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THE SUSCEPTIBILITY OF FIFTY STRAINS OF SHIGELLA TO EIGHT ANTIMICROBIAL AGENTS

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Abstract

Fifty strains of Shigella from patients in Nakorn Chiang Mai Hospital and McCormick Hospital were isolated during the period from December 15, 1970, to March 20, 1971. The identification of Shigella was carried out by biochemical reaction and serology. Of 50 isolated Shigella, altogether 32 strains of Sh. flexneri were identified. All of these strains were tested for antimicrobial sensitivity by the serial tube dilution methed with eight antimicrobial agents. The antimicrobial agents were Garamycin (Gentamycin), Terramycin (Oxytetracycline), Colimycin, Streptomycin, Chloramphenicol, Ampicillin, Kanamycin and Sulfadiazine. The results indicated that Shigella flexneri were sensitive to Gentamycin 12.4%, Colimycin 68.75%, Ampicillin 12.5%, Kanamycin 12.5%, Chloramphenicol 3.12% respectively. Fourteen strains of Sh. sonnei were sensitive to Colimycin 83.33% and to Ampicillin 7.14%. For 4 strains of Shigella dysenteriae, 75% of them have been shown to be sensitive to Colimycin and 25% of them were sensitive to Kanamycin. None of Shigella were sensitive to Sulfadiazine, Streptomycin and Terramycin.

INTRODUCTION

Intestinal tract infections are among the most common disease usually found in tropical countries, particularly in poor hygenic populations. The infections agents may be parasitic, such as Hookworm, Entamoeba histolytica, or may be bacterial.

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Among bacterial intestinal tract infections, Shigellosis or bacillary dysentery, caused by Shigella, is one of the most common disease in Thailand. Shigella is divided by biochemical reactions into two major groups, mannitol fermenters and non-mannitol fermenters, and into four groups by serological reaction, group A (**Shigella dysenteriae**), group B (**Shigella flexneri**), group C (**Shigella boydii**), and group D (**Shigella sonnei**).

The transmission of this disease may occur by direct contact through fecal-oral transmission; or indirectly, by eating contaminated foods, vegetables, or drinking contaminated water or milk (1,2,3). The disease begins with acute diarrhoeae, accompanied by fever and often vomitting, cramps and tenesmus. In severe cases the stool may contain blood, mucus and pus.

Many antibiotics are introduced in the treatment of Shigellosis. Formerly sulfaguanidine was very effective, however later many strains developed resistance. Some enteric bacilli could transfer resistance factors to Shigella and make it resistant to the antibiotics (4,5,6,), Therefore, the antimicrobial sensitivity should done before treatment. But sometime the doctor can not wait for the sensitivity results. In this case the drug of choice shoul be given. This experiment tried to study the antimicrobial susceptibility of Shihella and it will be useful for the doctor to choose the drug for treatment of Shigellosis.

MATERIALS AND METHODS

I. **Organism.** Shigella used in this experiment were isolated from the patients in Chiang Mai Hospital and McCormick Hospital. Most of the patients came to the hospital with the symptoms of diarrhoeae or dysentery.

II. **Antimicrobial agents.** Eight antimicrobial agents, sulfadiazine, colimycin, terramycin, garamycin, ampicillin, chloramphenicol, streptomycin, and kanamycin were tested for susceptibility of Shigella.

These agents were dissolved in sterile distilled water to give concentration of 2000 mcg or unit/ml and kept frozen in 4 ml aliquots. Before uging the stock solution was thawed and the remainder discarded after use.

III. **Susceptibility testing method.** Stock solutions of antimicrobial agents were thawed and diluted 1 : 10 with sterile distilled water, the final concentration being 200 mcg or unit/ml. The serial dilution of antimicrobial agents were done using tryptic case soy broth to make concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781 and 0.39 mcg or unit/ml, the final volume was 0.5 ml. Then 0.5 ml. of 1 : 1000 dilution of overnight culture of Shigella

in trypticase soy broth was added to each antimicrobial dilution. Incubate 37° C overnight and read MIC (Minimal Inhibitory Concentration).

RESULTS

A total of 50 strains of Shigella were isolated, 32 strains were **Sigella flexneri**, 14 strains were **Shigella sonnei**, and 4 strains were **Shigella dysenteriae**.

The results of the antimicrobial susceptibility are shown in Table I, II and III. The percentage of sensitive and resistance are shown in Table V. These results are compared with blood level of the antimicrobial agents (Table IV). None of the Shigella were sensitive to streptomycin, sulfadiazine and terramycin. Colimycin seems to be effective.

Table I Minimal Inhibitory Concentration (MIC.) of Shigella flexneri 32 strains to 8 Antimicrobial agents

Concentration of Antimicrobial agents mcg/ml or unit/ml	MIC. to Antimicrobial agents							
	Caramycin mcg/ml	Colimycin mcg/ml	Terramycin mcg/ml	Chloram-phenicol mcg/ml	Streptomycin mcg/ml	Ampicillin mcg/ml	Kanamycin mcg/ml	Sulfadiazine mcg/ml
> 100	–	10	–	1	5	–	–	32
100	–	2	2	6	1	–	–	–
50	–	7	5	15	8	1	1	–
25	–	7	15	6	3	1	3	–
12.5	–	5	8	–	14	4	8	–
6.25	5	1	2	3	1	14	16	–
3.125	23	–	–	–	–	8	4	–
1.562	4	–	–	–	–	4	–	–
0.781	–	–	–	–	–	–	–	–
0.39	–	–	–	–	–	–	–	–
< 0.39	–	–	–	–	–	–	–	–

Table II Minimal Inhibitory Concentration (MIC.) of Shigella sonnei 14 Strains to 8 Antimicrobial agents

Concentration of Antimicrobial agents mcg/ml or unit/ml	MIC. to Antimicrobial agents							
	Garamycin mcg/ml	Colimycin mcg/ml	Terramycin mcg/ml	Chloram-phenicol mcg/ml	Strepto-mycin mcg/ml	Ampicillin mcg/ml	Kanamycin mcg/ml	Sulfadia-zine mcg/ml
> 100	--	2	--	--	--	--	--	14
100	--	2	--	3	--	--	--	--
50	--	4	1	6	3	--	--	--
25	--	5	5	1	8	1	--	--
12.5	--	1	2	4	3	1	--	--
6.25	4	--	6	--	--	9	10	--
3.125	10	--	--	--	--	2	4	--
1.562	--	--	--	--	--	1	--	--
0.781	--	--	--	--	--	--	--	--
0.39	--	--	--	--	--	--	--	--
< 0.39	--	--	--	--	--	--	--	--

Table III Minimal Inhibitory Concentration (MIC.) of Shigella dysenteriae 4 strains to 8 Antimicrobial agents

Concentration of Antimicrobial agents mcg/ml or unit/ml	MIC. to Antimicrobial agents							
	Garamycin mcg/ml	Colimycin mcg/ml	Terramycin mcg/ml	Chloram-phenicol mcg/ml	Strepto-mycin meg/ml	Ampicillin mcg/ml	Kanamycin mcg/ml	Sulfadia-zine mcg/ml
> 100	--	1	--	--	1	--	--	4
100	--	--	--	--	--	--	--	--
50	--	1	4	--	--	--	--	--
25	--	2	--	3	--	--	--	--
12.5	--	--	--	1	3	1	--	--
6.25	1	--	--	--	--	3	3	--
3.125	3	--	--	--	--	--	1	--
1.562	--	--	--	--	--	--	--	--
0.781	--	--	--	--	--	--	--	--
0.39	--	--	--	--	--	--	--	--
< 0.39	--	--	--	--	--	--	--	--

Table IV. The limit of Minimal Inhibitory Concentrations for "Sensitive", "Intermediate" and "Resistant" strains.

Antibiotics	Sensitive (mcg/ml)	Intermediate (mcg/ml)	Resistant (mcg/ml)
Streptomycin	5.0	5.0–25	25
Kanamycin	5.0	5.0–25	25
Gentamycin	2.5	2.5–10	10
Chloramphenicol	5.0	5.0–25	25
Tetracycline	2.5	2.5–50	5.0
Colimycin	100U/ml	100–500U/ml	500 U/ml
Ampicillin	2.5	2.5–10	10

Table V Percentage of sensitive (S), intermediate (I), and resistance (R) to 8 antimicrobial agents of Shigella.

Antimicrobial agents		Sh. flexneri	Sh. sennei	Sh. dysenteriae
Streptomycin	%S	0	0	0
	%I	12.5	71.4	75
	%R	87.5	28.6	25
Ampicillin	%S	12.5	7.14	0
	%I	43.75	75.4	75
	%R	47.75	17.46	25
Kanamycin	%S	12.5	0	25
	%I	84.38	100	75
	%R	3.12	0	0
Sulfadiazine	%S	0	0	0
	%I	0	0	0
	%R	100	100	100
Garamycin	%S	12.5	0	0
	%I	87.5	100	100
	%R	0	0	0
Colimycin	%S	68.75	83.33	75
	%I	31.25	16.66	25
	%R	0	0	0
Terramycin	%S	0	0	0
	%I	0	0	0
	%R	100	100	100
Chloramphenicol	%S	3.12	0	0
	%I	18.25	0	75
	%R	78.63	100	25

DISCUSSION

Antimicrobial susceptibility is very important in antibiotic therapy (7). Each organism is different in its susceptibility to drugs. There are many methods for determining antibiotic susceptibility such as the disc agar diffusion method, the agar plate dilution method, and the test tube serial dilution method. The test tube serial dilution method seem to be the best with the result explessed in quantitative terms as MIC (Minimal Inhibitory Concentration), which is easy to compared with other antibiotics. However, this method is time consuming, laborious and needs skilled technicians. There are many factors affection the accuracy of the test : for example, medium pH, stability of antibiotics, inoculum, and and incubation time.

From our result, all of the 50 Shigella strains were resistant to sulfadiazine, streptomycin, and terramycin. This may be because these drugs have been used commonly for a long time, and patients usually buy and take them by themselves. So the posibility that the organisms have been exposed to and have developed resistance to these drugs is higher. Haltalin et. al. (8) have shown that only 25 of 52 strains were sensitive to sulfadiazine while 47 of 52 strains were sensitive to ampicillin. The other may have resulted from the small amount of our sample.

Colimycin and kanamycin seem to be the most effective drugs for Shigellosis, because only a few strain of Shigella were resistant to these drugs.

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