ANNUAL PROGRESS REPORT

SEATO Medic Study No. 66	Antibiotic Sensitivities and Cross Resistance Patterns of Pathogenic Bacteria Indigenous to Thailand
Project No. 3A 025601 A 811	Military Medical Research Program S. E.
Task 01:	Military Medical Research Program S. E.
Subtask 01:	Military Medical Research Program SEASIA (Thailand)
Reporting Installation:	US Army-SEATO Medical Research Laboratory, APO 146, San Francisco, California
	Division of Medical Research Laboratories
	Department of Bacteriology and Immunology
Period Covered by Report:	1 December 1963 to 31 March 1964
Principal Investigator:	Howard E. Noyes, Ph.D.
Associate Investigator:	Mrs. Panyasri Benjadol
Assistants:	Mrs. Malinee Thareesawasdi Miss Prani Senadisai
Reports Control Symbol:	MEDDH-288
Security Classification:	UNCLASSIFIED

ABSTRACT

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The objective of this study was to determine bacteriostatic levels of six antibiotics for enteric organisms recently isolated from patients with acute diarrhea. Enteric diseases constitute one of the most important medical problems relating to the effectiveness of troops operating in tropical areas. Reliance on rapid mobility of troops into combat areas will usually mean that the soldiers will contract diarrheal diseases at a critical time. The use of antibiotics to moderate the effects of outbreaks of diarrhea and eliminate carriers is related directly to the susceptibility of the etiologic agents. The problem of antibiotic-resistant organisms could be especially critical in Thailand where antibiotics are available without prescription, and in some instances, are relatively inexpensive.

Using the tube dilution technique it was found that 29 enteric isolates of a total of 468 strains representing 81 species and serotypes were resistant to greater than 100 mcg/ml of the six antibiotics studied. Salmonella paratyphi B and Salmonella montevideo accounted for 15 of the 29 resistant strains. This is significant because these two species are isolated frequently from patients with acute diarrhea. All Salmonella typhosa strains were sensitive to chloramphenicol although this drug has been used routinely for therapy of typhoid fever for many years in Thailand. Based on in vitro sensitivities, oxytetracycline was the most effective antibiotic tested with approximately 78 percent of the strains being sensitive to 50 mcg/ml or less. Listed in decreasing order of effectiveness the other antibiotics evaluated were chloramphenicol, neomycin sulfate, kanamycin sulfate, streptomycin sulfate and penicillin G.

BODY OF REPORT

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Objective: To determine bacteriostatic levels of six antibiotics for enteric diseases recently isolated from patients with acute diarrhea. Enteric diseases constitute one of the most important medical problems relating to the effectiveness of troops operating in tropical areas. Reliance on rapid mobility of troops into combat areas will usually mean that the soldiers will contract diarrheal diseases at a critical time. The use of antibiotics to moderate the effects of outbreaks of diarrhea and eliminate carriers is related directly to the susceptibility of the etiologic agents. The problem of antibiotic-resistant organisms could be especially critical in Thailand where antibiotics are available without prescription, and, in some instances, are relatively inexpensive

The purpose of this study was to determine bacteriostatic levels of six antibiotics for enteric organisms recently isolated from patients with acute diarrhea.

Description: All organisms in this study except 25 isolates of El Tor Ogawa from Viet Nam were isolated from stools or rectal swabs from hospitalized Thai nationals during late 1963, and early 1964. Isolation procedures were as follows. Rectal swabs were placed in Selenite-F enrichment broth and incubated for 6 - 18 hours before subculture to Salmonella - Shigella, MacConkey, and Eosin - methylene blue agar plates for isolation of enteric pathogens. Isolates with appropriate biochemical patterns were examined serologically in accordance with methods described by Edwards and Ewing.

Sensitivity tests were carried out in trypticase soy broth, pH 7.4, by use of the tube dilution method. The inoculum for each tube was approximately 10^6 organisms/ml and the bacteriostatic level was the lowest concentration of antibiotic preventing visable growth. The commercially available antibiotics used were chloramphenicol, kanamycin, neomycin sulfate, oxytetracycline, penicillin and streptomycin sulfate.

Progress: Relative usages of antimicrobial agents in the treatment of patients with acute diarrhea are shown in Table I which is based on specimens submitted during this study. In many instances the choice of drug was based on cost rather than medical judgement because many patients sought medical attention only after self-medication was unsuccessful.

Overall these in vitro studies indicate that the most effective antibiotic studied was oxytetracycline followed in order of decreasing effectiveness by chloramphenicol, neomycin sulfate, kanamycin, streptomycin sulfate and penicillin G. (Table II) However it should be emphasized that some strains of nearly every species were resistant to a given antibiotic and the choice of therapy should be based on antibiotic sensitivity studies.

Oxytetracycline - All but three of the 42 species of salmonella isolated tended to be sensitive to this antibiotic (TableIII). However these three are among the enteric pathogens isolated most frequently in Thailand. Most of the shigella strains were sensitive with Shigella flexneri being the least sensitive. More than half of the enteropathogenic Escherichia coli were sensitive with none of the 12 serotypes outstanding. Among the paracolons, most Paracolobactrum intermedium were sensitive; most Paracolobactrum coliforme were resistant and the isolates of Paracolobactrum aerogenoides were about equally divided. Most vibrios were very sensitive to oxytetracycline with the exception of one Ogawa

TABLE I

Antibiotic Therapy of Patients with Acute Diarrhea

Antibiotic	Number of patients
Sulfonamide	218
Neomycin sulfate	129
Streptomycin sulfate	122
Chloramphenicol	59
Tetracyclines	31
Others	11
No Antibiotic	245

TABLE II

Sensitivities of Enteric Isolates to Six Antibiotics

Antibiotic	Number of Isolates Sensitive (mcg/ml)								
	1.56	3.12	6.25	12.5	25	50	100	200	200
Oxytetracycline	162	81	62	23	13	17	4	15	84
Chloramphenicol	90	67	92	80	29	9	9	15	73
Neomycin Sulfate	8	24	48	105	138	55	27	5	52
Kanamycin	0	4	14	54	107	194	34	9	52
Streptomycin Sulfate	0	1	7	29	58	120	72	46	129
Penicillin G.	0	1	21	60	100	47	23	7	209

TABLE III

Sensitivity of Enteric Isolates to Oxytetracycline

Organisms	Number of isolates tested	Number of isolates sensitive (mcg/ml)								
		1.56	3.12	6.25	12.5	25	50	100	200	200
Salmonella paratyphi B	10	1	1	-	-	-	-	-	-	8
Salmonella derby	10	2	3	-	-	-	-	-	-	5
Salmonella typhimurium	6	-	1	4	1	-	-	-	-	-
Salmonella montevideo	10	-	-	2	-	-	-	-	-	8
Salmonella typhosa	8	6	1	-	-	1	-	-	-	-
Salmonella lexington	10	2	6	2	-	-	-	-	-	-
Salmonella anatum	10	5	1	3	1	-	-	-	-	-
Other salmonella	82	26	26	19	5	2	1	-	-	3
Shigella dysenteriae	6	3	1	1	-	-	-	-	-	1
Shigella flexneri	17	3	1	2	-	3	4	1	1	2
Shigella boydii	3	1	2	-	-	-	-	-	-	-
Shigella sonnei	15	1	2	4	3	1	1	-	1	2
Alkalescens-Dispar	6	-	3	-	-	1	-	-	-	2
Escherichia coli (Enteropathogenic)	66	3	15	12	5	1	3	-	7	20
Paracolobactrum aerogenoides	10	1	1	2	-	-	2	1	3	-
Paracolobactrum coliforme	10	-	-	1	-	1	1	-	-	7
Paracolobactrum intermedium	7	-	2	2	2	-	-	-	-	1
Proteus mirabilis	9	1	-	-	-	-	-	-	2	6
Pseudomonas sp.	10	7	3	-	-	-	-	-	-	-
Aerobacter aerogenes	10	-	1	-	3	2	1	1	-	2
Intermediate coliforme	10	-	1	1	2	-	1	1	-	4
Escherichia freundii	10	-	3	2	-	-	-	-	-	5
Bethesda Ballerup	10	1	3	4	-	1	-	-	-	1
Providencia	10	1	1	1	-	-	-	-	1	6
El Tor Ogawa (Viet Nam)	25	24	1	-	-	-	-	-	-	-
El Tor Ogawa	26	24	1	-	-	-	1	-	-	-
El Tor Inaba	24	22	1	-	1	-	-	-	-	-
Non-agglutinating Vibrios	32	28	-	-	-	-	3	-	-	1

serotype and 4 non-agglutinating vibrios. All but one strain of Proteus mirabilis were resistant and all strains of pseudomonas were sensitive. Strains of the remaining species ranged the entire gamut from very sensitive to completely resistant.

Chloramphenicol - In general the in vitro findings with chloramphenicol paralleled those of oxytetracycline (Table IV) except that somewhat higher levels of chloramphenicol were required. Exceptions were (1) Salmonella derby strains were more sensitive, (2) more strains of Sh. flexneri were highly resistant, and (3) most strains of pseudomonas were resistant to chloramphenicol. None of the strains of S. typhosa have become highly resistant to chloramphenicol although it has been the drug of choice for typhoid fever in Thailand for many years. It was noted that vibrios isolated in Thailand tended to be more resistant to chloramphenicol than isolates from Viet Nam.

Neomycin and Kanamycin - These antibiotics can be discussed together because of the closeness of their antibacterial spectrum, i.e. 53 of 61 strains resistant to greater than 100 mcg/ml of kanamycin were equally resistant to neomycin. While the pattern of cross-resistance is apparent in Tables V and VI, neomycin consistently was more inhibitory than kanamycin. Some strains of all genera studied were sensitive to clinically achievable levels of neomycin, but this was less true for kanamycin. In the absence of specific sensitivity studies there is nothing in this data to indicate a choice of kanamycin over neomycin.

Streptomycin - Only 37 of the 462 strains tested were inhibited by 12.5 mcg/ml this antibiotic and most of these were randomly distributed among the enteropathogenic E. coli and various salmonella species (Table VII). There was no indication that any species or serotype was consistently sensitive to streptomycin.

Penicillin G. - This antibiotic was included in the study because it has been shown to be bacteriostatic for a number of gram negative pathogens. The results in Table VIII show that practically all the organisms except a few salmonella and shigella strains were resistant to penicillin G. Of particular interest was the complete resistance of Proteus mirabilis, a species reported to have many strains sensitive to Penicillin G.

Summary: The antimicrobial sensitivities of Gram - negative bacteria isolated from patients with acute diarrhea were assayed against oxytetracycline, chloramphenicol, penicillin, streptomycin, neomycin and kanamycin. Oxytetracycline and chloramphenicol in that order were the most inhibitory for most species and from this standpoint they are clearly the antibiotics of choice until laboratory results are available.

TABLE IV

Sensitivity of Enteric Isolates to Chloramphenicol

Organisms	Number of isolates tested	Number of isolates sensitive (mcg/ml)								
		1.56	3.12	6.25	12.5	25	50	100	200	200
Salmonella paratyphi B	10		-	-	1		1		-	8
Salmonella derby	10		1	5	3	1	-	-	-	-
Salmonella typhimurium	6	-	3	2	1	-	-	-	-	-
Salmonella montevideo	10	-	-				-	-	-	10
Salmonella typhosa	8	3	2	2	1	-	-		-	-
Salmonella lexington	10	-	4	3	2	1	-		-	-
Salmonella anatum	10	-	1	3	5	1	-		-	
Other Salmonella	82	-	11	37	25	6	1	1	-	1
Shigella dysenteriae	6	2	2	2	-	-	-	-	-	
Shigella flexneri	17	6	2	-	-	1	-	-	5	3
Shigella boydii	3		3	-	-	-	-	-	-	
Shigella sonnei	15	1	2	4	3	1	-	-	-	4
Alkalescens-Dispar	6	-	1	2	2	-	-	-	-	1
Escherichia coli (Enteropathogenic)	66	-	6	10	17	4	-	1	3	25
Paracolobactrum aerogenoides	10		1	-	1	2	-		1	5
Paracolobactrum coliforme	10	-		1	1	-	2	1	1	4
Paracolobactrum intermedium	6	1	3	-	2	-	-	-	-	-
Proteus mirabilis	9	-	-		2	6		1	-	
Pseudomonas sp.	10	-	1				1	2	3	3
Aerobacter aerogenes	10	-	1	2	4	2			-	1
Intermediate coliforme	10	-		1		1	1	1	2	4
Escherichia freundii	10	-	3	2	2	-		-	-	3
Bethesda Ballerup	10			9		-	-	-	-	1
Providencia	10	-	-	1	5	3	1	-	-	
El Tor Ogawa (Viet Nam)	25	25	-	-	-	-	-	-	-	
El Tor Ogawa	28	16	9	1	1	-	-	-	-	
El Tor Inaba	24	9	11	3	-		1		-	
Non-agglutinating Vibrios	34	27	-	2	2	-	1	2	-	

TABLE V

Sensitivity of Enteric Isolates to Neomycin Sulfate

Organisms	Number of isolates tested	Number of isolates sensitive (mcg/ml)								
		1.56	3.12	6.25	12.5	25	50	100	200	200
Salmonella parathyphi B	10	-	-	-	2	-	-	-	-	8
Salmonella derby	10	-	-	-	3	-	2	-	-	5
Salmonella typhimurium	6	-	-	2	2	1	1	-	-	-
Salmonella montevideo	10	-	-	-	-	3	-	-	-	7
Salmonella typhosa	8	3	3	1	1	-	-	-	-	-
Salmonella lexington	10	-	-	-	6	-	3	1	-	-
Salmonella anatum	10	-	-	-	-	4	1	5	-	-
Other salmonella	82	-	4	9	34	11	8	10	2	4
Shigella dysenteriae	6	-	-	1	-	3	1	1	-	-
Shigella flexneri	17	1	-	1	8	5	-	2	-	-
Shigella boydii	3	-	-	1	2	-	-	-	-	-
Shigella sonnei	15	-	-	-	2	3	9	-	-	1
Alkalescens-Dispar	6	-	-	2	2	-	-	2	-	-
Escherichia coli (Enteropathogenic)	66	-	3	11	22	9	4	-	-	17
Paracolobactrum aerogenoides	10	-	4	5	1	-	-	-	-	-
Paracolobactrum coliforme	9	-	-	-	8	-	-	-	-	1
Paracolobactrum intermedium	8	-	-	2	4	1	1	-	-	-
Proteus mirabilis	9	-	-	-	-	3	6	-	-	-
Pseudomonas sp.	10	-	1	-	2	3	4	-	-	-
Aerobacter aerogenes	10	-	4	6	-	-	-	-	-	-
Intermediate coliforme	10	1	-	2	1	1	-	-	-	5
Escherichia freundii	10	-	2	1	3	-	1	-	-	3
Bethesda Ballerup	10	2	3	2	1	2	-	-	-	-
Providencia	10	-	-	-	-	2	3	3	2	-
El Tor Ogawa (Viet Nam)	25	-	-	-	-	23	2	-	-	-
El Tor Ogawa	29	1	-	2	1	22	3	-	-	-
El Tor Inaba	25	-	-	-	1	23	1	-	-	-
Non-agglutinating Vibrios	33	-	-	-	4	19	5	3	1	1

TABLE VI

Sensitivity of Enteric Isolates to Kanamycin

Organisms	Number of isolates tested	Number of isolates sensitive (mcg/ml)								
		1.56	3.12	6.25	12.5	25	50	100	200	200
Salmonella paratyphi B	10	-	-	-	-	2	-			8
Salmonella derby	10	-	-	-	-	3	2	-	-	5
Salmonella typhimurium	6	-	-	-	1	4	1		-	
Salmonella montevideo	10	-	-	-	-	1	2			7
Salmonella typhosa	8	-	2	1	4	1	-		-	
Salmonella lexington	10	-	-	-	1	4	4	-	-	1
Salmonella anatum	10	-	-	-	-	-	9	1	-	
Other salmonella	82	-	-	2	9	27	37	4	-	3
Shigella dysenteriae	6	-	-	-	-	3	1	1	1	
Shigella flexneri	17	-	-	3	6	6	-	1	1	
Shigella boydii	3	-	-	-	1	2	-	-	-	
Shigella sonnei	15	-	-	-	2	7	3	2	-	1
Alkalescens-Dispar	6	-	-	-	1	2	1			2
Escherichia coli (Enteropathogenic)	66	-	-	1	6	18	17	6	-	18
Paracolobactrum aerogenoides	10	-	-	3	2	4	-	1	-	
Paracolobactrum coliforme	10	-	1	-	3	3	2		-	1
Paracolobactrum intermedium	8	-	-	-	5	1	1	1	-	-
Proteus mirabilis	9	-	-	-	-	-	3	6	-	
Pseudomonas sp.	10	-	-	-	1	2	-	2	3	2
Aerobacter aerogenes	10	-	-	3	7	-	-		-	
Intermediate coliforme	9	-	1	1	1	-	2	-	4	
Escherichia freundii	10	-	-	-	-	2	3	2	-	3
Bethesda Ballerup	10	-	-	-	1	3	4	2	-	
Providencia	10	-	-	-	-	-	9	-	-	1
El Tor Ogawa (Viet Nam)	25	-	-	-	-	-	25	-	-	
El Tor Ogawa	29	-	-	-	2	1	26	-	-	
El Tor Inaba	25	-	-	-	-	3	19	3	-	
Non-agglutinating Vibrios	34	-	-	-	1	8	23	2	-	

TABLE VII

Sensitivity of Enteric Isolates to Streptomycin Sulfate

Organisms	Number of isolates tested	Number of isolates sensitive (mcg/ml)								
		1.56				25	50	100	200	
Salmonella paratyphi B	10	-	-	-	-	1	-	1	-	8
Salmonella derby	10	-	-	-	-	1	-	3	2	3
Salmonella typhimurium	6	-	-	-	-	1	-	4	1	
Salmonella montevideo	10	-	-	-	-		-		10	
Salmonella typhosa	8	-	-	-	-	3	4	1	-	
Salmonella lexington	10	-	-	-	-		1	5	2	2
Salmonella anatum	10	-	-	-	-		-	2	3	5
Other salmonella	82	-	1	2	6	21	31	12	9	
Shigella dysenteriae	6	-	-	-	-	2	1	1	1	1
Shigella flexneri	17	-	-	1	3	1	-	1	4	7
Shigella boydii	3	-	-	1	-	1	1			
Shigella sonnei	15	-	-	-	-	8	1	1	1	4
Alkalescens-Dispar	6	-	-	-	-		1	1	2	2
Escherichia coli (Enteropathogenic)	66	-	-	3	12	11	4	2		34
Paracolobactrum aerogenoides	10	-	-	-	4	1	-			5
Paracolobactrum coliforme	10	-	-	-	-	1	2		1	6
Paracolobactrum intermedium	6	-	-	-	-	1	1	1	1	2
Proteus mirabilis	9	-	-	-	-		-	4	1	4
Pseudomonas sp.	10	-	-	-	1	2	1	-	-	6
Aerobacter aerogenes	10	-	-	-	3	2	-			5
Intermediate coliforme	10	-	-	-	-	-	-		2	8
Escherichia freundii	10	-	-	-	-	-	2	2	1	5
Bethesda Ballerup	10	-	-	-	-		1	3	-	6
Providencia	10	-	-	-	-		2	2		6
El Tor Ogawa (Viet Nam)	25	-	-	-	-		25	-		
El Tor Ogawa	28	-	-	-	-		21	4	1	2
El Tor Inaba	25	-	-	-	-		15	6	2	2
Non-agglutinating Vibrios	34	-	-	-	-	1	5	16	2	10

TABLE VIII

Sensitivity of Enteric Isolates to Penicillin G.

Organisms	Number of isolates tested	Number of isolates sensitive (mcg/ml)								
		1.56	3.12	6.25	12.5	25	50	100		200
Salmonella paratyphi B	10	-	-	-	-	2	-	-		
Salmonella derby	10	-	-	-	7	1	2	-		
Salmonella typhimurium	6	-	-	-	2	3	-	-		
Salmonella montevideo	10	-	-	-	-	-	-	-		
Salmonella typhosa	8	-	-	5	2	-	-	-	1	
Salmonella lexington	10	-	-	1	5	2	-	-	-	1
Salmonella anatum	10	-	-	1	3	3	3	-		
Other salmonella	82	-	-	7	30	35	6	2	1	
Shigella dysenteriae	6	-	-	-	2	1	-	1	-	1
Shigella flexneri	17	-	-	3	4	6	1	-	1	2
Shigella boydii	3	-	-	-	-	3	-	-	-	
Shigella sonnei	15	-	-	-	-	1	9	5		
Alkalescens-Dispar	6	-	-	2	-	-	1	1	-	2
Escherichia coli (Enteropathogenic)	66	-	-	-	2	14	21	6	2	21
Paracolobactrum aerogenoides	10	-	-	-	-	-	-	-	-	10
Paracolobactrum coliforme	10	-	-	-	1	3	2	2		2
Paracolobactrum intermedium	8	-	-	-	-	1	-	1		6
Proteus mirabilis	9	-	-	-	-	-	-	2	-	7
Pseudomonas sp.	10	1	-	-	-	-	-	-	-	9
Aerobacter aerogenes	10	-	-	-	-	-	-	-		10
Intermediate coliforme	10	-	-	-	-	-	-	-		10
Escherichia freundii	10	-	-	-	-	-	-	-		10
Bethesda Ballerup	10	-	-	-	-	-	-	-	-	10
Providencia	10	-	-	-	-	-	-	-		10
El Tor Ogawa (Viet Nam)	25	-	-	-	-	-	-	-	-	25
El Tor Ogawa	29	-	1	1	1	-	-	1	-	25
El Tor Inaba	25	-	-	-	1	23	1	-	-	
Non-agglutinating Vibrios	34	-	-	-	-	1	1	2	2	28

Conclusion: Twenty-nine strains of enteric pathogens were resistant to greater than 100 mcg/ml of each antibiotic studied, presumably the result of the unrestricted availability of antibiotics in Thailand. Additional antibiotics, not generally available in this area should be tested against these resistant strains and one or more effective antimicrobials should be stockpiled and utilized judiciously.