

Table III
DOCTORS AND NURSES IN LABOR ROOM

Persons	Specimens	Salmonellae	Shigellae	Enteropathogenic E. coli
210	214	7	0	2

Serotypes of Pathogenic Organisms Isolated

Salmonellae

Salmonella group E	3
Salmonella group D	2
Salmonella group C ₂	1
S. lexington	<u>1</u>
Total	7 ====

Enteropathogenic E. coli

E. coli 0119:B14	2
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Table IV
SAMPLES TAKEN FROM LABOR ROOM

Source	Organisms Isolated			
	1 st Sampling Time	2 nd Sampling Time	3 rd Sampling Time	4 th Sampling Time
Instruments tray	<u>Pseudomonas sp.</u> <u>Alkaligenes sp.</u> <u>Proteus mirabilis</u>			
Rubber pad	<u>Non-patho. E. coli</u> <u>Pseudomonas sp.</u>			
Baby Balance	Intermediate coliform			
Rectal exam. gloves	No growth			
Basin	<u>Non-patho. E. coli</u> <u>Alkaligenes sp.</u> Inter. coliform <u>P. aerogenoides</u>			
Apron	<u>Pseudomonas sp.</u>	<u>E. freundii</u> <u>Aerobacter sp.</u> <u>P. aerogenoides</u>	Staph. coag. neg <u>Alkaligenes sp.</u> <u>Aerobacter sp.</u>	<u>Non-patho. E. coli</u> <u>P. aerogenoides</u> <u>P. coliforme</u>
Towel	<u>Pseudomonas sp.</u>			
Basinette	<u>E. freundii</u> <u>P. aerogenoides</u> <u>P. intermedium</u> <u>Pseudomonas sp.</u>	<u>Staph. coag. pos.</u> <u>Aerobacter sp.</u> <u>P. coliforme</u> <u>P. intermedium</u>		
Side of the crib	<u>Aerobacter sp.</u> <u>P. aerogenoides</u>			
Side of the labor table	<u>Non-patho. E. coli</u> <u>Aerobacter sp.</u> <u>P. coliforme</u> <u>P. intermedium</u>	Staph. coag. neg <u>B. subtilis</u> <u>Proteus species</u> <u>Alkaligenes sp.</u>		
Endotracheal tube	Staph. coag. neg <u>Alkaligenes sp.</u> <u>B. subtilis</u>			
Cat gut tray	<u>Pseudomonas sp.</u>			
Faucet for cleaning rectal exam. glove	<u>Pseudomonas sp.</u>	<u>Aerobacter sp.</u> <u>Pseudomonas sp.</u>		
Long forceps container	<u>Pseudomonas sp.</u> <u>Alkaligenes sp.</u>			

Table IV (Continued)

Source	Organisms Isolated		
	1 st Sampling Time	2 nd Sampling Time	3 rd Sampling Time
Stirrup	Non-patho. <u>E. coli</u>		
Forcep used for umbilical dressing	<u>Aerobacter sp.</u> <u>P. aerogenoides</u>		
Endotracheal tube tray	Non-patho. <u>E. coli</u>		
Forcep used for picking eye swab cotton ball	Staph. coag. neg <u>Aerobacter sp.</u> <u>Pseudomonas sp.</u>		
Thermometer tray	<u>Pseudomonas sp.</u> <u>P. aerogenoides</u> <u>Aerobacter sp.</u>	No growth	
Resuscitator tray	Staph. coag. neg <u>Aerobacter sp.</u> <u>Pseudomonas sp.</u> <u>P. coliforme</u> <u>P. aerogenoides</u> Non-patho. <u>E. coli</u>		
Water bottle for babies	Staph. coag. neg <u>B. subtilis</u> <u>Pseudomonas sp.</u> <u>Pseudomonas sp.</u> <u>Alkaligenes sp.</u> <u>P. aerogenoides</u>		
Oxygen Rubber tube	Staph. coag. neg <u>Pseudomonas sp.</u>		
Laryngoscope	<u>Alkaligenes sp.</u>		
Water in the oxygen bottle	<u>Pseudomonas sp.</u>	<u>Pseudomonas sp.</u>	<u>Pseudomonas sp.</u>
De Lee tube	No growth		
Saline bottle	Non-patho. <u>E. coli</u> <u>Aerobacter sp.</u> <u>P. aerogenoides</u>		

Table V

SPECIMENS FROM PATIENTS IN THE LABOR ROOM

Persons	Specimens	Salmonella	Shigella	Enteropathogenic E. coli
35	36	2	0	1

Organisms IsolatedSalmonellae

S. paratyphi B	1
S. derby	<u>1</u>
Total	<u>2</u> =====

Enteropathogenic E. coli

E. coli 025:B19

Table VI
DOCTORS AND NURSES ON PREMATURE WARD

Persons	Specimens	Salmonellae	Shigellae	Enteropathogenic E. coli
170	175	3	3	3

Serotypes of Pathogenic Organisms Isolated

Salmonellae

S. typhosa	1
S. manhattan	1
Salmonella group C ₂	1
Total	3
	=====

Shigellae

Sh. sonnei form II	1
Sh. boydii 7	1
Sh. flexneri 6	1
Total	3
	=====

Enteropathogenic E. coli

E. coli 025:B19	2
E. coli 0124:B17	1
Total	3
	=====

Table VII
SAMPLES TAKEN FROM PREMATURE WARD

Source	Organisms Isolated				
	1st Sampling Time	2nd Sampling Time	3rd Sampling Time	4th Sampling Time	5th Sampling Time
Thermometer trays	Non-patho. <u>E. coli</u> <u>P. mirabilis</u> <u>Pseudo. sp.</u>	Non-patho. <u>E. coli</u> <u>Alkaligenes sp.</u> <u>Pseudo. sp.</u>	<u>Staph. coag. neg.</u> <u>P. mirabilis</u> <u>Alkaligenes sp.</u> <u>P. aerogenoides</u>	Non-patho. <u>E. coli</u> <u>E. freundli</u> <u>Pseudo. sp.</u>	<u>S. derby</u> <u>E. coli</u> <u>A. aerogenes</u> <u>P. aerogenoides</u> <u>Pseudo. sp.</u>
Rectal thermometers	Non-patho. <u>E. coli</u> <u>P. aerogenoides</u> <u>Klebsella</u> <u>aerobacter</u>	<u>P. coliforme</u> <u>P. aerogenoides</u> <u>P. intermedium</u>	<u>P. coliforme</u> <u>P. aerogenoides</u> <u>Inter. coliforme</u> <u>Staph. coag. neg.</u>	<u>Klebsella</u> <u>aerobacter</u>	No growth
Medicine Glasses	No growth	No growth	Non-patho. <u>Aerobacter sp.</u> <u>P. aerogenoides</u>	<u>Staph. coag. pos.</u> <u>Aerobacter sp.</u> <u>Pseudo. sp.</u>	
Milk	No growth	No growth	<u>P. coliforme</u>		
Medicine spoons	Non-patho. <u>E. coli</u> <u>Aerobacter sp.</u> <u>Inter. coliforme</u> <u>P. aerogenoides</u> <u>Alkaligenes sp.</u> <u>Pseudo. sp.</u>	<u>Staph. coag. pos.</u> <u>Klebsella</u> <u>aerobacter</u> <u>Pseudo. sp.</u>	Non-patho. <u>E. coli</u>		<u>Alkaligenes sp.</u>
Paper towels	<u>Staph. coag. neg.</u>	<u>Aerobacter sp.</u> <u>Pseudo. sp.</u> <u>Alkaligenes sp.</u> <u>Staph. coag. neg.</u>	Non-patho. <u>E. coli</u>	No growth	
Water in oxygen bottle	<u>Staph. coag. neg.</u> <u>Pseudo. sp.</u>	<u>Pseudo. sp.</u>	Non-patho <u>E. coli</u> <u>Aerobacter sp.</u> <u>Pseudo. sp.</u>	<u>Pseudo. sp.</u>	<u>Staph. coag. pos.</u> <u>Pseudo. sp.</u> <u>E. subtilis</u>
Water in incubators	<u>Staph. coag. neg.</u> <u>Aerobacter sp.</u>	Non-patho. <u>E. coli</u> <u>Aerobacter sp.</u>	<u>Aerobacter sp.</u> <u>P. coliforme</u> <u>P. aerogenoides</u>	<u>Alkaligenes sp.</u> <u>Staph. coag. neg.</u>	<u>Aerobacter sp.</u> <u>Pseudo. sp.</u>
Nipple trays	<u>Alkaligenes sp.</u>				

Table VIII
SPECIMENS FROM MOTHERS OF BABIES POSITIVE FOR S. DERBY

Persons	Specimens	Salmonella	Shigella	Enteropathogenic E. coli
6	15	1	0	0

Organisms Isolated

S. bergedorf	1
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Table IX
ENTEROPATHOGENIC E. COLI ISOLATES ASSOCIATED WITH
SALMONELLA

S. derby E. coli 025:B19	3 cases
S. derby E. coli 0126:B16	1 case
S. derby E. coli 0125:B15	1 case
S. paratyphi B E. coli 025:B19	1 case
S. paratyphi B E. coli 0119:B14	1 case
Total	7 cases

was no S. paratyphi B isolated from these personnel or from fomites in the labor room. Table IV shows the organisms isolated from the samples taken in the labor room. In addition, 35 patients in the labor room were examined by mean of rectal swabs (Table V). Only 2 salmonellae representing S. paratyphi B and S. derby were identified. Neither of the isolates were sources of infection because they were not found at time when the outbreaks occurred and the patients were not the mothers of premature babies. Both mothers were considered asymptomatic carriers.

In the S. derby outbreak from September to December 1964 it was found that about 80 percent of the infected premature babies were infected during the first few days of life. Examinations were made of 175 rectal swabs from 170 personnel including doctors, nurses and rotating student nurses. Results in Table VI show that 3 salmonellae representing 3 species, 3 shigellae representing 3 species and 3 enteropathogenic E. coli representing 2 serotypes were found. No S. derby, the causal enteropathogen was isolated from these personnel.

The environment was examined by culturing swabs from various sites in the premature ward. Results in Table VII indicate that coliform organisms were generally distributed. S. derby was isolated from the rectal thermometer tray and might have been the source of the infection even though disinfectant solution (Zephiran solution 1:4000) was reportedly changed every 12 hours. Used rectal thermometers were placed in the same tray and supposed to be washed and cleaned in 70% alcohol before used again. These data suggest that rectal thermometers could have been responsible for the direct spread of the infection among the premature babies.

None of 15 specimens from 6 mothers were positive for S. derby although one was positive for Salmonella bergedorf (Table VIII).

Results in Table II show that enteropathogenic E. coli serotypes were isolated from 4 percent of all specimens. Serotype 0119:B14 was found most frequently. Most of the babies infected with enteropathogenic E. coli were not infected early during their hospitalization. There were 7 cases from which enteropathogenic E. coli were isolated together with salmonellae (Table IX). From the epidemiologic study, it was found that adults were carrier of the enteropathogenic E. coli as well as babies themselves.

Antibiotic sensitivity tests were done on some isolates of S. paratyphi B. The available antibiotics used were chloramphenicol, oxytetracycline and neomycin sulfate. Most of the organisms were sensitive to all three antibiotics with only 3 strains resistant to greater than 50 mcg/ml of chloramphenicol. Based on finding at that time neomycin sulfate was the antibiotic of choice and was used in the premature ward at that time.

During the second outbreak, sensitivity tests of S. derby indicated that the organism was sensitive only to colimycin sulfate. This antibiotic was recommended to the attending physicians in a dosage of 100,000 units per kg. per day

for 5 days for prophylactic treatment and for 7 to 10 days for therapeutic treatment. Because of the prolonged outbreak and the finding of the source of infection within the premature ward, all premature babies were treated with colimycin sulfate and increased dosages were used for the chronic cases.

After the likely source of the infection within the premature ward was found, aseptic precautions were intensified by cleaning walls, equipment, mopping floors with lysol solution (1:200) every 2 weeks and vaporizing formalin in the temporarily evacuated ward for 16-24 hours. In addition the diarrheal babies were separated from the normal babies and the newborn babies were placed in a new premature ward. Isolates of S. derby gradually decreased after these corrective measures were initiated.

Summary and Conclusion: About 15 percent of the rectal swabs were positive for enteric pathogens. In a study of enteropathogenic organisms in premature babies, 12 percent yielded salmonellae, 0.25 percent yielded shigella and 2.5 percent yielded enteropathogenic E. coli. There were two diarrheal outbreaks caused by S. paratyphi B and S. derby respectively during the period covered by this report. The infant mortality rate was about 5 percent in infants from whom the pathogenic organisms were isolated. Since most of the pathogens were isolated during the first few days of life, epidemiologic studies were carried out on personnel and the environment of the premature ward and the labor room. It was found out that the rectal thermometer tray might have been the source of the infection. Aseptic precautions were carried out along with the separation of diarrheal babies from normal babies. The number of isolates of salmonellae decreased.

Conclusion: The combination of intensive laboratory studies and conscientious cooperation on the part of hospital authorities decreased the severity of these outbreaks. The inocula for new outbreaks were shown to be present in hospital personnel and new mothers. Constant surveillance is necessary to preclude repeated outbreaks.