

## Bio-assay of Chloramphenical in Enteric Fever Cases.

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### Summary:-

This paper reports our experience in monitoring Chloramphenical therapy during the outbreak of typhoid fever in 2040 and 2041 at Kathmandu. The species isolated were salmonella typhi, salmonella paratyphi A, and one salmonella paratyphi C and one salmonella para B. Cases of enteric fever were treated with adequate doses of chloramphenical i. e. 2gm daily till temp. drops in adults and 50mg- 100mg/kg body weight in children. Temp. did not get controlled even in 7-10 days as expected. Also four relapse cases were reported. It was a very worrying feature for us. It posed problems: a) whether the patient is not taking adequate dose as prescribed or b) the chloramphenical is resistant as reported elsewhere or c) the antibiotic is not properly absorbed. We made an enquiry and found that the patients were taking the medicine as prescribed. Also, we performed the sensitivity test of the isolates and found that all the isolates except one were sensitive to chloramphenical. Prompted by this we felt that it is necessary to do bio-assay in order to find out whether the drug is being properly absorbed or not. Most of the leading brands of chloramphenical available in the market were tested to find out the therapeutic blood level. The therapeutic level of chloramphenical in blood is 5- 20ug./ml. (but usual adequate level is 5- 10ug/ml.)

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## Introduction:-

The causative organism of enteric fever is salmonella sp. of which the main source of infection is feco - oral contamination. In a country like our, chloramphenical is widely considered to be the first drug of choice for enteric fever. So, study with different brands of oral and parenteral chloramphenical was carried out.

## Chloramphenical:-

This was prepared in 1947 from streptomycin in U. S. A. and synthetically made in 1949. It is highly soluble in alcohol and poorly soluble in water. Chloramphenical is bacteriostatic; it interferes with protein synthesis by inhibiting the enzyme, which transfers the peptide chain to the new amino acid on the ribosome.

After oral administration of chloramphenical, absorption from the alimentary tract is almost complete. It is widely distributed approximately even throughout the body. But concentration in the pleural space & CSF, in the absence of inflammation are about 30 - 50% of plasma conc. Although it is metabolised in the liver its biliary concentration is not more than 5% & is excreted in the urine where only 5 - 10% of the drug appears unchanged.

## Methods & Materials:-

**Bacteriological Investigation-** Sub-culture from blood culture were performed at 37° C. on Macconkey agar using a standard loop technique.

The bio-assay indicates the conc. of chloramphenical in the blood of the patient having pre & post medication. For this, we applied the well diffusion technique as described by Garrod. Firstly pour the Nutrient agar plate with standard known bacteria sensitive to 'C'. In this case, we have used staph. ox NCTC. After pouring, different wells were made for clinical materials and for different conc. of chloramphenical. Taking a known standard chloramphenical, different dilutions were made as 3.5ug/ml., 7.5ug/ml., 15ug/ml. & 30ug/ml. Equal amounts of serum & antibiotic were put in different wells & allowed the plate to stand on the table for one to two hours for diffusion of serum & antibiotic before the growth of organisms. Then, the plate was incubated at 37° C over night. The plate is read next day for zone of inhibition in different wells of serum & antibiotic conc. Graph was made to find out the conc. of chloramphenical level in serum.

Initially the antibiotic assay of chloramphenical was done as pre & post administration of the chloramphenical. The pre was just before the next dose & post were one hour & two hours after the administration of drug. As the antibiotic level were not adequate in the post two hrs. after administration of drug, another trial of post administration drug of 1 hr., 2hrs. & 3 hrs. were done.

### Result;-

In both the trials, the antibiotic level of the serum after the administration of drug up to 3 hrs. were less than 3ug/ml. Total No. of assay done is 60, out of them 14 showed required MIC & three showed more than 25ug/ml. in chloramphenical therapy. These were in a special brand of chloramphenical only. The respective results are given in the table (1)

### Discussion;-

This series confirms certain facts about cases of enteric fever. In the year 2040 total no. of blood culture done is 2442, total no. of positive cases of salmonella typhi is 254 & salmonella paratyphi 'A' is 63. In the year 2041, total no. of blood culture done is 4251, of which positive cases of salmonella sp. is 468 & salmonella paratyphi 'A' is 176, salmonella para 'C' 1

There are some cases of clinically suspected enteric fever, but blood culture was negative in them. In such cases, we tried the bone marrow culture for salmonella. Total No. of bone marrow culture done is 10 out of them two positive for salmonella typhi & two for salmonella paratyphi 'A' with this result it shows the importance of bone marrow culture as an ancillary method of diagnosis in blood culture negative cases.

Cases of enteric fever were treated with chloramphenical. But proper response was not observed i.e. temp. did not fall in 5 - 7 days as expected. So, bio-assay of different proprietors brands of chloramphenical was carried out. It revealed that the most of the brands of chloramphenical except few had blood levels less than the desired therapeutic level.

### Conclusion ;-

All the proprietary brand did not give the same blood level of the antibiotic & this could be the main cause of varied nature of response of patient to antibiotic. 'Chloromycetin' gave the consistently uniform desired blood level.

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No.	Sex	Age	INHIBITION ZONE in cm.						Conc. in mg/ml from graph approximately.			Remarks	
			Chloramphenical Conc in mg/ml				Serums.						
			3.5	7.5	15	30	SI	SII	SIII	SI	SII	SIII	
1.	M	30	1.5	1.9	2.2	2.5	1.2	1.4		2	3		
2.	F		1.3	1.6	1.8	2.4	1.1	1.3		3	3.5		
3.	M	8	1.3	1.6	1.8	2.4	.9	1.1		3	3		
4.	F	8	1.3	1.6	1.8	2.4	1.4	1.5		5	5		
5.	M	22	.75	1.6	2.2	2.5	.75	2.1		3	5		
6.	M	35	1.9	2.2	2.9	3.3	.8	1.5	3.9	3	3	30	
7.	F	7	1.1	1.5	2.1	2.5	.8	1.0	1.3	3	3	5	
8.	F	23	1.1	1.6	2.1	2.5	.8	1.0	1.4	3	3	5	
9.	M	20	1.1	1.4	2.2	2.6	.9	1.1	1.2	3	5	5	
10.	F	60	1.1	1.4	2.2	2.6	1.2	1.3	1.3	5	5	5	
11.			.7	.9	1.5	2	.7		1.3	3		11	
12.	M	11	.7	.9	1.5	2	.7	.7	.7	3	3	3	
13.	M	17	.7	.9	1.5	2	.7	.7	.7	3	3	3	
14.	M	12	.7	.9	1.5	2	.7	.7	.7	3	3	3	
15.			.7	.9	1.5	2	.6	.8	.8	3	5	5	
16.	F	28	.7	.9	1.5	2	.7	.7	.7	3	3	3	
17.	F	9	.65	.9	1.6	1.9	.65	.65	1.3	3	3	11	
18.	M	19	.65	.9	1.6	1.9	.65	.65	.65	3	3	3	
19.	M	27	.65	.9	1.6	1.9	.6	.65	.65	3	3	3	
20.	F	23	.65	.9	1.6	1.9	.65	.65	.65	3	3	3	
21.	F	18	.65	.9	1.6	1.9	.65	.65	.6	3	3	3	
22.	M		.65	.9	1.6	.9	1.65	.65	.65	3	3	3	
23.	F	23	.65	.9	1.6	.9	1.65	.65	.65	3	3	3	
24.	F	33	.65	.9	1.6	.9	1.65	.65	.65	3	3	3	
25.	M	27	"	"	"	"	"	"	"	"	"	"	
26.	F	18	"	"	"	"	"	"	"	"	"	"	
27.	M	23	"	"	"	"	"	"	"	"	"	"	
28.	M	20	"	"	"	"	"	"	"	"	"	"	
29.	M	10	"	"	"	"	"	"	"	"	"	"	

30.	M	52	"	"	"	"	"	"	"	"	"	"
31.	M	17	1.5	1.9	2.2	2.5	1.5	2.3	2.8	3.5	17	30
32.	M	17	1.3	1.5	2	2.5	1.3	1.3	1.8	3	3	12
33.	M	18	1.3	1.5	2	2.5	1.3	1.3	1.3	3	3	3
34.	M	21	1.1	1.3	2	2.6	1.3	1.9	1.9	5	12	12
35.	M	18	1.1	1.3	2.3	2.9	1.3	1.85	2.3	5	11	15
36.	F	17	1.1	1.3	2.3	2.9	1.1	1.2	1.2	3	5	5
37.	M	55	1.1	1.3	2.3	2.9	1.0	1.5	2.9	3	10	30
38.	F	20	1.1	1.3	2.3	2.9	1.1	1.1	1.1	3	3	3
39.	M	25	1.1	1.3	2.3	2.9	1.1	1.1	1.2	3	3	3
40.	M	38	1.9	2.2	2.4	2.9	1.9	2	2.4	3	3	3
41.	F	13	1.9	2.2	2.4	2.9	1.9	1.9	1.9	3	3	3
42.	M	18	1.9	2.2	2.4	2.9	2.9	1.9	1.9	3	3	3
43.	M	22	"	"	"	"	"	"	"	"	"	"
44.	F	19	"	"	"	"	"	"	"	"	"	"
45.	M	16	"	"	"	"	"	"	"	"	"	"
46.	M	22	"	"	"	"	"	"	"	"	"	"
47.	F	18	"	"	"	"	"	"	"	"	"	"
48.	F	23	"	"	"	"	"	"	"	"	"	"
49.	M	19	"	"	"	"	"	"	"	"	"	"
50.	M	25	"	"	"	"	"	"	"	"	"	"
51.	M	27	"	"	"	"	"	"	"	"	"	"
52.	M	45	"	"	"	"	"	"	"	"	"	"
53.	E	29	"	"	"	"	"	"	"	"	"	"
54.	M	26	"	"	"	"	"	"	"	"	"	"
55.	M	19	"	"	"	"	"	"	"	"	"	"
56.	M	20	6.3	1.6	1.8	2.4	1.3	1.3	1.6	3	3	3
57.	M	34	1.3	1.5	2.5	2.6	1.3	2.1	2.5	3	3	3