

AD-774 363

CONCENTRATIONS OF AMPICILLIN AND
CHLORAMPHENICOL IN THE SERUM OF
PATIENTS WITH ACUTE 'SALMONELLA'
ENTERIC FEVER

Isis A. Mikhail, et al

Naval Medical Research Unit No. 3
FPO New York 09527

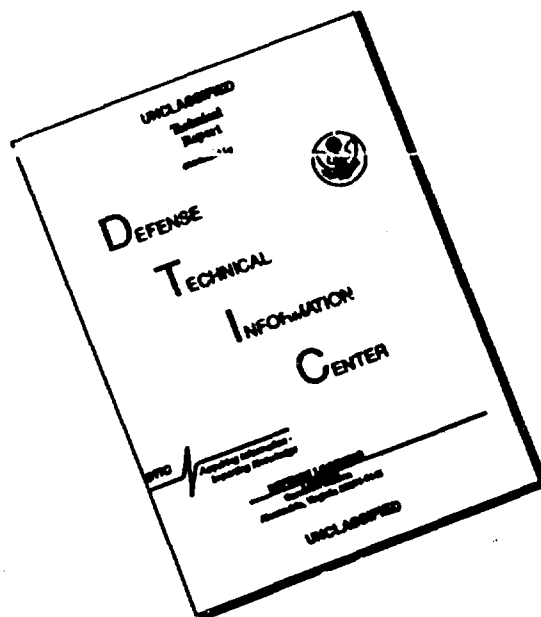
23 June 1972

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

DISCLAIMER NOTICE



**THIS DOCUMENT IS BEST
QUALITY AVAILABLE. THE COPY
FURNISHED TO DTIC CONTAINED
A SIGNIFICANT NUMBER OF
PAGES WHICH DO NOT
REPRODUCE LEGIBLY.**

UNCLASSIFIED
Security Classification

AD 774363

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) U.S. Naval Medical Research Unit No. 3 FPO New York 09527		2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	
		2b. GROUP	
3. REPORT TITLE CONCENTRATIONS OF AMPICILLIN AND CHLORAMPHENICOL IN THE SERUM OF PATIENTS WITH ACUTE <u>SALMONELLA</u> ENTERIC FEVER			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Technical/Scientific report			
5. AUTHOR(s) (First name, middle initial, last name) Isis A. Mikhail, D.C. Kent, K. Sorensen, W.R. Sanborn, and J. Smith			
6. REPORT DATE 23 June 1972		7a. TOTAL NO. OF PAGES 3	7b. NO. OF REFS 14
8a. CONTRACT OR GRANT NO.		9a. ORIGINATOR'S REPORT NUMBER (S) NAMRU-3-TR.46-73	
b. PROJECT NO.		9b. OTHER REPORT NO (S) (Any other numbers that may be assigned this report) Acc.878	
c. MR041.20.01-0261			
d.			
10. DISTRIBUTION STATEMENT Distribution of this report is unlimited			
11. SUPPLEMENTARY NOTES Published in: Antimicrob. Ag. Chemother. 2(5):336-339, 1972		12. SPONSORING MILITARY ACTIVITY Bureau of Medicine and Surgery Department of the Navy Washington, D.C. 20300 20372	
13. ABSTRACT Concentrations of ampicillin and chloramphenicol were measured in the serum of patients being treated for acute infections by <u>Salmonella typhi</u> or <u>S. paratyphi A</u> . Ampicillin was administered by the oral or intramuscular routes, whereas chloramphenicol was given orally only. Ampicillin concentrations were highest 1 hr after initiation of intramuscular therapy and 2 hr after oral administration. The serum concentration of ampicillin was significantly higher when it was given intramuscularly than when it was given orally. However, serum concentrations in orally treated patients were approximately the same at 1 hr after the fifth dose as they were 1 hr after the first dose, in contrast to intramuscularly treated patients, in whom the serum concentration of ampicillin was significantly lower 1 hr after the fifth dose. On the other hand, serum concentrations of chloramphenicol were significantly higher after the fifth dose than after the first dose. The rates of cure, relapse, and drug treatment failure were similar for all treatment regimens, and the drug concentrations attained in serum were generally above the minimal inhibitory concentration for the infecting salmonellae.			

DD FORM 1 NOV 65 1473 (PAGE 1)

17N 0101 807 8801

UNCLASSIFIED

Security Classification

UNCLASSIFIED

Security Classification

14	KEY WORDS	LINK A		LINK B		LINK C	
		ROLE	WT	ROLE	WT	ROLE	WT
	<u>Salmonella</u> Ampicillin Chloramphenicol Enteric Fever <u>Salmonella typhi</u> <u>S. paratyphi A.</u> Serum concentration Therapy						

DD FORM 1 NOV 65 1473 (BACK)

(PAGE 2)

ia

UNCLASSIFIED

Security Classification

Concentrations of Ampicillin and Chloramphenicol in the Serum of Patients with Acute *Salmonella* Enteric Fever

ISIS A. MIKHAIL, D. C. KENT, K. SORENSEN, W. R. SANBORN, AND J. SMITH

U.S. Naval Medical Research Unit No. 3, Cairo, Egypt

Received for publication 23 June 1972

Concentrations of ampicillin and chloramphenicol were measured in the serum of patients being treated for acute infections by *Salmonella typhi* or *S. paratyphi* A. Ampicillin was administered by the oral or intramuscular routes, whereas chloramphenicol was given orally only. Ampicillin concentrations were highest 1 hr after initiation of intramuscular therapy and 2 hr after oral administration. The serum concentration of ampicillin was significantly higher when it was given intramuscularly than when it was given orally. However, serum concentrations in orally treated patients were approximately the same at 1 hr after the fifth dose as they were 1 hr after the first dose, in contrast to intramuscularly treated patients, in whom the serum concentration of ampicillin was significantly lower 1 hr after the fifth dose. On the other hand, serum concentrations of chloramphenicol were significantly higher after the fifth dose than after the first dose. The rates of cure, relapse, and drug treatment failure were similar for all treatment regimens, and the drug concentrations attained in serum were generally above the minimal inhibitory concentration for the infecting salmonellae.

Although chloramphenicol has been the antibiotic of choice for treating acute *Salmonella* enteric fever (13, 14), prolonged therapy occasionally has caused serious complications (6, 11), and ampicillin has been suggested as alternate therapy (3, 5, 10). In vitro, ampicillin has exhibited slightly greater inhibition of salmonellae than did chloramphenicol (9). Studies with healthy human volunteers have demonstrated that maximal serum concentrations of ampicillin occur approximately 2 hr after oral administration, and clinically significant levels remain for at least 6 hr (4). Nevertheless, a 23% failure rate has been observed, and it has been suggested that intramuscular ampicillin might be more effective (8).

Since absorption rates by the oral route might be different in acutely ill enteric fever patients than in healthy persons, this study was initiated to determine ampicillin concentrations in the serum of such patients after oral or parenteral therapy. Comparisons of drug levels and patient response, as well as comparisons with chloramphenicol treatment, were also made.

MATERIALS AND METHODS

Subjects. The 39 patients studied exhibited clinical symptoms of acute enteric fever and were admitted to

the Abbassia Fever Hospital, Cairo, Egypt. *Salmonella* infection in each case was confirmed by blood culture. Patients were between the ages of 6 and 25 years, with a mean age of 14 years. There were 28 males and 11 females. Careful questioning revealed no specific therapy prior to admission and, furthermore only those patients were included in this study whose initial serum specimen contained no antimicrobial activity against the test bacterial strains. Fever onset time prior to admission ranged from 2 to 30 days, with a mean of 10 days.

Infecting strains. *Salmonella* spp. isolated from the blood were tentatively identified by standard biochemical and serological procedures (2) and were subsequently confirmed at the Services des Bacteriophages, Institut Pasteur, Paris, France. The minimal inhibitory concentration (MIC) of ampicillin and chloramphenicol for 26 strains of the 39 salmonella isolates was determined by the inocula-replicating method (12).

Treatment schedules. Patients were treated by one of three therapeutic regimens in rotational order of admission: intramuscular ampicillin (IMA), oral ampicillin (OA), or oral chloramphenicol (OC). The daily ampicillin dosage was 100 mg/kg, divided into four doses given every 6 hr. After 72 hr, IMA therapy was discontinued, and therapy was continued orally. The chloramphenicol oral dose was 50 mg/kg daily, given as above. Treatment was continued for 7 days after body temperature had returned to normal.

Specimens. Antibiotic concentrations in serum were determined immediately before initiation of therapy and at 0.5, 1, 2, 4, 24, 25, and 28 hr thereafter. No further specimens were examined because it was believed that any differences in absorption rates would be evident in this period.

Antibiotic assay. Antibiotic concentrations in the sera were bioassayed by an agar-well diffusion method with the use of simultaneous parallel comparison with standards (1). Antibiotic concentrations in the standards ranged from 0.1 to 80 $\mu\text{g/ml}$ for ampicillin and from 5 to 1,280 $\mu\text{g/ml}$ for chloramphenicol. Standards were prepared in pooled, pretested normal human serum each time patient sera were received, and the standards and specimens were stored together at -70°C until tested. Each assay involved three replications of the test serum and standards. Results were analyzed by "Method 2" of Bennett et al. (1).

A *Bacillus subtilis* (ATCC 6633) spore suspension was used to assay ampicillin (1), and *Sarcina lutea* (ATCC 9341) was used to assay chloramphenicol (7). The latter test was modified to employ Sensitivity Test Medium (Oxoid), since this agar yielded more clearly defined inhibition zones than the 1.5% nutrient agar (Difco) originally described.

RESULTS

Serum concentrations of antibiotic. Maximal mean ampicillin concentrations were obtained 2 hr after initial OA administration (3.0 $\mu\text{g/ml}$) and 1 hr after the initial IMA (24.6 $\mu\text{g/ml}$) dose (Fig. 1). By 4 hr after the first dose by either treatment method, serum concentrations had decreased, but the IMA serum concentrations were significantly higher than with OA ($P < 0.0001$). At 24 hr, they were still significantly different ($P < 0.0001$). The serum level after IMA was significantly lower (15.9 $\mu\text{g/ml}$) 1 hr after the fifth dose than 1 hr after the first dose (24.6 $\mu\text{g/ml}$; $P = 0.0086$). In contrast, similar comparison 4 hr after the first dose (4.8 $\mu\text{g/ml}$) and 4 hr after the fifth dose (7.2 $\mu\text{g/ml}$) showed a significantly higher concentration after the latter IMA dose ($P < 0.0001$). The serum concentration after OA at the same intervals revealed no significant differences (1 hr versus 25 hr, $P = 0.35$; 4 hr versus 28 hr, $P = 0.54$).

After each OC dose, peaks in serum concentration were noted. There were significant increases in serum concentrations of chloramphenicol between the 1st and 25th hr and the 4th and 28th hr (i.e., 1 and 4 hr after the first and fifth doses, $P < 0.0001$).

Etiological and clinical findings. A drug failure patient was defined as a patient who did not respond clinically to therapy within 10 days, although bacteriologically the blood was negative. Relapses were defined as symptoms, fever, and bacteremia reappearing after cessation of therapy. There were four drug failures and one

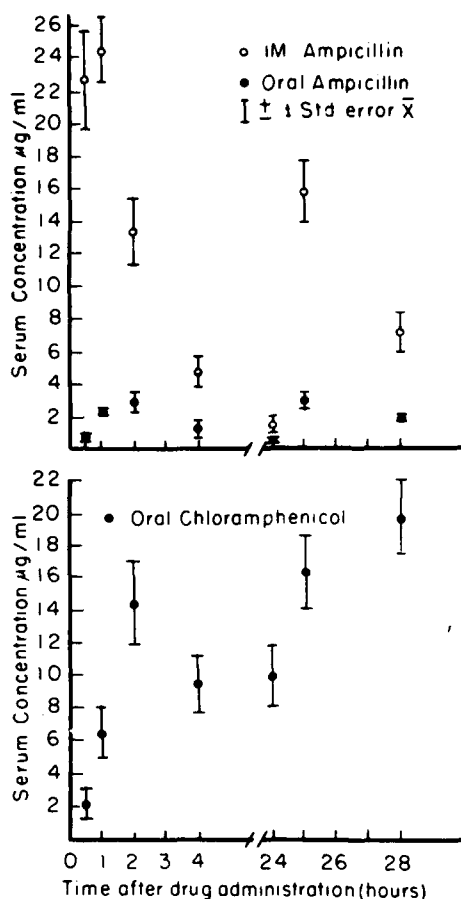


FIG. 1. Drug concentrations in the serum of enteric fever patients.

relapse on ampicillin therapy, and there were two relapses after chloramphenicol therapy (Table 1).

Although the cure rate for *S. typhi* enteric fever with OA appeared better than for IMA, there was no significant difference ($P = 0.56$). When the results of all patients with *S. typhi* enteric fever treated by ampicillin (20 patients with 17 cures) were pooled, a cure rate of 85% was found. This rate was not significantly different from the 78% (seven of nine) cure rate for chloramphenicol ($P = 0.74$). There were insufficient paratyphoid A cases for statistical analysis.

Serum drug concentrations versus relapse and drug failure. The pattern of antibiotic concentrations in the serum of relapse and drug failure patients were compared with the 99% confidence interval (CI) of the mean antibiotic concentra-

TABLE 1. Enteric fever etiology and therapeutic results

Etiology	Drug	Route	No. of patients		
			Fail-ure	Re-lapse	Cure
<i>S. typhi</i>	Ampicillin	Oral	1	0	10 (91%)
	Ampicillin	im ^a	2	0	7 (78%)
	Chloramphenicol	Oral	0	2	7 (78%)
<i>S. paratyphi</i> A	Ampicillin	Oral	1	1	3 (60%)
	Ampicillin	im	0	0	3 (100%)
	Chloramphenicol	Oral	0	0	2 (100%)

^a Intramuscular.

tions for all patients in each treatment group (Table 2). The ampicillin concentration in the serum of the relapse patient receiving oral therapy was within the 99% CI at each sampling period, excepting the 30-min sample period. The ampicillin concentration in the serum of drug failure patient 203 was below the 99% CI at the 4- and 25-hr sample periods, and on patient 1149 it was below the 99% CI at the 28-hr sample. The ampicillin concentration in the serum of drug failure patient 174 on intramuscular therapy was below the 99% CI at the 2-, 4-, and 28-hr sample periods. It was below the 99% CI at the 30-min, 1-, 2-, 4-, and 28-hr sample periods in patient 231. In one relapse patient on OC, the drug level was below the 99% CI during the first hour sampling

period. However, antibiotic concentrations in most serum samples tested were above the MIC of the infecting strain involved in relapse and drug failure. The exceptions were the 30-min and 1-hr samples of one relapse patient treated with chloramphenicol and the 30-min sample in one OA subject (Table 2). The MIC for the strains involved in relapse and drug failures was similar to that for the strains isolated from patients who were cured (*S. typhi*, 0.1 to 0.2 µg of ampicillin/ml and 3.1 µg of chloramphenicol/ml; *S. paratyphi* A, 0.4 to 0.8 µg of ampicillin/ml and 3.1 µg of chloramphenicol/ml).

DISCUSSION

The maximal concentrations of ampicillin in the serum of enteric fever patients after oral administration occurred within 2 hr. This result was nearly identical with that observed in healthy human volunteers (4). Thus, it appeared that acute enteric fever patients absorbed ampicillin at a normal rate. Although maximal ampicillin serum concentrations occurred even more quickly after intramuscular injection and were much higher than after oral treatment, patterns of concentration change with the two routes of administration were generally the same. Cure and failure rates were also the same. Therefore, the premise that parenteral therapy would be superior to oral therapy is apparently disproved.

In this study, ampicillin given by either route appeared to be equally as effective as oral chlor-

TABLE 2. Ampicillin and chloramphenicol concentrations in the serum of enteric fever patients experiencing drug failure (DF) or relapse (R) compared with the 99% confidence interval (CI) of the mean serum concentrations in all patients

Therapy	Patient	Infecting <i>Salmonella</i>	MIC (µg/ml)	Antibiotic concn in serum (µg/ml)							
				0 hr	0.5 hr	1 hr	2 hr	4 hr	24 hr	25 hr	28 hr
Ampicillin, oral	203 (DF)	<i>S. typhi</i>	0.1	0	1.20	2.45	1.75	0.24	0.51	0.91	1.15
	1149 (DF)	<i>S. paratyphi</i> A	0.4	0	1.35	2.55	3.20	1.10	0.63	3.75	0.52
	1170 (R)	<i>S. paratyphi</i> A	0.8	0	0.10	2.05	5.30	1.08	0.93	4.10	2.80
	99% CI ^a		—	0	0.36-1.35	1.75-3.20	1.60-4.47	0.54-2.52	0.10-1.07	1.26-4.95	0.54-3.21
Ampicillin, intramuscular	174 (DF)	<i>S. typhi</i>	0.1	0	40.00	18.50	7.10	0.10	ND	13.00	1.30
	231 (DF)	<i>S. typhi</i>	0.1	0	0.82	15.50	3.35	1.10	0.15	15.50	0.65
	99% CI		—	0	13.40-32.28	18.05-31.13	9.22-21.73	2.33-7.22	0.03-2.93	9.77-22.06	3.37-11.04
Chloramphenicol, oral	226 (R)	<i>S. typhi</i>	3.1	0	ND	ND	ND	ND	13.00	15.70	21.00
	1175 (R)	<i>S. typhi</i>	3.1	0	0	0	10.70	11.50	8.40	23.50	19.50
	99% CI		—	0	0.54-5.18	1.71-11.40	6.66-22.49	4.38-14.84	4.09-16.06	9.72-23.25	12.92-26.59

^a Mean, all patients.^b Not done.

amphenicol. Cure rates and failure rates were not significantly different.

Comparison of the serum antibiotic patterns in drug failure and relapse patients with those of cured patients revealed that some values in the former group were lower than the 99% CI of the mean for all patients. Nevertheless, in most instances serum antibiotic concentrations were in excess of the MIC for the infecting salmonellae, and it would seem that the bacteria should have been inhibited. In most of these patients, the peaks of serum ampicillin concentration were lower than those seen in cured patients. Although this may have been a factor in drug failure or relapse, it would not seem to be sufficient cause in itself for treatment failure. Thus, the reasons for these lower concentrations, as well as the basic causes of treatment failures, remained obscure.

LITERATURE CITED

1. Bennett, J. V., J. L. Brodie, E. J. Benner, and W. M. M. Kirby. 1966. Simplified, accurate method for antibiotic assay of clinical specimens. *Appl. Microbiol.* 14:170-177.
2. Edwards, P. R., W. H. Ewing. 1968. Identification of enterobacteriaceae, 6th ed. Burgess Publishing Co., Minneapolis, Minn.
3. Edwards, W. H. 1970. Antimicrobial therapy of *Salmonella* infections. *J. Egypt. Pub. Health Ass.* 45:206-217.
4. Kundsén, E. T., G. N. Rolinson, and S. Stevens. 1961. Absorption and excretion of Penbritin. *Brit. Med. J.* 2:198-200.
5. Patel, K. M. 1964. Ampicillin in typhoid fever. *Brit. Med. J.* 1:907.
6. Paul, R. M. 1963. Plasma concentration of chloramphenicol and bone marrow suppression. *Blood* 21:363-372.
7. Randall, W. A., A. Kirshbawn, J. K. Nielsen, and D. Wintermere. 1949. Diffusion plate assay for chloramphenicol and aureomycin. *J. Clin. Invest.* 28:940-942.
8. Robertson, R. P., M. F. Abdel Wahab, and F. O. Raasch. 1968. Evaluation of chloramphenicol and ampicillin in *Salmonella* enteric fever. *N. Engl. J. Med.* 278:171-176.
9. Rolinson, G. N., and S. Stevens. 1961. Microbiological studies on a new broad-spectrum penicillin, "Penbritin." *Brit. Med. J.* 2:191-196.
10. Sleet, R. A., G. Sangster, and J. M. Murdoch. 1964. Comparison of ampicillin and chloramphenicol in treatment of paratyphoid fever. *Brit. Med. J.* 1:148-150.
11. Smiley, R. K., G. E. Cartwright, and M. M. Wintrobe. 1952. Fatal aplastic anaemia following chloramphenicol (Chloromycetin) administration. *J. Amer. Med. Ass.* 149:914-918.
12. Steers, E., E. L. Foltz, and B. S. Graves. 1959. Inocula-replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiot. Chemother.* 9:307-311.
13. Woodward, T. E., J. E. Smadel, and H. L. Ley. 1950. Chloramphenicol and other antibiotics in the treatment of typhoid fever and typhoid carriers. *J. Clin. Invest.* 29:87-99.
14. Woodward, T. E., J. E. Smadel, H. L. Ley, R. Green, and D. S. Mankikar. 1948. Preliminary report on the beneficial effect of chloromycetin in the treatment of typhoid fever. *Ann. Intern. Med.* 29:131-134.