# Azithromycin for Bacterial Watery Diarrhea: A Reanalysis of the AntiBiotics for Children With Severe Diarrhea (ABCD) Trial Incorporating Molecular Diagnostics

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***Background.*** Bacterial pathogens cause substantial diarrhea morbidity and mortality among children living in endemic settings, yet antimicrobial treatment is only recommended for dysentery or suspected cholera.

***Methods.*** AntiBiotics for Children with severe Diarrhea was a 7-country, placebo-controlled, double-blind efficacy trial of azithromycin in children 2–23 months of age with watery diarrhea accompanied by dehydration or malnutrition. We tested fecal samples for enteric pathogens utilizing quantitative polymerase chain reaction to identify likely and possible bacterial etiologies and employed pathogen-specific cutoffs based on genomic target quantity in previous case-control diarrhea etiology studies to identify likely and possible bacterial etiologies.

***Results.*** Among 6692 children, the leading likely etiologies were rotavirus (21.1%), enterotoxigenic *Escherichia coli* encoding heat-stable toxin (13.3%), *Shigella* (12.6%), and *Cryptosporidium* (9.6%). More than one-quarter (1894 [28.3%]) had a likely and 1153 (17.3%) a possible bacterial etiology. Day 3 diarrhea was less common in those randomized to azithromycin versus placebo among children with a likely bacterial etiology (risk difference [RD]$_{likely}$, −11.6 [95% confidence interval {CI}, −15.6 to −7.6]) and possible bacterial etiology (RD$_{possible}$, −8.7 [95% CI, −13.0 to −4.4]) but not in other children (RD$_{unlikely}$, −0.3% [95% CI, −2.9% to 2.3%]). A similar association was observed for 90-day hospitalization or death (RD$_{likely}$, −3.1 [95% CI, −5.3 to −1.0]; RD$_{possible}$, −2.3 [95% CI, −4.5 to −.01]; RD$_{unlikely}$, −0.6 [95% CI, −1.9 to .6]). The magnitude of risk differences was similar among specific likely bacterial etiologies, including *Shigella*.

***Conclusions.*** Acute watery diarrhea confirmed or presumed to be of bacterial etiology may benefit from azithromycin treatment.

***Clinical Trials Registration.*** NCT03130114.

**Keywords.** bacterial diarrhea; azithromycin; molecular diagnostics; pediatric diarrhea; *Shigella*.

Diarrheal illness is the second leading cause of death in children aged <5 years [1]. Diarrhea deaths disproportionately occur in low- and middle-income countries [2]. The World Health Organization (WHO) recommends oral rehydration therapy, therapeutic zinc, and continued feeding for children with diarrhea, reserving antibiotics only for children with dysentery (presumptive shigellosis) or suspected *Vibrio cholerae* infection [3]. Despite these guidelines, children presenting to health facilities with moderate to severe diarrhea continue to die in the 3 months following diarrhea presentation, at an almost 9-fold higher rate than age- and community-matched children without diarrhea [4]. Children aged <2 years are also at high risk of linear growth faltering in the months following moderate to severe diarrhea [4, 5]. Importantly, linear growth faltering is on the pathway to stunting, which is associated with health and economic consequences that can extend into adulthood [6, 7].

The AntiBiotics for Children with severe Diarrhea (ABCD) study was a multicountry, randomized, double-blinded, placebo-controlled trial that tested whether a 3-day course of azithromycin reduced mortality or improved growth when administered to children presenting to facilities with acute watery diarrhea and at least 1 of the following: dehydration, severe stunting, or moderate wasting [8]. ABCD found no evidence of a mortality benefit but a small benefit in linear growth and risk of hospitalization (a secondary outcome) following a 3-day course of azithromycin [9].

Bacterial pathogens such as *Shigella* spp, enterotoxigenic *Escherichia coli* encoding heat-stable toxin (ST-ETEC), *Campylobacter* spp, and typical enteropathogenic *E coli* (tEPEC) are estimated to be responsible for less than half of watery diarrhea cases [10, 11] but are disproportionately associated with linear growth faltering [12, 13] and/or death [4, 14]. Since azithromycin is a broad-spectrum antibiotic with demonstrated efficacy against gram-negative bacteria such as *Shigella* spp [15, 16], *Campylobacter* spp [17, 18], and ETEC in adult travelers [19], we postulated that its potential benefit was diluted in ABCD by the presence of a significant proportion of children without a bacterial cause of their diarrhea. Utilizing molecular methods of pathogen detection and ascribing possible and likely etiologies based on pathogen quantity, which has been shown to associate with diarrhea in large case-control studies [10, 20], we sought to determine the effect of azithromycin on risk of death, hospitalization, diarrhea duration, and linear growth in children with and without bacterial etiologies.

## METHODS

The ABCD methods and primary outcome results, which did not incorporate enteric pathogen testing, are described elsewhere [8, 9]. In summary, children in Bangladesh, India, Kenya, Malawi, Mali, Pakistan, and Tanzania aged 2–23 months presenting to health facilities with acute watery diarrhea were screened for eligibility between June 2017 and July 2019. Enrolled children had some or severe dehydration as defined by the WHO [21]; moderate wasting (11.5 cm ≤ mid-upper arm circumference [MUAC] <12.5 cm [if ≥6 months of age] or −2 < weight-for-length $z$-score [WLZ] < −3 [all ages]); and/or severe stunting (length-for-age $z$-score [LAZ] < −3). In addition, children had no indication for an antibiotic (ie, clinically defined dysentery, severe acute malnutrition, signs of other infections requiring antibiotics) and had not received antibiotics in the 14 days prior to presentation. Once enrolled, children were acutely managed, and a clinical history, physical examination, and caregiver interview were conducted. Whole stool or a flocked rectal swab if stool not passed (Pediatric FLOQswab, Copan Diagnostics, Murrieta, California, catalog number 5U002S) was collected prior to randomization and stored at −80°C. Children were randomized to a 3-day course of azithromycin (10 mg/kg/day) or placebo, and all doses were directly observed by the study team.

Follow-up visits were conducted at home or at a facility both 2 and 3 days after enrollment for directly observed therapy and a brief clinical assessment of adverse events and 90 days after enrollment to collect vital status, anthropometry (length, weight, MUAC), and history of hospitalization. An additional visit was conducted at 180 days postenrollment to assess vital status. Four months after trial initiation, 2 questions related to the diarrheal episode (presence of diarrhea and whether or not the diarrhea was worsening, staying the same, or improving) were added to the day 2/day 3 follow-up visit questionnaires.

Fecal samples from the first approximately 1000 children enrolled at each site were tested by quantitative polymerase chain reaction (qPCR) with a customized TaqMan array card (TAC) following the procedures described previously [22]. In brief, total nucleic acid was extracted from either stool or swab using the QIAamp Stool Fast DNA Mini kit (Qiagen, Valencia, California) [23]. Twenty microliters of the total nucleic acid from whole stool or 46 μK from rectal swab was mixed with AgPath One Step reverse-transcription PCR reagents (Thermo Fisher, Carlsbad, California) in a 100 μL reaction, then loaded into the TAC and run in a ViiA 7 or QuantStudio 7 Flex Real Time PCR system (Thermo Fisher). External controls, MS2 for RNA targets and phocine herpesvirus for DNA targets, were incorporated to monitor the extraction and amplification performance. One extraction blank was included per batch of extraction to rule out laboratory contamination. A cycle threshold (Ct) value of 35, determined previously as the analytical limit of detection [24], was applied. The data were validated only when the corresponding external control and extraction blank yielded valid results. Because we hypothesized that rectal swabs would be less sensitive than whole stool samples due to lower specimen quantity, we also

obtained 433 paired rectal swab and whole stool samples from children enrolled at the Kenya site. Pathogen Ct values derived from rectal swabs were then adjusted by the mean Ct difference for each pathogen between the paired rectal swabs and whole stools (Supplementary Table 1 and Supplementary Figure 1). The *ipaH* gene, which is amplified both for *Shigella* and enteroinvasive *Escherichia coli* (EIEC), was assumed to be *Shigella* based on metagenomic sequencing and low rates of EIEC diarrhea found in similar settings/populations [25, 26].

We developed pathogen-specific cutoffs for assigning likely diarrhea etiology based on the quantity of pathogen DNA/RNA in the stool sample (ie, pathogen burden). Cutoffs were calculated by adapting statistical models previously developed in 2 large multisite studies of diarrhea in similar settings that included nondiarrheal controls, the 7-site Global Enteric Multicenter Study (GEMS) and the 8-site Malnutrition and the Consequences for Child Health and Development (MAL-ED) cohort study [10, 20]. Specifically, in a post hoc reanalysis of GEMS data, we performed qPCR testing of stool from 4077 children with moderate to severe watery diarrhea and 1:1 age-, sex-, village-, and season-matched controls to fit a multivariable conditional logistic regression model, where the outcome was case versus control status and the predictors were the quantity of nucleic acid for each enteric pathogen, with a random slope for each study site. For MAL-ED, we used qPCR testing of 6315 watery diarrheal stools and 28 444 monthly nondiarrheal stools to fit a generalized linear mixed model with an outcome of diarrheal stool versus nondiarrheal stool, and the predictors were sex, age in months, the quantity of nucleic acid for each pathogen, a random slope for each site, and a random effect for each individual child.

To calculate cutoffs (Supplementary Table 2), for each of GEMS and MAL-ED, we first estimated quantity-specific odds ratios (ORs) for Ct values from 35, the analytical limit of detection for the assay, to 15 by 0.001 increments by taking the median ORs for each Ct value from 10 000 random permutations from a normal distribution derived from the model coefficients and the variance-covariance, drawn equally from each of the site-specific models. We then calculated the episode-specific attributable fraction (AFe), where $AFe_i = 1/j * (1 - 1/OR_i)$ and $OR_i$ is the quantity-specific median OR. We then fit a loess regression to the relationship between Ct and AFe for all values of AFe between 0.2 and 0.8 inclusive and picked the highest Ct value with an AFe $\geq 0.5$ (ie, majority attribution) as the cutoff (Supplementary Figures 2 and 3). Finally, we took the mean of the Ct values for each pathogen from GEMS and MAL-ED to arrive at the cutoffs for the present study. Only pathogens deemed attributable to diarrhea (AFe $\geq 0.5$ in either GEMS or MAL-ED) were considered in the present analysis as no Ct cutoffs are available for pathogens not associated with diarrhea in case-control analyses.

Ct values less than or equal to the aforementioned established cutoffs were considered "likely" etiology. Ct values greater than the cutoffs but <35 were considered "possible" etiologies. "Unlikely" etiology was defined as a Ct value $\geq 35$. To interrogate the effect of bacterial etiology on azithromycin efficacy, we compared outcomes between children in the 2 randomization arms within subgroups defined by likely, possible, and unlikely bacterial etiology. Bacteria considered as a likely or possible etiology included *Campylobacter* spp, tEPEC, ST-ETEC, *Salmonella* spp (typhoidal and nontyphoidal *Salmonella*), *Shigella* spp, and *Vibrio cholerae.* Children with a possible bacterial etiology in whom a virus or parasite was present at likely etiologic levels were reclassified in the unlikely bacterial etiology group. In additional exploratory analysis, we evaluated the effect of azithromycin in subsets of children with specific bacterial etiologies.

We evaluated the effect of azithromycin, in the above-defined subgroups, on the following outcomes: presence of diarrhea 3 days after enrollment, the combined outcome of death or hospitalization by day 90, and change in LAZ between enrollment and day 90. Day 3 diarrhea was not included in the prespecified outcomes of the main trial but was collected prospectively after the etiology substudy was conceived to be consistent with etiology-specific antibiotic trials that use diarrhea as a primary endpoint. Furthermore, death or hospitalization by day 90 was a secondary outcome in the main trial. The outcomes for this subgroup analysis were chosen based on the biologic plausibility that there might be a differential effect of azithromycin by bacterial etiology and had at least 80% power to detect an effect of azithromycin in the subgroup of children with a likely bacterial etiology, assuming approximately 25% would fall in this subgroup. We also reported on the outcome of death by day 90 and day 180; however, these outcomes were highly underpowered in the primary trial and were therefore anticipated to be underpowered in this subgroup analysis.

The effect of azithromycin on dichotomous outcomes was modeled using log-binomial (or Poisson if models did not converge [27]) mixed-effects models with an identity link (estimating risk difference) and ΔLAZ modeled with generalized linear mixed-effects models (estimating mean difference in difference). Each model included an indicator variable for bacterial etiology (likely, possible, unlikely) and randomization arm, as well as country (as an indicator variable) to account for country-stratified randomization, and interaction terms between randomization arm and each level of bacterial etiology. Evidence of effect modification by bacterial etiology was determined by likelihood ratio test. An α = .05 was used to determine statistical significance in this exploratory secondary analysis.

Ethical review committees from the WHO and each respective country institution approved the trial protocol, and informed consent was obtained from all caregivers of included participants. All analyses were done in Stata version 16.0 and R software.
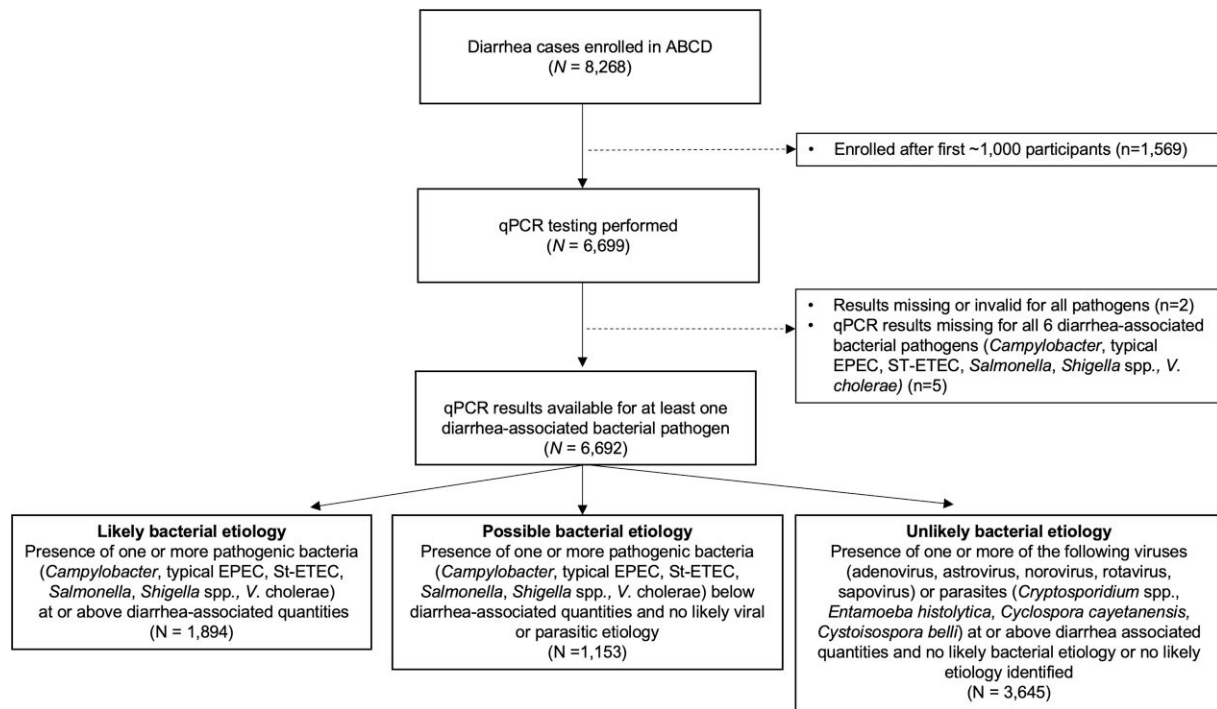
**Figure 1.** Participant flowchart describing children included in the subanalysis of diarrhea etiology among children enrolled in the AntiBiotics for Children with severe Diarrhea (ABCD) trial. Abbreviations: ABCD, AntiBiotics for Children with severe Diarrhea; EPEC, enteropathogenic *Escherichia coli*; qPCR, quantitative polymerase chain reaction; ST-ETEC, enterotoxigenic *Escherichia coli* encoding heat-stable toxin.

## RESULTS

Of the 8268 children enrolled in the ABCD trial between July 2017 and July 2019, the first 6699 (81.0%) children's fecal samples were qPCR-tested, 6692 (99.9%) of which had valid qPCR results (Figure 1). Approximately one-third of fecal samples were rectal swabs (Bangladesh [17.2%], India [42.4%], Kenya [56.5%], Malawi [42.4%], Mali [61.2%], Pakistan [38.0%], Tanzania [14.8%]). The median age of children was 12 months (interquartile range [IQR], 7–17 months) and 53.9% were male (Table 1). Children experienced a median of 2 days of diarrhea (IQR, 1–3 days) before presenting to the health facility, 54.4% were dehydrated, 32.9% were stunted, and 46.4% had moderate wasting. Child characteristics were balanced between the 2 randomization arms.

The 5 leading likely etiologies of diarrhea were rotavirus (21.1%), ST-ETEC (13.3%), *Shigella* (12.6%) *Cryptosporidium* (9.6%), and norovirus GII (8.6%) (Figure 2). Rotavirus was the leading likely etiology in each age category of 2–5 months, 6–11 months, and 12–23 months. The second and third leading likely etiologies were norovirus and ST-ETEC in 2- to 5-month-olds, *Cryptosporidium* and ST-ETEC in 6- to 11-month-olds, and *Shigella* and ST-ETEC among children aged 12–23 months. Site-specific attributable fractions are presented in Supplementary Table 3A and 3B and closely matched the distribution of pathogens determined using the Ct cutoffs (Supplementary Figure 4).

More than one-quarter of children (1894 [28.3%]) had a likely bacterial cause of their diarrhea, and likely bacterial causes were balanced across the 2 arms (Supplementary Figure 5). The effect of azithromycin on the outcome of diarrhea on day 3 was modified by the presence of a bacterial etiology (interaction $P < .001$): Among children with a likely bacterial etiology, 11.8% in the azithromycin arm had diarrhea 3 days after randomization compared to 23.3% of children in the placebo group (risk difference [RD], −11.6 [95% confidence interval {CI}, −15.6 to −7.6]; Table 2). Among children with a possible bacterial etiology, 7.1% in the azithromycin arm had 3-day diarrhea versus 15.8% of placebo-treated children (RD, 8.7% [95% CI, −13.0% to −4.4%]). There was no difference between randomization arms on day 3 diarrhea among the subset of children without a bacterial etiology (RD, −0.3% [95% CI, −2.9% to 2.3%]). During the 90 days of follow-up, azithromycin was associated with fewer deaths/hospitalizations among children with a likely (RD, −3.1% [95% CI, −5.3% to −1.0%]) and possible (RD, −2.3% [95% CI, −4.5% to −.01%]) bacterial cause of diarrhea, which was not observed in children with an unlikely bacterial etiology (RD, −0.6 [95% CI, −1.9 to .6]), but testing for effect modification was not statistically significant (interaction $P = .10$). Magnitudes of association were similar when considering rectal swab samples only (Supplementary Table 4) and considering death and hospitalization as separate outcomes

**Table 1. Participant Characteristics at Study Enrollment by Randomization Arm (N = 6692)**

| Characteristic | Overall (N = 6692) | | Azithromycin (n = 3347) | | Placebo (n = 3345) | |
|---|---|---|---|---|---|---|
| Enrollment site | | | | | | |
| Bangladesh (Jun 2017–Jan 2019) | 998 | (14.9%) | 500 | (14.9%) | 498 | (14.9%) |
| India (Sep 2017–May 2019) | 998 | (14.9%) | 501 | (15.0%) | 497 | (14.9%) |
| Kenya (Dec 2017–Jul 2019) | 1014 | (15.2%) | 502 | (15.0%) | 512 | (15.3%) |
| Malawi (Jan 2018–Jul 2019) | 691 | (10.3%) | 343 | (10.3%) | 348 | (10.4%) |
| Mali (Sep 2017–Jun 2019) | 1000 | (14.9%) | 498 | (14.9%) | 502 | (15.0%) |
| Pakistan (Sep 2017–Mar 2019) | 995 | (14.9%) | 498 | (14.9%) | 497 | (14.9%) |
| Tanzania (Sep 2017–Jul 2019) | 996 | (14.9%) | 505 | (15.1%) | 491 | (14.7%) |
| Child age | | | | | | |
| 2–5 mo | 967 | (14.5%) | 473 | (14.1%) | 494 | (14.8%) |
| 6–11 mo | 2799 | (41.8%) | 1408 | (42.1%) | 1391 | (41.6%) |
| 12–23 mo | 2926 | (43.7%) | 1466 | (43.8%) | 1460 | (43.6%) |
| Male sex | 3604 | (53.9%) | 1800 | (53.8%) | 1804 | (53.9%) |
| Duration of diarrhea prior to presentation, d, median (IQR) | 2 | (1–3) | 2 | (1–3) | 2 | (1–3) |
| No. of unusually loose or watery stools in last 24 h, median (IQR) | 6 | (5–9) | 6 | (5–9) | 6 | (5–9) |
| Dehydration status | | | | | | |
| None | 3053 | (45.6%) | 1545 | (46.2%) | 1508 | (45.1%) |
| Some | 3285 | (49.1%) | 1638 | (48.9%%) | 1647 | (49.2%) |
| Severe | 354 | (5.3%) | 164 | (4.9%) | 190 | (5.7%) |
| LAZ, median (IQR) | −1.4 | (−2.4 to −0.6) | −1.4 | (−2.3 to −0.6) | −1.4 | (−2.4 to −0.6) |
| Stunting[a] | | | | | | |
| None | 4491 | (67.1%) | 2257 | (67.4%) | 2234 | (66.8%) |
| Moderate | 1209 | (18.1%) | 593 | (17.7%) | 616 | (18.4%) |
| Severe | 992 | (14.8%) | 497 | (14.9%) | 495 | (14.8%) |
| MUAC, cm, median (IQR) | 12.9 | (12.3–13.9) | 12.9 | (12.3–13.9) | 12.9 | (12.3–13.9) |
| WLZ[b], median (IQR) | −1.34 | (−2.1 to −0.4) | −1.4 | (−2.2 to −0.4) | −1.3 | (−2.1 to −0.4) |
| Wasting[c] | | | | | | |
| None | 3589 | (53.6%) | 1761 | (52.6%) | 1828 | (54.7%) |
| Moderate | 3103 | (46.4%) | 1586 | (47.4%) | 1517 | (45.4%) |
| No. of children <5 y of age in household, median (IQR) | 1 | (1–2) | 1 | (1–2) | 1 | (1–2) |
| Wealth quintile | | | | | | |
| Lowest | 864 | (12.9%) | 443 | (13.2%) | 421 | (12.6%) |
| Second | 1092 | (16.3%) | 546 | (16.3%) | 546 | (16.3%) |
| Middle | 1071 | (16.0%) | 552 | (16.5%) | 519 | (15.5%) |
| Fourth | 1779 | (26.6%) | 839 | (25.1%) | 940 | (28.1%) |
| Highest | 1886 | (28.2%) | 967 | (28.9%) | 919 | (27.5%) |
| Fecal sample type | | | | | | |
| Whole stool | 4094 | (61.2%) | 2025 | (60.5%) | 2069 | (61.9%) |
| Rectal swab | 2598 | (38.8%) | 1322 | (39.5%) | 1276 | (38.2%) |

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: IQR, interquartile range; LAZ, length-for-age z-score; MUAC, mid-upper arm circumference; WLZ, weight-for-length z-score.

[a]No stunting defined as LAZ ≥ −2; moderate stunting defined as −3 ≤ LAZ < −2; severe stunting defined as LAZ < −3.

[b]Three participants missing WLZ at enrollment.

[c]No wasting defined as MUAC ≥12.5 cm (if ≥6 months) or WLZ ≥ −2; moderate wasting defined as 11.5 cm ≤ MUAC <12.5 cm (if ≥6 mo) or −2 < WLZ < −3. Three participants missing WLZ at enrollment.

(Supplementary Table 5). Azithromycin treatment was associated with a modest improvement in ΔLAZ among children with an unlikely bacterial etiology (difference in difference, 0.06 [95% CI, .02–.10]), but the effect was smaller and imprecise in the children with a likely or presumed bacterial etiology and the effect modification could have been due to chance (interaction $P = .6$).

Among the 1894 children with a likely bacterial etiology, we further explored the difference between randomization arms on all outcomes among those with diarrhea likely caused by ST-ETEC (n = 889), *Shigella* (n = 845), tEPEC (n = 215),

*V cholerae* (n = 174), and *Salmonella* (n = 52). Only 6 children had *Campylobacter* quantities at likely etiologic levels, so effects were not explored. The proportion of children with diarrhea on day 3 was lower in children randomized to azithromycin compared to placebo in all subsets (other than *Salmonella*) and improved the change in LAZ for children with diarrhea attributed to *Shigella* and ST-ETEC (Table 3). Specifically, among children with *Shigella*, linear growth faltered less in those treated with azithromycin (mean ΔLAZ, −0.13 [standard deviation {SD}, 0.52]) than children treated with placebo (mean ΔLAZ,
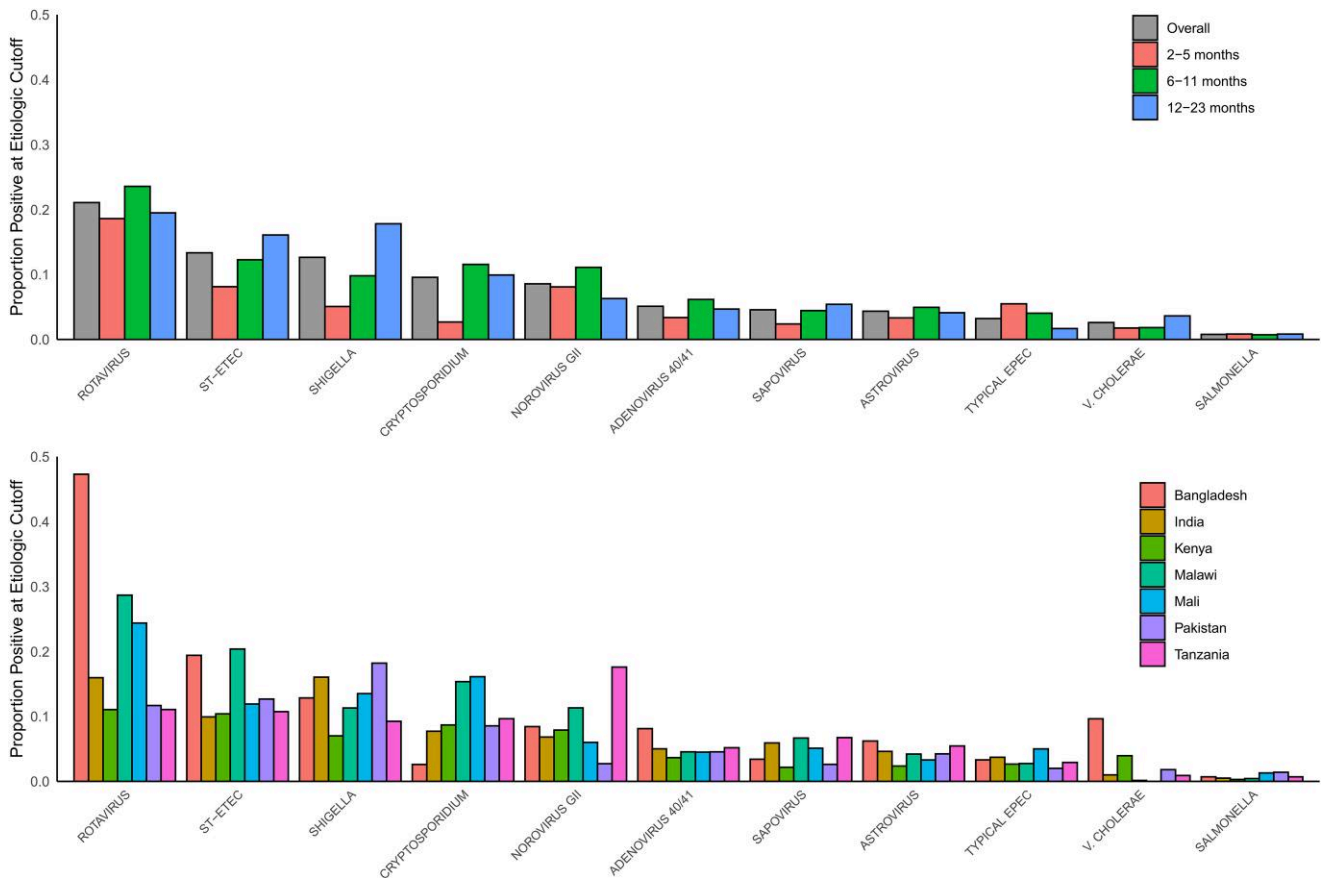
**Figure 2.** Likely etiologies (determined by pathogen-specific diarrhea-attribution cycle threshold cutoffs) among AntiBiotics for Children with severe Diarrhea (ABCD) study participants by site and age. Showing etiologies with 10% prevalence or more. Abbreviations: EPEC, enteropathogenic *Escherichia coli*; ST-ETEC, enterotoxigenic *Escherichia coli* encoding heat-stable toxin.

−0.21 [SD, 0.54]; difference in difference, 0.11 [95% CI, .04–.17]). Although there was not a statistically significant difference in terms of risk of death or hospitalization by day 90 between children randomized to azithromycin versus placebo in any of the bacteria groups, the magnitude of the effect of the point estimate ranged from 1 to 5 fewer deaths per 100 children in the azithromycin-treated group compared to the placebo group (Table 3 and Supplementary Table 6). Notably, 12.3% of the placebo-treated children with tEPEC were hospitalized or died during the 90-day follow-up, as did 7.6% of placebo-treated children with *Shigella* diarrhea and 7.2% with ST-ETEC diarrhea. In the unlikely bacterial etiology group, 4.1% of children died or were hospitalized during the 90-day follow-up period.

## DISCUSSION

In this subgroup analysis of a multicountry, placebo-controlled, randomized trial of azithromycin in conjunction with WHO standard of care to treat acute watery diarrhea, azithromycin was associated with shorter diarrhea duration and fewer deaths/hospitalizations in the subset of children with a likely bacterial etiology, with some residual benefit observed in children with bacteria detected at quantities below the pathogen-specific etiologic cutoffs. More than one-quarter of all enrolled children had a bacterial etiology identified, a proportion nearly identical (32%) to that observed among acute watery diarrhea cases in the GEMS study [10]. Antibiotic treatment efficacy studies of presumed or confirmed bacterial diarrhea have frequently used diarrhea presence after 2–6 days as a primary endpoint [15, 28–31] and here we show a benefit to azithromycin treatment of bacterial diarrhea in terms of the clinical outcome of diarrhea on day 3. Fewer than 9 children would be needed to treat to avert 1 case of day 3 diarrhea, and this effect was consistent across *Shigella*, ST-ETEC, tEPEC, and *V cholerae*, the most commonly identified bacterial etiologies.

While an effect of azithromycin on the secondary combined outcome of death or rehospitalization 90 days after diarrhea presentation was observed in the primary trial analysis [9], the absolute risk reduction was small (86 children would need to be treated to avert 1 death or hospitalization by day

**Table 2. Effect of Azithromycin Within Subgroups Defined by Likely, Possible, and Unlikely Diarrhea Attribution to 1 or More of the Following Bacteria: *Shigella* spp, Enterotoxigenic *Escherichia coli* Encoding Heat-Stable Toxin, Typical Enteropathogenic *E coli*, *Vibrio cholerae*, *Salmonella* spp, and/or *Campylobacter* spp**

| Outcome and Subgroup | Proportion With the Indicated Outcome/Mean Outcome | | Risk Difference or Difference in Difference[a] (95% CI) | Evidence of Effect Modification[b] |
| | AZM | Placebo | | |
| --- | --- | --- | --- | --- |
| Diarrhea on day 3[c] (n = 4835) | | | | <.001 |
| Likely bacterial etiology (n = 1358) | 79/672 (11.8%) | 160/686 (23.3%) | −11.6% (−15.6% to −7.6%) | |
| Possible bacterial etiology (n = 815) | 28/392 (7.1%) | 67/423 (15.8%) | −8.7% (−13.0% to −4.4%) | |
| Unlikely bacterial etiology (n = 2662) | 173/1349 (12.8%) | 172/1313 (13.1%) | −0.31% (−2.9% to 2.3%) | |
| Death or hospitalization by day 90 (n = 6692) | | | | .10 |
| Likely bacterial etiology (n = 1894) | 42/930 (4.5%) | 74/964 (7.7%) | −3.1% (−5.3% to −1.0%) | |
| Possible bacterial etiology (n = 1153) | 16/566 (2.8%) | 30/587 (5.1%) | −2.3% (−4.5% to −.01%) | |
| Unlikely bacterial etiology (n = 3645) | 63/1851 (3.4%) | 73/1794 (4.1%) | −0.62% (−1.90% to .61%) | |
| ΔLAZ[d], mean (SD) (n = 6406) | | | | .58 |
| Likely bacterial etiology (n = 1813) | −0.18 (0.55) | −0.21 (0.56) | 0.03 (−.02 to .08) | |
| Possible bacterial etiology (n = 1106) | −0.13 (0.67) | −0.16 (0.61) | 0.03 (−.04 to .11) | |
| Unlikely bacterial etiology (n = 3487) | −0.13 (0.61) | −0.19 (0.59) | 0.06 (.02–.10) | |

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: AZM, azithromycin; CI, confidence interval; ΔLAZ, difference in length-for-age z-score[LAZ] between enrollment and day-90; SD, standard deviation.

[a]Effect of azithromycin on outcome, adjusted for site (indicator) and baseline value (for ΔLAZ model). Risk difference per 100 child-years (for proportions) and difference of difference reported for mean values (ΔLAZ).

[b]*P* value from likelihood ratio test comparing nested models with and without additive interaction term between randomization arm and bacterial etiology group.

[c]Question added to questionnaire after recruitment began, therefore missing for some.

[d]Change between day 90 visit and enrollment visit.

90), a benefit likely diluted by enrollment of children with non-bacterial diarrhea. The present analysis supports this hypothesis in that the number needed to treat to prevent death or hospitalization by day 90 in children with likely bacterial diarrhea was >33. This effect size is comparable or greater than the effect size used to support the routine use of amoxicillin to treat nonsevere pneumonia, based on the outcome of treatment failure that also included death and/or hospitalization, albeit as the prespecified primary endpoint and not limited to bacterial pneumonia [32]. Clinical management guidelines in high-resource settings also indicate antibiotic treatment for some enteric bacteria, such as *Shigella* [33], and children with underlying malnutrition or other host vulnerabilities common in resource-limited settings may experience the greatest benefit of such treatment.

It is clear from this and other studies that dysentery captures only a small fraction of bacterial diarrhea [20, 34], and thus syndromic guidelines limiting antibiotics to children with dysentery leave many with bacterial diarrhea untreated. Indiscriminate use of antibiotics for diarrhea could promote antibiotic resistance; therefore, better diagnostic tools with high accuracy for bacterial diarrhea at the point-of-care are needed. Such tools could include rapid molecular or lateral flow tests, accurate biomarkers, or improved clinical scoring systems [35–38]. Rapid molecular testing for enteric bacteria was recently trialed in Botswana and, while underpowered, found a modest benefit of this test-and-treat strategy in preventing future diarrheal episodes [39]. A test-and-treat strategy

could also limit antibiotic use; between 23% and 77% of children with watery diarrhea are treated with antibiotics empirically [12, 40, 41].

We observed a small residual clinical benefit of azithromycin treatment in diarrhea with bacteria identified but at a lower quantity than the prespecified cutoff for etiology attribution. The etiologic cutoffs derived from large observational studies were designed to favor specificity over sensitivity to confirm etiologies, and this finding suggests that some underattribution of bacterial etiology likely occurred. The cutoff used for *Campylobacter* was particularly stringent, due to subclinical carriage being particularly common in the GEMS and MAL-ED studies [10, 20] and a poor association between quantity detected and diarrhea, leading to possible underclassification of *Campylobacter*-attributed diarrhea. Post hoc analyses could possibly use treatment effect to further evaluate the test characteristics of these cutoffs and better identify the subset of diarrhea that benefits from antimicrobial therapy. It is also possible that the benefit of azithromycin treatment includes reduction in subclinical bacterial infections, an explanation consistent with the observed reduction in *Shigella* infections, and all-cause mortality reductions, among children randomized to mass azithromycin distribution in Niger [42, 43].

Interestingly, the modest effect of azithromycin on short-term linear growth was not observed in children with the combined groups of likely or possible bacterial etiology. Modest benefit was observed in some specific bacterial subgroups, such as *Shigella* and ST-ETEC. *Shigella* and ST-ETEC diarrhea

**Table 3. Effect of Azithromycin Among Subsets of Children With Specific Pathogenic Bacteria at Likely Etiologic Levels[a]**

| Outcome | Diarrhea on Day 3[b] | | | Death or Hospitalization by Day 90 | | | ΔLAZ[c] | | |
|---|---|---|---|---|---|---|---|---|---|
| | AZM | Placebo | Risk Difference (95% CI)[d] | AZM | Placebo | Risk Difference (95% CI)[d] | AZM, Mean (SD) | Placebo, Mean (SD) | Difference in Difference (95% CI)[e] |
| Likely ST-ETEC diarrhea (n = 889) | 33/300 (11.0%) | 67/316 (21.2%) | **−11.2 (−16.8 to −5.6)** | 22/431 (5.1%) | 33/458 (7.2%) | −1.5 (−4.6 to 1.5) | −0.17 (0.52) | −0.23 (0.54) | 0.08 (.01−.14) |
| Likely *Shigella* diarrhea (n = 845) | 36/301 (12.0%) | 82/316 (26.0%) | −14.0 (−20.0 to −8.0) | 19/411 (4.6%) | 33/434 (7.6%) | −2.9 (−6.0 to 0.27) | −0.13 (0.52) | −0.21 (0.54) | 0.11 (.04−.17) |
| Likely typical EPEC diarrhea (n = 215) | 10/80 (12.5%) | 24/82 (29.3%) | −15.6 (−28.3 to −3.0) | 6/109 (5.5%) | 13/106 (12.3%) | −4.9 (−11.7 to 1.9) | −0.27 (0.52) | −0.21 (0.54) | −0.01 (−.16 to .14) |
| Likely *Vibrio cholerae* diarrhea (n = 174) | 12/63 (19.1%) | 22/56 (39.3%) | −20.2 (−36.2 to −4.3) | 3/84 (3.6%) | 7/90 (7.8%) | −5.6 (−13.7 to 2.4) | −0.31 (0.5) | −0.13 (0.64) | −0.16 (−.34 to −.03) |
| Likely *Salmonella* diarrhea (n = 52) | 2/17 (11.8%) | 4/22 (18.2%) | −6.4 (−29.8 to 16.9) | 0/25 (0%) | 1/27 (3.7%) | … | −0.09 (0.47) | −0.24 (0.81) | 0.07 (−.23 to .38) |
| Likely *Campylobacter* diarrhea (n = 6) | 0/2 (0%) | 1/2 (50%) | … | 0/3 (0%) | 0/3 (0%) | … | 0.003 (0.17) | −0.30 (0.31) | … |

Data are presented as no./No. (%) unless otherwise indicated.

Abbreviations: AZM, azithromycin; EPEC, enteropathogenic *Escherichia coli*; SD, standard deviation; ST-ETEC, enterotoxigenic *Escherichia coli* encoding heat-stable toxin.

[a] *Shigella*: cycle threshold (Ct) <28.7; ST-ETEC: Ct <25.4; typical EPEC: Ct <18.1; *Vibrio cholerae*: Ct <32.6; *Salmonella*: Ct <31.9; *Campylobacter*: Ct <16.3.

[b] Question added to questionnaire after recruitment began, therefore missing for some.

[c] Change between day 90 visit and enrollment visit.

[d] Effect of azithromycin on outcome, adjusted for site (indicator). Risk difference per 100 child-years.

[e] Effect of azithromycin on outcome, adjusted for site (indicator) and baseline length-for-age z-score. Difference of mean ΔLAZ.

have been consistently associated with short-term linear growth faltering [12, 13], and antibiotic treatment for *Shigella* was observed to have linear growth–promoting benefits in GEMS [12]. A modest, statistically significant benefit was observed in the subset of children without a bacterial etiology. This finding was surprising and needs to be assessed in other studies to exclude the possibility of a spurious result. If true, a potential mechanism could relate to a role for azithromycin on reducing inflammation in the context of environmental enteropathy [44] [45–47].

Strengths of this analysis include the large sample size of children with etiology-specific diarrhea (including *Shigella*, ST-ETEC, and tEPEC) as well as the merits of the original trial including it being double-blind, placebo-controlled, multicountry, implementing WHO standards of care, and with ascertainment of short- and longer-term outcomes representing a spectrum of diarrhea convalescence. Limitations include those related to any post hoc analysis; namely, that observed effects were due to other sources of bias, such as type 1 error that could occur by the inclusion of an outcome (day 3 diarrhea) that was not prespecified in the original trial and the use of a secondary outcome in the original trial (the combined outcome of death or hospitalization). We also had limited power for the primary outcome of mortality in subgroups, limiting our ability to formally test for effect modification by bacteria etiology in this primary endpoint. Assignment of likely and possible etiology is complicated with sensitive diagnostic methods, particularly in settings where asymptomatic carriage is high, but we attempted to overcome this by utilizing pathogen quantity cutoffs that erred on specificity. Other factors, such as age, underlying malnutrition, and previous enteric pathogen exposure also likely impact whether or not a given pathogen caused diarrhea, in addition to pathogen quantity. We did not have antimicrobial susceptibility data to confirm whether azithromycin was active against specific bacterial isolates. While azithromycin remains active against most *Shigella*, ETEC, *Campylobacter*, *V cholerae*, and *Salmonella* spp [48], it is possible that rates of azithromycin resistance in these gram-negative bacteria reduced the effect of azithromycin that we would have otherwise observed. The emergence of azithromycin resistance in enteric bacteria such as *Shigella* [49–51] makes it imperative that consideration of expanded use of azithromycin for bacterial watery diarrhea be weighed against the potential for increased resistance.

This reanalysis of the ABCD trial addresses evidence gaps in the use of antibiotic therapy, in conjunction with other WHO-indicated standards of care, for watery diarrhea of bacterial etiology among children in low- and middle-income settings. If confirmed, the use of azithromycin for acute watery diarrhea in young dehydrated or undernourished children living in settings of high bacterial diarrhea burden may be warranted if the subset of children with diarrhea who benefit from azithromycin can be identified.

## Supplementary Data

## Notes

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