Comparative Effectiveness of Co-trimoxazole and Tetracycline in the Treatment of Cholera[1]

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The purpose of the study reported here was to compare the bactericidal effectiveness of tetracycline and co-trimoxazole (a combination of sulfamethoxazole and trimethoprim) in treating cholera. The study, an open-ended random trial using adult patients with cholera cases confirmed by stool culture, was carried out in March 1993 at the Cholera Treatment Unit (CTU) of the Hospital de Apoyo Departamental María Auxiliadora in Lima, Peru.

A total of 107 subjects were divided into two groups (A and B). The 50 in Group A received 500 mg of tetracycline orally every 6 hours for 3 days; the 57 in Group B received co-trimoxazole (160 mg of trimethoprim and 800 mg of sulfamethoxazole) orally every 12 hours for 3 days. The two groups were comparable in terms of age, sex, duration of symptoms prior to hospital admission, time at which antibiotic treatment was initiated, and clinical evolution. Control stool cultures of specimens obtained after treatment showed Vibrio cholerae *O-1 present in 2% of the Group A and 12.3% of the Group B patients, and also showed* V. cholerae *non-O-1 present in 2% of the Group A patients and 3.5% of the Group B patients. Overall, it was concluded that both therapeutic treatment regimens were effective and that the strains of* V. cholerae *observed in the southern sector of the city of Lima were still susceptible to both antibiotics.*

Vibrio cholerae is a gram-negative bacterium that is generally susceptible to various antibiotics including tetracycline, chloramphenicol, trimethoprim, streptomycin (*1*), and most of the β-lactamic antibiotics including penicillin and ampicillin (2). Of these, tetracycline is the one most used to treat cholera and is the treatment of choice in many places (*3–5*).

In recent years *V. cholerae* has shown increasing plasma-mediated resistance to tetracycline and other antibiotics, a phenomenon that until the last decade had not reached significant levels (*1*). However, in 1990 Saraswathi and Duodhor reported 38.4% resistance to tetracycline among subjects in Bombay, India (*6*). In 1992 Ferreira reported that of cholera infections studied in Angola, 10% were resistant to tetracycline, 26% to chloramphenicol, 74% to kanamycin, and 86% to multiple antibiotics including ampicillin, streptomycin, spectinomycin, and the combination of trimethoprim and sulfamethoxazole known as co-trimoxazole (*7*). Also in 1992, Ramamurthy et al. reported that of cholera infections studied among subjects in Calcutta, India, 63% showed resistance to tetracycline, 83% to co-trimoxazole, and 96% to furazolidone (*8*).

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Indiscriminate use of antibiotics may facilitate proliferation of resistant strains, such as occurred in Tanzania where tetracycline use for treatment and prophylaxis of *V. cholerae* infection was followed by growth of resistance from 0% to 76% in a 5-month period (*9*). A similar phenomenon appears to have occurred in Guayaquil, Ecuador, where a study conducted by Weber et al. in July 1991 (only five months after the appearance of *V. cholerae* in the Americas) showed resistance to multiple antibiotics in 36% of the cases studied (*10*). These data suggest a need to maintain constant surveillance in order to detect the appearance of *V. cholerae* strains resistant to the antibiotics of choice. In a similar vein, they also point up the importance of regularly assessing the clinical effectiveness of these medications and ensuring the availability of other therapeutic options.

Co-trimoxazole has been shown effective in treating *V. cholerae* infections as well as infections caused by enterotoxigenic *Escherichia coli*, primarily in areas where resistance to tetracycline has been recorded, and is considered a good alternative for treating cholera (*4*).

The aim of the study reported here was to compare the bactericidal effectiveness of tetracycline and co-trimoxazole for treating cholera patients in Lima, Peru.

MATERIALS AND METHODS

A prospective, longitudinal, simple-blind study was conducted in March 1993 on patients in the Cholera Treatment Unit (CTU) of the Hospital de Apoyo Departamental María Auxiliadora in Lima. Inclusion criteria were as follows: patients of either sex over 15 years old with liquid stools not containing mucus or blood and with a stool culture taken on admission (i.e., prior to administration of treatment) that was positive for *V. cholerae* were considered for inclusion in the study if they had not taken any antibiotics during the 3 days preceding admission to the CTU. Pregnant women and subjects with underlying chronic disease were not included in the study.

The initial study design called for 50 patients to be included in each treatment group. Accordingly, 100 slips of paper containing the name of a specific pharmaceutical (50 labeled co-trimoxazole and 50 labeled tetracycline) were prepared, so as to assign each patient in a random fashion to one group or the other in the order of their arrival at the CTU, with all those not meeting the inclusion criteria being eliminated in the process. However, 20% of the patients failed to return for control purposes following treatment, and this made it necessary to continue the selection and allotment process until each group once again contained 50 patients. Since distribution was made on a random basis, a total of 50 was not reached for the tetracycline group until the co-trimoxazole group had swelled to 57. In order not to influence the study results, it was decided that no patient would be withdrawn from the latter group. Accordingly, the final sample consisted of 107 patients, all of whom gave their oral consent to participate in the study.

Group A patients received 500 mg of tetracycline orally every 6 hours for 3 days, while Group B patients received co-trimoxazole (160 mg trimethoprim and 800 mg sulfamethoxazole) orally every 12 hours for 3 days. Administration of the antibiotics was supervised during the patients' stay in the CTU, after which each patient assumed responsibility for continuing the treatment at home.

The study included all CTU patients with acute diarrheal disease who, at the time of their admission, satisfied the above-mentioned criteria. A swab sample was taken of each patient's first diarrheal stool subsequent to admission to the hospital, and the samples were transported to the laboratory in tubes containing Cary-Blair medium.

All patients received rehydration treatment, either orally, intravenously, or by both routes, as determined by their degree of dehydration in accordance with the case management protocol adopted by the CTU (*11*).

The hourly volume of diarrhea was estimated for each of the following periods: the first 4 hours of hospitalization; from the fourth to the eighth hour; from the eighth to the twelfth hour; and from the twelfth hour on. Each patient was discharged when his or her diarrhea volume fell to 400 cm^3 per hour or less. All the patients were asked to return to the CTU 48 hours after completing the full three-day antibiotic treatment so that control stool samples could be obtained. These latter samples were taken by rectal swab and were conveyed to the laboratory in tubes containing Cary-Blair medium. Once at the laboratory, the samples were incubated in peptonized broth for 6 to 8 hours and were cultured on agar plates containing thiosulfate, citrate, bile salts, and sucrose (TCBS). Subsequent identification of the microorganisms cultured was made by means of standard biochemical methods (*12, 13*) and specific antisera for the Ogawa and Inaba serotypes.

The susceptibility of the bacterial strains isolated to specific antibiotics was assessed using the Kirby-Bauer technique (*14*). *E. coli* strain ATCC 25922, *Staphylococcus aureus* strain ATCC 25923, and *Pseudomonas aeruginosa* strain ATCC 27853 were used as controls. The culturing and subsequent susceptibility tests were done at the enteropathogen reference laboratory of the National Institute of Health (*Instituto Nacional de Salud*—INS) in Lima.

During final evaluation of the results, only those patients who had satisfactorily completed the treatment regimen and returned for the control visit within the previously established time period were considered. Treatment was deemed effective in those cases where the control stool culture yielded negative results.

In general, the results are expressed in terms of the mean ± 1 standard deviation (SD), although in cases where the data are quite dispersed, the median and range of values obtained are also stated.

RESULTS

Of the 107 patients whose cholera diagnoses were confirmed by stool culture 72 (67.3%) were male and 35 (32.7%) were female. The mean age was 36 ± 16 years for the group receiving tetracycline (Group A) and 40 ± 15 years for the group receiving co-trimoxazole (Group B). Table 1 shows the sex and age distributions of patients in the two groups.

It should be noted that all 107 strains of vibrios isolated prior to treatment were *V. cholerae* serovar O-1, El Tor biotype, with 102 of these belonging to the Ogawa serotype, 3 being of the Inaba serotype, and 2 being of the rugose type. In the susceptibility test (where the diameter of the growth inhibition disc produced by an antibiotic is used to assess whether a strain exhibits complete susceptibility, intermediate susceptibility, or resistance to the antibiotic) all of the strains cultured prior to treatment were found completely susceptible to co-trimoxazole; and while nearly all were found completely susceptible to tetracycline, 7 (6.5%) exhibited only intermediate susceptibility (Table 2). No resistant strains were detected.

The mean duration of the interval between onset of initial symptoms and hos-

Table 1. Distribution by age and sex of patients treated with tetracycline (Group A) and co-trimoxazole (Group B). Lima, March 1993.

Patients	Group A	Group B	Total
Sex.			
Male	36	36	72
Female	14	21	35
Age			
(mean ± 1 SD)	*36.2 ± 16.3*	*39.6 ± 14.8*	

Table 2. *In vitro* susceptibility to tetracycline and co-trimoxazole of *Vibrio cholerae* strains isolated by stool culture prior to administration of treatment. Lima, March 1993.

Degree of susceptibility	Antibiotic	
	Tetracycline	Co-trimoxazole
Complete susceptibility	100	107
Intermediate susceptibility	7	0
Resistance	0	0
Total	107	107

pital admission was 12 ± 10 hours. This interval was less than 24 hours for 88% of the patients in Group A and 93% of those in Group B, a difference that was not statistically significant.

Likewise, no significant differences were found between the Group A and Group B patients with regard to degree of dehydration or response to rehydration treatment. Mean arterial blood pressure on admission was 31.5 mm Hg, with a range of 0 mm Hg (no systolic pressure) to 113 mm Hg. The average overall requirement for intravenous saline solution was 8.9 ± 4.9 L, while the average overall requirement for oral rehydration solution was 23.3 ± 13.4 L.

No statistically significant differences were found between the Group A and Group B members with regard to volume of diarrhea per hour. Regarding antibiotic treatment (Table 3), the same percentage (70%) of Group A and Group B members began receiving antibiotic treatment within 12 hours of admission to the CTU, although the mean for Group A was 11.5 ± 6.7 hours (with a range of 3 to 34 hours) while for Group B it was 12.6 ± 6.5 hours (with a range of 5 to 30 hours). The medians for groups A and B were 9 and 10 hours, respectively.

The sample for the control stool culture was taken an average of 2 ± 1 days after completion of the antibiotic treatment. As Table 4 shows, the results were positive for *V. cholerae* O-1 in one Group A patient (2.0%) and seven Group B patients (12.3%), and were also positive for *V. cholerae* non-O-1 in one Group A patient (2.0%) and two Group B patients (3.5%). A bacteriologic cure was obtained for 98% of the Group A patients (treated with tetracycline) and for 87.7% of the Group B patients (treated with co-trimoxazole), all those whose stools yielded negative results or yielded positive results only for *V. cholerae* non-O-1 being considered cured. This means that therapeutic failure was six times more frequent in the second group (12.3%) than in the first (2%).

Table 3. Hours elapsed between admission of patients to the Cholera Treatment Unit (CTU) and administration of the initial dose of tetracycline or co-trimoxazole. Lima, March 1993.

Time (in hours)	Tetracycline		Co-trimoxazole	
	(No.)	(%)	(No.)	(%)
3–7	12	24	10	17
8–12	23	46	30	53
13–17	6	12	4	7
18–22	5	10	8	14
23–27	2	4	4	7
>27	2	4	1	2
Total	50	100	57	100
Average ± 1 SD	11.5 ± 6.7		12.6 ± 6.4	
Median	9.0		10.0	

Table 4. Results of control stool cultures made with samples following treatment with tetracycline (Group A) or co-trimoxazole (Group B). Lima, March 1993.

Results	Group A		Group B		Total
	No.	%	No.	%	(No.)
V. cholerae O-1	1	2	7	12.3	8
V. cholerae non-O-1	1	2	2	3.5	3
Negative	48	96	48	84.2	96
Total	50	100	57	100.0	107

As Table 5 indicates, the mean length of hospital stay was 25 ± 11 hours for Group A (tetracycline) patients and 31 ± 13 hours for Group B (co-trimoxazole) patients. This difference is statistically significant ($P = 0.006$). Fourteen percent of the Group B patients remained hospitalized for over 48 hours.

Seven of the eight *V. cholerae* O-1 strains recovered following treatment were subjected to susceptibility testing. One strain was not viable at the time the evaluation was carried out. One of the remaining strains was found resistant to co-trimoxazole, while three exhibited intermediate susceptibility to tetracycline. The strain that was resistant to co-trimoxazole was recovered from a patient who had been treated with this medication (Table 6).

DISCUSSION AND CONCLUSIONS

The percentages of resistant *V. cholerae*, as reported in the literature, vary in accordance with the geographic areas and the particular epidemiologic situations involved (*6–9, 15*). As this suggests, appropriate monitoring of the behavior of the various strains of *V. cholerae* as a function of the various antibiotics normally used against them is essential for choosing the most effective therapeutic regimen.

Beginning in 1984, reports on bacteriologic susceptibility to co-trimoxazole in Peru have noted resistance developed by *E. coli* and some species of *Shigella*, at an average level of 40% (*16–20*). However,

Table 5. Number of hours during which patients treated with tetracycline (Group A) and co-trimoxazole (Group B) remained hospitalized in the Cholera Treatment Unit. Lima, March 1993.

Hospitalization (in hours)	Group A		Group B	
	No.	%	No.	%
0–12	6	12	2	3
13–24	22	44	18	32
25–36	14	28	17	30
37–48	7	14	12	21
49–60	1	2	7	12
>60	0	0	1	2
Total	50	100	57	100
Average ± 1 SD*	24.5 ± 11.2		31.2 ± 13.2	

*$P = 0.006$.

Table 6. *In vitro* susceptibility to tetracycline and co-trimoxazole of *V. cholerae* strains isolated by control stool culture from samples obtained after completion of antibiotic treatment. Lima, March 1993.

Degree of susceptibility	Tetracycline (No.)	Co-trimoxazole (No.)
Complete susceptibility	4	6
Intermediate susceptibility	3	0
Resistance	0	1
Total	7	7

reports dealing with the strains of *V. cholerae* present in Peru's first two epidemic outbreaks, occurring in 1991 and 1992, showed that 100% of the responsible organisms were susceptible to co-trimoxazole (*17, 21*).

The study reported here was carried out during the third epidemic outbreak of cholera in Peru and 2 years after the initial outbreak in 1991. Although bacteriologic cure was more frequent in the Group A (tetracycline) patients than in the Group B (co-trimoxazole) patients, the difference was not statistically significant when viewed in the context of the sample size.

This study was designed to assess the effectiveness of treatment against those *V. cholerae* strains (in all cases *V. cholerae* O-1) isolated by stool culture before antibiotic treatment began. It should be stressed that finding a strain resistant to co-trimoxazole in the control stool culture demonstrated that resistance can occur even after administration of short-duration treatment, such as that used to deal with a case of cholera.

Another finding worthy of note was the recovery of *V. cholerae* non-O-1 from three patients following treatment. In all three cases, the initial stool culture had revealed the presence of *V. cholerae* O-1 El Tor Ogawa, and the patient's diarrhea was attributed to this strain. The three cases, one in Group A and two in Group B, are not considered examples of therapeutic failure because the initial etiologic agent was not found in the control stool culture. This finding could have either of two explanations: (1) development of a new infection occurring at the time of the cholera epidemic, or (2) loss of agglutinable surface antigens by the responsible microbe, a phenomenon observed in certain cases of classical *V. cholerae* infection (*22*).

A number of differences observed in the clinical evolution of the patients are worthy of comment. Patients treated with tetracycline were discharged earlier, on the average, than those treated with co-trimoxazole. The reason was that the Group A patients' diarrhea volume tended to decline faster. Specifically, between the 12th hour of hospitalization and discharge, 70% of the Group A patients' diarrhea volume declined to 400 cm^3 per hour or less, while this occurred in only 54% of the Group B patients. Nevertheless, 42% of the patients in Group B had nearly comparable diarrhea volumes (400–600 cm^3 per hour) between the 12th hour of hospitalization and time of discharge, indicating that there was no substantial difference in this regard between the two groups. The time at which antibiotic treatment was initiated does not appear to explain the differences in clinical evolution, as 70% of the patients in each group received the initial dose of antibiotic 12 hours or less after admission to the CTU.

Overall, the study indicated that both tetracycline and co-trimoxazole, from the bacteriologic and clinical standpoints, remain effective treatments for cholera cases occurring in the southern sector of the city of Lima. However, it is recommended that an active monitoring system, based on testing to determine susceptibility to common antibiotics, be kept in place in order to detect possible selection and emergence of resistant *V. cholerae* strains.

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