RESEARCH PAPER

Single Dose Azithromycin *Versus* Ciprofloxacin for Cholera in Children: *A Randomized Controlled Trial*

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Objective: To compare the clinical and bacteriological success of single dose treatment with azithromycin and ciprofloxacin in children with cholera.

Design: Randomized, open labelled, clinical controlled trial**.**

Setting: Tertiary care hospital.

Participants: 180 children between 2-12 years, having watery diarrhea for ≤24 hr and severe dehydration, who tested positive for Vibrio cholerae by hanging drop examination or culture of stool.

Intervention: Azithromycin 20 mg/kg single dose (*n*=91) or Ciprofloxacin 20 mg/kg single dose (*n*=89). Dehydration was managed according to WHO guidelines.

Main outcome measures: Clinical success (resolution of diarrhea within 24 hr) and bacteriological success (cessation of excretion of *Vibrio cholerae* by day 3). Secondary outcome variables included duration of diarrhea, duration of excretion of *Vibrio cholerae* in stool, fluid requirement, and proportion of children with clinical or bacteriological relapse.

Results: The rate of clinical success was 94.5% (86/91) in children treated with Azithromycin and 70.7% (63/89) in those treated with Ciprofloxacin [RR (95% CI)=1.34 (1.16-1.54); *P*<0.001]. Bacteriological success was documented in 100% (91/91) children in Azithromycin group compared to 95.5% (85/89) in Ciprofloxacin group [RR (95% CI)=1.05 (1.00-1.10); *P*=0.06]. Patients treated with Azithromycin had a shorter duration of diarrhea [mean(SD) 54.6 (18.6) *vs* 71.5 (29.6) h; mean difference (95% CI) 16.9 (9.6–24.2); *P*<0.001] and lesser duration of excretion of *Vibrio cholerae* [mean(SD) 34.6 (16.3) vs 52.1 (29.2) h; mean difference (95% CI) 17.5 (0.2–24.7), *P*<0.001] in children treated with Azithromycin vs Ciprofloxacin. The amount of intravenous fluid requirement was significantly less among subjects who received Azithromycin as compared to those who received Ciprofloxacin [mean(SD) 4704.7(2188.4) vs 3491.1(1520.5) mL; Mean difference (95% CI) 1213(645.3–1781.9); *P*<0.001]. Proportion of children with bacteriological relapse was comparable in two groups [6.7% (6/89) vs 2.2% (2/91); RR (95% CI) 0.95 (0.89–1.01); *P*=0.16]. None of the children in either group had a clinical relapse.

Conclusion: Single dose azithromycin is superior to ciprofloxacin for treating cholera in children.

Key words: *Azithromycin, Antibiotic, Cholera, Ciprofloxacin, Management.*

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WHO recommends a 3-5 day course of furazolidone, trimethoprimsulphamethoxazole or erythromycin for treatment of cholera in children; tetracycline may be used for those more than 8 years of age(1-3). However, strains of *V. cholerae* resistant to these drugs have been identified in Bangladesh and elsewhere(4). Ciprofloxacin was found to be effective in treatment of cholera with a good *in vitro* activity, long half life, high stool concentration after ingestion and safety for use in children(5). Single dose ciprofloxacin has been widely studied in adults(6,7) but studies in children(8) are limited. Its mechanism of action is different from penicillin, erythromycin and tetracycline, hence it can be used

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for organisms resistant to the traditionally recommended antibiotics. In recent years, strains of *V. cholerae* resistant to fluoroquinolones have also been identified from various parts of India(9-11). Identification of clinically efficacious alternative antibiotics is therefore necessary for use in children with cholera.

Azithromycin, a synthetic macrolide antibiotic is an emerging antibiotic with action against *V. cholerae*(12). Single dose treatment with azithromycin has a potential advantage of ease of administration, good comp-liance, and reduced cost of treatment. Studies on treatment of cholera in children with single dose azithromycin are limited to comparisons with erythromycin(13,14). We compared the efficacy of single dose of azithromycin to ciprofloxacin for treatment of cholera in children and hypothesized that azithromycin is at least as effective as ciprofloxacin in treatment of cholera.

METHODS

The study was designed as a randomized, open labelled, clinical controlled trial; and was conducted in a tertiary care hospital of India, from March 2006 to February 2007. Clearance was obtained from the institutional ethical committee. The study protocol was fully explained to the parents/guardian, and informed written consent was obtained.

***Sample size*:** The sample size was calculated for an equivalence study. Clinical success for treatment with single dose ciprofloxacin was estimated at 94% in a previous study(8). To reach a predictive power of 80%, with an alpha error of 5% and a beta error of 20%, 87 patients were required in each treatment group to show that the difference in the rates of clinical success between the treatment groups did not exceed 10%(15).

***Enrolment*:** Children between 2-12 years, having watery diarrhea for 24 hr or less, with features of severe dehydration as per WHO criteria(3), were eligible to be included. Of these, only those who demonstrated *Vibrio cholerae* in stool either by a hanging drop preparation or culture, were finally analyzed. Children with severe undernutrition (weight for age less than 60% of 50th percentile of CDC 2000 standards), a coexisting systemic illness, blood in stool; and those having received an antibiotic/antidiarrheal within preceding 24 hours, were excluded.

***Data collection*:** Baseline data were collected: this included name, age, address, telephone number, duration of illness, frequency of diarrhea and vomiting prior to admission, and presence of associated symptoms including abdominal pain, fever, and abdominal distension. A history of previous antibiotic/antidiarrheal ingestion in the last 24 hrs was elicited. Occupation, education and monthly income of parents were recorded and a socioeconomic status was assigned based on revised Kuppuswamy classification(16). Evaluation was done for general hygiene, vitals, and signs of dehydration(2). The present weight was recorded on a standardized weighing scale to the nearest 0.5 kg. Height was measured to the nearest 0.1 cm. The same observer obtained all the measurements.

***Randomization and allocation*:** Eligible children were allotted a study number. These numbers corresponded to the order of patients entering the trial. Children were randomized to receive a single dose of oral azithromycin (20 mg/kg) or ciprofloxacin (20 mg/kg). A simple randomization was done using a computer generated random number table on a master list.

Allocation of the treatment group was concealed by having the names of both the study drug stored in identical sealed envelope, which were opened after a patient had been enrolled in the study and assigned a study number. Randomized children were immediately rehydrated with intravenous Ringer's lactate solution (30 mL/kg in first ½ hour followed by 70 mL/kg over next 2½ hours). A stool sample was obtained for hanging drop examination and culture for *Vibrio cholerae*, as soon as the child passed stools after admission. The patient was reassessed for hydration after 3-4 hours and managed further as per the WHO Guidelines(2).

The assigned Study drug was orally administered after initial rehydration, under supervision. Eligible subjects received either a single dose of azithromycin (20 mg/kg) or ciprofloxacin (20 mg/kg). Both the drugs were available in 100 mg, 250 mg and 500 mg tablets and the dose was rounded to

nearest 50 mg. The dose was repeated if the child vomited within 10 minutes of drug administration.

Each Study day was defined as 24 hour counted from the administration of study drug. Children remained in the Study center for 72 hours (day 3) or until resolution of watery diarrhea, whichever was later. The parents were asked to bring their child back for a follow-up visit on day 7. If the patient failed to return on the follow-up visit, the parents were contacted by telephone and asked to come on the next day.

Clinical monitoring was performed on multiple occasions on the day of admission and subsequently at the end of day 1, 2, 3 and 7. A record was kept of frequency of stool and vomiting for every 24 hrs. The amount of intravenous fluid and ORS administered was also recorded at the end of each Study day. A stool sample or rectal swab was obtained at the end of day 1, 2, 3 and at follow-up visit (day 7). We also noted for any possible adverse effects of the drug administered like hypersensitivity reaction, phototoxicity, tendinopathy and joint pain or swelling.

***Microbiological evaluation*:** The motility of *V. cholerae* was seen by hanging drop preparation(17). Stool sample was transported in alkaline peptone water or Cary Blair media and processed. The stool samples were cultured in bile salt agar, MacConkey agar and thiosulphate citrate bile sucrose agar. Plates were incubated at 37ºC for 24 hours. The samples were inoculated in fresh alkaline peptone water for enrichment and subseqent plating. Bacteriological analysis was done by standard laboratory techinques(18) and *V. cholerae* isolates were serotyped by slide agglutination test using specific antisera (Denca Saken). Antimicrobial susceptibility testing of the strains was performed by standard methods.

Outcome measures: The primary outcome variables were (*i*) *clinical success:* defined as resolution of diarrhea within 72 hours after the start of therapy; and (*ii*) bacteriological success: defined as absence of *Vibrio cholerae* in the stool sample from day 3 onwards. Resolution of diarrhea was considered when the child has passed two consecutive formed stools or had not passed stool for 12 hours.

Secondary outcome variables included (*i*) total duration of diarrhea (recovery time) defined as time elapsed from the entry into study till resolution of diarrhea in hours; (*ii*) total requirement of ORS and/or intravenous therapy; (*iii*) duration of excretion of *V. cholerae* in stool; (*iv*) proportion of children with clinical relapse (defined when there was cessation of diarrhea for 1 day or longer followed by return of diarrhea), or bacteriological relapse (defined as a positive stool culture following a negative culture report).

***Statistical analysis*:** Data were analysed using SPSS version 13.0. All quantitative variables (between the groups) were compared by unpaired *t*-test; categorical variables were compared by Chi-square test or Fisher's exact test. $P<0.05$ was considered as significant. Variables which were measured repeatedly were analysed with repeated measure ANOVA at 1% level of significance to allow for multiple comparisons.

RESULTS

Four hundred seven children were included in the study and were randomized to receive azithromycin (n=205) or ciprofloxacin (n=202). Of these, 180 children who tested positive for *V. cholerae* by hanging drop examination or culture of the stool were finally included in the analysis, and designated as "Study subjects". A total of 89 Study subjects received ciprofloxacin and 91 received azithromycin (***Fig.* 1**). Baseline characteristics of the study subjects were comparable between the groups (***Table* I**).

Outcome variables between the two groups are compared in ***Table* II**. Symptomatic improvement was assessed by comparing the frequency of diarrhea and vomiting. The frequency of stool and vomiting was significantly lower in children who received azithromycin as compared to the ciprofloxacin group during the first 72 hours. The rate of decline in frequency of stool and vomiting was however comparable between ciprofloxacin and azithromycin groups (***Fig.* 2*a*, 2*b***).

The follow-up loss in first 72 hours of hospital stay was only 3.3%. However, the follow-up loss beyond day 3 was 18.8%, which was significant. An

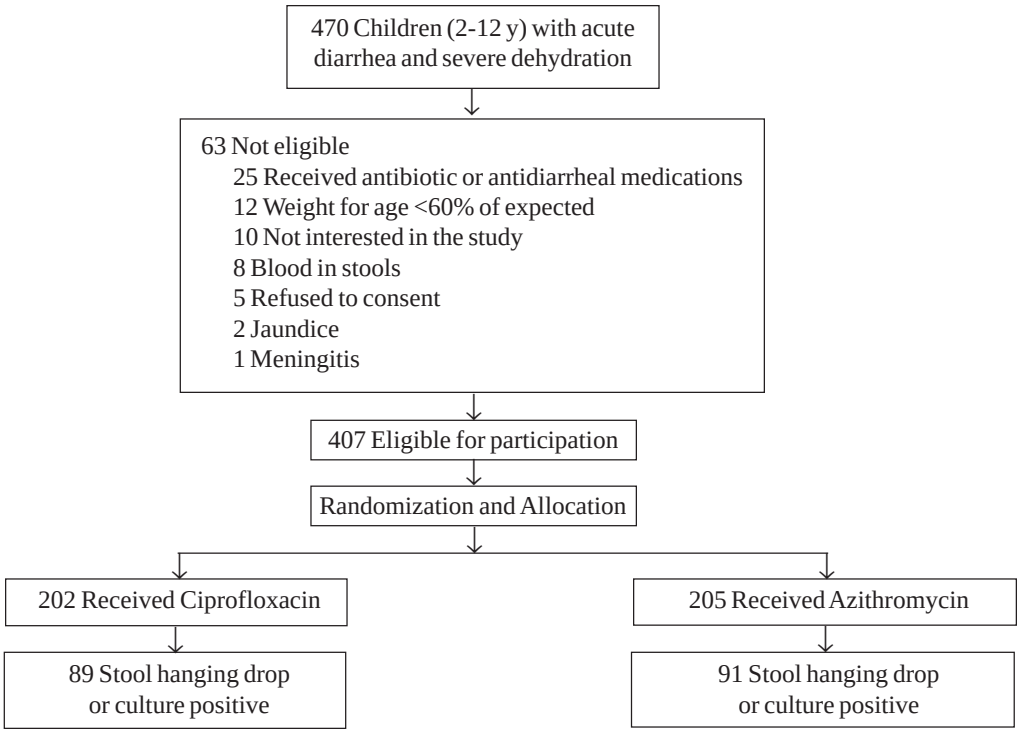


FIG. 1 *Study Flow Chart.*

intention to treat analysis was used for subjects lost to follow-up. Baseline patient characteristics were compared for subjects lost to follow up (*n*=34) with those who completed the study (*n*=146) .

DISCUSSION

Our study concluded that single dose azithromycin is superior to single dose ciprofloxacin for the treatment of cholera in children. The rate of clinical success was significantly more in patients treated with Azithromycin as compared to those treated with Ciprofloxacin, although the rate of bacteriological success was comparable in the two groups. Subjects who received Azithromycin had a significantly lesser duration of diarrhea, shorter duration of excretion of *V. cholerae*, and lower requirement of intravenous fluids. Rate of bacteriological relapse was found to be comparable and none of the subjects in either group had clinical relapse.

The results pertaining to superiority of single dose azithromycin over ciprofloxacin are consistent with a previous study(7) in adults. However, the rates of clinical and bacteriological success with azithromycin are much higher in our study (95-100%) as compared to earlier studies with azithromycin(7,13), which reported a success rate of between 70-75%. The discrepancy in the success rates could be attributed to differing definitions of success adopted in these trials and differences in baseline characteristics of the enrolled population. We adopted a 72 hours cut off for defining success instead of 48 hours used in the previous studies(7,8,13). Another possible explanation is that the strains of *Vibrio cholerae* in their study setting could have been earlier exposed to azithromycin. This could lead to emergence of resistance to azithromycin(19,20). As our study population was not exposed to the drug for diarrheal illnesses, chances of *V. cholerae* exposure to the drug were scanty, which could probably explain such high rates of clinical and bacteriological success.

Quinolone antimicrobials, especially nalidixic acid, are widely used in India for treatment of gastrointestinal infections. Therefore, it could be expected that *V. cholerae* strains would have received considerable exposure to these agents and exposure to nalidixic acid could have been a selective force for quinolone resistance in India.

TABLE I BASELINE COMPARISON OF PATIENT CHARACTERISTICS IN THE TWO GROUPS

Patient characteristics	Ciprofloxacin (*n*=89)	Azithromycin (*n*=91)	*P* value
Mean (SD)			
Age (months)	64 (33.9)	70 (37.7)	0.24
Weight (kg)	17.4 (7.2)	18.6 (7.7)	0.25
Height (cm)	102.5 (17.8)	105.3 (18.1)	0.28
Loose stools*	15.2 (4.5)	14.4 (4.7)	0.24
Duration of diarrhea (h)	17.9 (7)	18 (6.8)	0.94
Frequency of vomiting	9.7 (6.1)	9.7 (6.2)	0.97
Proportion (%)			
Male Sexs	52 (58.4%)	51 (56.1%)	0.75
Residence			
Rural	3 (3.3%)	8 (8.8%)	0.01
Urban	29 (32.5%)	13 (14.2%)	
Urban slum	57 (64%)	70 (76.9%)	
Socioeconomic status (SES)			
Lower SES	19 (21.3%)	20 (21.9%)	0.17
Middle SES	51 (57.3%)	41 (45.1%)	
Upper SES	0 (0%)	1 (1.1%)	
Source of drinking water			
Treated	61 (68.5%)	68 (74.7%)	0.36
Untreated	28 (31.5%)	23 (23.3%)	
Safe water storage practices	20 (22.5%)	22 (24.1%)	0.78
Open field latrine	25 (28.1%)	25 (27.5%)	0.93
Flush latrine	64 (71.9%)	66 (72.5%)	
Proper hand washing	53 (59.5%)	54 (59.3%)	0.97
Children breastfed	87 (97.7%)	88 (96.7%)	0.67
Frequency of symptoms [*n* (%)]			
Vomiting	78 (87.6%)	81 (89%)	0.77
Pain abdomen	32 (35.9%)	30 (32.9%)	0.67
Abdominal distension	5 (5.6%)	2 (2.2%)	0.27
Fever	4 (4.4%)	3 (3.2%)	0.72

*Means compared with Student's t-test; proportions compared by Chi-square test/Fisher's exact test; *Prior to admission.*

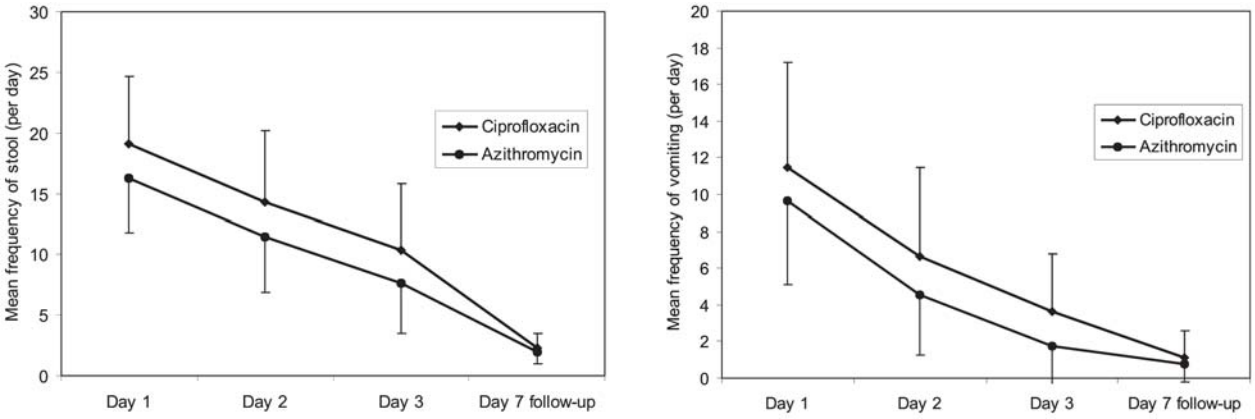
Hence, ciprofloxacin resistance might have emerged in direct response to selective pressure exerted by nalidixic acid coupled with disproportionate use of fluoroquinolones for all bacterial infections in our country. A consistent increase in median inhibitory concentration (MIC) of *V. cholerae* strains to ciprofloxacin has been reported(21,22). The findings are troublesome as a further increase in MIC may render ciprofloxacin ineffective in management of cholerae caused by such multi-drug resistant strains of *V. cholerae*. Estimation of MIC in our study could have answered many such queries. Considering the emergence of fluoroquinolone resistance in our study setting(8), we could possibly explain that although the sensitivity of ciprofloxacin reaches 99.4% in our study, the strains probably required higher doses of ciprofloxacin and for a longer duration to be clinically and bacteriologically effective.

The strength of our study was its robust design. The sample was statistically sound, practical, suited the convenience and provided credibility to our results. The only study on efficacy of azithromycin for treatment of cholera in children has limitation of small sample size(16), and this study did not analyse the outcome measures in terms of clinical or bacteriological success. Our study setting was based on a tertiary care hospital catering to all ailments of an urban population. This ensures a true picture of diarrheal disease burden as compared to referral centers catering to only diarrheal disease, as is the case in most of the previous pediatric studies on cholera. Our study had certain limitations; the intervention was not masked, there was moderate follow-up loss and the volume of diarrhea and vomiting was not ascertained. The population from urban slums is migratory, and it is typical for them to change homes, which is primarily dictated by job requirements. We acknowledge this as a reality for such trials conducted in the developing world, accounting for our follow-up losses. However, this did not affect the results as shown by comparable baseline characteristics of study subjects and those lost to follow-up.

We conclude that single dose azithromycin is a useful alternative for treating cholera in children. Considering the clinical efficacy and lack of

TABLE II COMPARISON OF OUTCOME VARIABLES IN CIPROFLOXACIN AND AZITHROMYCIN GROUPS

Outcome variable	Ciprofloxacin (*n*=89)	Azithromycin (*n*=91)	Relative Risk(95% CI)	*P* value
Number (%)				
Clinical success	63 (70.6%)	86 (94.5%)	1.33 (0.65–0.86)	<0.001
Bacteriological success	85 (95.5%)	91 (100%)	1.04 (0.91–0.99)	0.06
Bacteriological relapse	6 (6.7%)	2 (2.2%)	0.95 (0.89–1.01)	0.16
Clinical relapse	Nil	Nil	–	–
Mean (SD)			Mean difference(95% CI)	
Duration of diarrhea (h)	71.5 (29.6)	54.6 (18.6)	16.9 (9.6–24.2)	<0.001
Duration of excretion of *Vibrio cholerae* (h)	52.1 (29.2)	34.6 (16.3)	17.5 (10.3-24.7)	<0.001
ORS requirement (mL)	3473.8 (1341.7)	3644.4 (1374.9)	-170.6 (-577.6–236.3)	0.41
IV fluid requirement (mL)	4704.7 (2188.4)	3491.1 (1520.5)	1213 (645.3–1782.0)	<0.001



(*a*) (*b*)

FIG. 2 *Comparison of mean frequency of (a) diarrheal stools and (b) vomiting between Ciprofloxacin and Azithromycin groups on day 1, day 2, day 3, and at follow-up visit (day 7).*

resistance to azithromycin, we advocate that it should be considered as an option for first line treatment of childhood cholera in areas where *V. cholerae* infection are caused by susceptible strains.

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Competing interests: Azithromycin was provided by FDC India limited.

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WHAT IS ALREADY KNOWN?

- Azithromycin is efficacious for treatment of cholera in adults.

WHAT THIS STUDY ADDS?

- Azithromycin is superior to ciprofloxacin for treatment of cholera in children.

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