Clinical Study
Influence of Zinc Supplementation in Acute Diarrhea Differs by the Isolated Organism

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1. Introduction

In 2004, the World Health Organization (WHO) and the United Nations Children’s Fund (UNICEF) recommended, in a joint statement, the use of zinc supplementation for the treatment of acute diarrhea in developing countries [1]. This recommendation was based on strong biological and epidemiological evidence which suggested that zinc supplementation can significantly reduce the overall duration of diarrhea and is also likely to reduce stool volume and frequency [2]. However, there exists significant heterogeneity in the effects of zinc on diarrhea-related outcomes observed across published randomized controlled trials [3–5]. Potential contributors to this heterogeneity are currently not fully understood.

There is some evidence to suggest that the beneficial effect of zinc may not be equivalent against the common causative organisms. Roy et al. [6] had first demonstrated that the extent to which mucosal permeability is affected in different diarrheas depends on the causative organisms—in general, diarrheas caused by invasive organisms show higher permeability. Second and consistent with this observation, Canani et al. [7] observed that zinc-induced promotion of ion absorption across the gut is evident in response to the ion secretion caused by Vibrio cholerae toxin but not the Escherichia coli heat-stable enterotoxin. Third, Surjawidjaja et al. [8] showed that although zinc sulphate can inhibit the growth of enteropathogens in vitro, the lethal dose required to kill 50% of the organisms (LD50) widely varies across the species of the causative organisms. Consequently it is possible that the overall beneficial effect of zinc supplementation observed in a trial may depend on the spectrum of the causative organisms within that study. The influence of zinc supplementation on diarrhea could thus be dependent on...
the organisms present in the gut. Using microbiological and clinical data from a three-arm randomized controlled trial of zinc supplementation, we therefore determined whether differential organisms can partially contribute to the effect of zinc. Due to limited resources, we were unable to conduct serotyping for pathogenic organisms causing diarrhea. In this report, therefore, we demonstrate the modulation of effect of zinc supplementation by bacterial isolates in the stool and rotavirus infection.

2. Patients and Methods

2.1. Study Subjects. This dataset comes from a double-blind, randomized, placebo-controlled clinical trial in children aged 6–59 months attending the Indira Gandhi Government Medical College and Hospital, in Nagpur, India with >3 unformed stools in the prior 24 hours; duration of diarrhea <72 hours; and ability to accept oral fluids or feeds. Details about the study subjects, the trial design, and its rationale are provided elsewhere [9]. Briefly, all children aged 6 months to 59 months attending study center with more than three unformed stools in the prior 24 hours with a total duration of diarrhea at recruitment of up to 72 hours and who were able to accept oral fluids were recruited in the study. The exclusion criteria were chronic or severe complicating illness, known positive HIV status, kwashiorkor, residing outside a radius of 30 km around the hospital, participating in another study, or already enrolled in this study. The trial is registered with the International Standard Randomized Controlled Trial register with the unique identifier ISRCTN85071383. The Ethics Committee of Indira Gandhi Government Medical College, Nagpur, and the Human Research Ethics Committee of the University of Newcastle, New South Wales, Australia (HREC Approval no: H-500-0203) approved the study protocol and the treatment effects monitoring committee monitored the trial for safety.

2.2. Study Protocol. Each recruited child was sequentially assigned to one of the following three treatment arms using a randomization protocol fixed a priori: placebo (Pl, \( n = 271 \)) arm, zinc (Zn, \( n = 264 \)) only arm, and zinc and copper (Zn + Cu, \( n = 273 \)) arm. Participants in the Zn arm received the therapeutic dose 2 mg/kg/day of zinc while participants in the Zn + Cu arm received the same dose of zinc as well as 0.2 mg/kg/day of copper. Microbiological investigations were conducted with a sterile container having a plastic spoon attached to the inside of the screw cap for stool collection. The feecal sample first underwent a naked eye examination for consistency, presence of mucous and blood. In the laboratory, the sample underwent gross and microscopic examination of wet and iodine preparations. Kenyon's method of acid fast staining for parasitic cyst was also done. For bacterial isolation sample was inoculated on sheep blood agar, MacConkey Bile Salt Agar, selenite F broth, Skirrow's campylobacter medium, alkaline peptone water, and thiosulphate citrate bile salts agar. The bacterial isolates were identified by standard bacteriological techniques [10]. Enzyme-linked immunosorbent assay (ELISA; Premier Rotaclone, Meridian Bioscience, Inc, Cincinnati, OH) was used for rotavirus antigen detection.

2.3. Outcomes and Predictors. In this study we examined whether zinc supplementation either alone or in combination with copper was associated with improvement in two diarrhea-related outcomes—the likelihood of continued diarrhea beyond 3 days since initiation of therapy, and the volume of stool collected during hospital stay. This latter outcome was divided into two categories based on the median stool volume in the study subjects. This study was conducted as a set of prespecified subgroup analyses that specifically aimed to tease out possible heterogeneity of zinc supplementation effects across the spectrum of the organisms isolated from stools.

2.4. Statistical Analyses. We followed the existing guidelines for reporting subgroups analyses from randomized controlled trials [11, 12]. Recognizing that the interpretations and recommendations based on subgroups analyses heavily depend on a critical and careful statistical analysis, we conducted our analyses in two steps.

In the first step, we attempted to establish that our analyses will have sufficient statistical power while addressing the issues of multiple comparisons and of treatment (zinc) \( \times \) modifier (isolated organisms) interactions. For this we used the following three complementary analytical techniques. (a) The first is the method of multifactor dimensionality reduction (MDR) [13] to examine the likely interactions among isolated organisms and zinc supplementation for the two diarrhea related organisms. MDR is a nonparametric, exploratory approach that identifies and characterizes the potential interactions in a large number of discrete predictors with a binary outcome and is commonly used to identify gene-gene interactions. (b) We next looked for significant bivariate interaction of each major isolated organism with zinc supplementation for both the outcomes. For this, we used the interaction contrast statistic (\( \Delta \)) described by Gönen [14]. We tested the statistical significance of this interaction using the Breslow-Day test for heterogeneity and also determined the statistical power of our study to make inferences about these interactions. To estimate the power we made use of the \( \theta \) statistic [14]. This was estimated using the arcsine transformation as 2sin\(^{-1}\)(\( p^{(1/2)} \)) where \( p \) is proportion of subjects developing the outcome of interest for a given combination of the therapy received and the isolated organism. The \( \theta \) statistic has a standard normal distribution with variance \( 1/n \), where \( n \) is the number of observations. (c) We next conducted stepwise multiple logistic regression analysis [15] for each outcome where the primary objective was to arrive at a reduced list of significant interactions based on the organisms identified in step (b). For stepwise regression we used the backward elimination strategy with a conventional probability retention criterion of 0.2. In the full model, we considered the main effects and all interactions (bivariate as well as higher-order) among the therapy and diarrheal isolates. One cardinal issue in subgroup analyses is the multiplicity of comparisons. To address this, we counted
the total number of comparisons and reported the likelihood of false positive identification of significant interactions.

In the second step of the analysis, we aimed to test the robustness of our findings in the light of possible correlations with other predictors of zinc effectiveness. In this step, we used multivariate unconditional logistic regression modeling. In these multivariate models we adjusted for the following covariates: age, gender, presence of stunting (weight-for-age z-score < −2), presence of wasting (length-for-age z-score < −2), wealth index score, hand washing score, baseline plasma zinc level, baseline plasma copper level, and baseline hemoglobin level. Statistical significance was examined at a type I error rate of 0.05.

MDR analysis used the MDR software (http://sourceforge.net/projects/mdr/). All other analyses were conducted using Stata 10.2 (Stata Corp, College Station, TX) software package.

3. Results

3.1. Description of Outcomes and Predictors. Of the total 808 study subjects, microbiological data was available for 801 (99.1%) children. From these 801 children a total of 913 organisms were isolated—548 (68.4%) children had a single organism isolated, 195 (24.3%) children had mixed isolates with at least 2 organisms, while no organisms could be isolated in 58 (7.3%) children. In the order of commonness the following organisms were isolated in the study subjects: E. coli 451 (56.3%), Klebsiella spp 276 (34.5%), rotavirus 169 (21.1%), parasitic ova and cysts in stool 121 (15.1%), Shigella spp 8 (1%), Salmonella spp 5 (0.6%), and Campylobacter jejuni 2 (0.2%). Interestingly, of the 169 children with rotavirus infection, E. coli-rotavirus combination was observed in 100. At baseline, children with weight for age < −2 z-score were 19%, 57.5%, and 32.7% in those with rotavirus infection, E.coli and Klebsiella isolates in the stool samples. Correspondingly the proportions with serum zinc <60 μg/dl were 25.2%, 56.4%, and 35%, respectively. Adherence to supplemnations was similar in the three groups at 92.2%, 90.4%, and 88%, respectively. Three hundred and six (38.2%) children had diarrhea that extended beyond 72 hours after initiation of therapy. The median stool volume for all the study subjects was 685 ml. Based on this cut-off we dichotomized the study children in to those who had low stool volume (<685 ml) and those who had high stool volume (≥685 ml).

3.2. Interactive Effects among Isolated Organisms and Zinc Supplementation. We first conducted MDR analyses to identify and characterize potential interactions among zinc supplementation and four major isolates: E. coli, Klebsiella, rotavirus, and parasites. Our results indicated (Figure 1) that for the outcome of diarrhea >3 days E. coli was not found to be contributory to any interaction while Klebsiella was noncontributory to the outcome of high stool volume. Presence of rotavirus was associated with a higher proportion of children with diarrhea >3 days especially when they received zinc supplementation as indicated by taller red bars in cells 4, 8, and 12 in Figure 1. On the other hand, when Klebsiella but not rotavirus was isolated from the stools, zinc supplementation appeared to be associated with a reduced likelihood of diarrhea >3 days (compare the red and blue bars in cells 6, 13, and 14 of Figure 1). Although MDR included parasites as another potential source of interactions for the outcome of diarrhea >3 days, its role did not appear to be definitive. Using this set interactions identified by MDR, its odds ratio for accurate prediction of the outcome was 2.34 (95% CI 1.73–3.17, P = 2.6 × 10⁻⁸) with an overall accuracy of 63.1%. For the outcome of high stool volume, MDR analysis again identified presence of rotavirus as a potential deterrent to the beneficial effect of zinc (magenta bars in cells 4, 8, 12, and 16 in Figure 1). In general, presence of E. coli in the stool was associated with an increased risk of high stool volume (magenta bars in cells 7, 8, and 13–16 in Figure 1). Again the role of parasites, although identified by MDR as a possible dimension, was equivocal. For this outcome, the OR for correct prediction based on MDR results was 2.33 (95% CI 1.73–3.14, P = 1.7 × 10⁻⁸) with an overall accuracy of 59.4%.

We next examined the bivariate interactions between each of the major isolates and zinc supplementation for both the outcomes (Figure 2). For diarrhea >3 days, the interaction constant was positive for E. coli and rotavirus, significantly negative for Klebsiella, and uninformative for parasites. This indicated that zinc supplementation may provide detrimental results in the presence of E. coli and rotavirus but may be associated with a shorter period of diarrhea in the case of Klebsiella isolates. The Breslow-Day test also identified E. coli, rotavirus, and Klebsiella to be interacting significantly with zinc supplementation for this outcome. As shown in Figure 2 (grey bars) our study had moderate to sufficient power to detect significant interactions. Although similar directionalities for interactions was also observed for the outcome of high stool volume, statistical significance as well as sufficient power was only achieved for the E. coli isolates. Since parasites did not demonstrate any significant interaction with zinc supplementation and had negligible statistical power for either of the outcomes, we omitted this source of interaction from all further analyses.

Lastly, we considered the importance of the interactions in a multivariate context. We studied four main effects (zinc, E. coli, Klebsiella, and, rotavirus), six bivariate interactions (listed as covariates 5 through 10 in Figure 3), and four three-way interactions (covariates 11 through 14 in Figure 3). To control for multiple comparisons in this step of the analysis we employed the method of stepwise logistic regression with a conventional retention criterion of 0.2. In the final models, we observed that for the outcome of diarrhea >3 days zinc supplementation provided two statistically significant and directionally opposite interactions: with rotavirus and (OR > 1) and with Klebsiella (OR < 1). In contrast, for the outcome of high stool volume there were two synergistic and significant interactions of zinc supplementation: with E. coli and with rotavirus (both OR > 1). Thus, there were four significant interaction terms that involved zinc supplementation in our stepwise multivariate regression procedure. As shown in Figure 3, there were a total of 10
Results of multifactor dimensionality reduction analyses

### Outcome: diarrhea for > 3 days

| Zinc supplementation | Rotavirus | No | Yes |
|----------------------|-----------|----|-----|
| No                   | Zinc     | 10.46 | 24.83 | 5.45 | 14.08 | 5.66 |
| Yes                  | Zinc     | 12.09 | 8.82 | 1.82 | 5.98 | 3.03 |

### Outcome: high stool volume

| Zinc supplementation | Rotavirus | No | Yes |
|----------------------|-----------|----|-----|
| No                   | Zinc     | 11.63 | 7.81 | 16.34 | 22.17 |
| Yes                  | Zinc     | 9.65 | 13.35 | 21.29 | 27.2 |

Figure 1: Investigation into the interaction of isolated organisms with the effect of zinc on two diarrhea-related outcomes—risk of continued diarrhea for 3 or more days (left column) and high stool volume (right column) based on the results from multifactor dimensionality reduction (MDR) analyses. Results show the proportion of subjects with (red and magenta bars) and without (blue and green bars) the indicated outcome for a given combination of zinc supplementation and isolated organisms. The background of each cell representing a specific combination is gradient, coded as shown in the key at the bottom of the MDR grid. Numbers at the top of the bars are proportions and numbers in yellow are cell identifiers.

### Bivariate interactions of isolates with zinc supplementation

| Outcome: diarrhea for > 3 days |
|-------------------------------|
| E. coli | 0.34 | 0.62 | 0.5389 | 0.5677 | 0.0531 |
| Klebsiella | 0.0237 | 0.0393 | 0.0255 | 0.7267 |
| Rotavirus | 0.11 | 0.09 | 0.2 |
| Parasites | 0.0531 | 0.0127 | 0.0107 | 0.0209 |

Figure 2: Statistical evaluation of the bivariate interactions between zinc supplementation and each of the four major isolates for two diarrhea-related outcomes—risk of continued diarrhea for 3 or more days (left column) and high stool volume (right column). The interaction was quantified using the interaction contrast statistic [14], the significance was tested using the Breslow-Day (B-D P) test for heterogeneity, and statistical power of interpretation in the present study was determined using the θ statistic described by Gönen [14]. Interaction contrast exceeding or below zero indicates a detrimental or protective interaction, respectively.
interaction terms in the full model for each outcome, and thus, a total of 20 interactions were considered in the analysis. At a type I error rate of 0.05, this would mean that one of the significant interactions detected in our study is likely to be a false positive.

### 3.3. Robustness of Treatment Heterogeneity: Multivariate Models

We next set out to investigate whether the significant interactions observed held the significance in the face of several other potential contributors to the two outcomes. Consistent with our previous observations [9], we found that zinc supplementation either alone or in combination with copper supplementation did not influence either of the study outcomes after adjusting for baseline covariates (rows titled “Overall” in Tables 1 and 2). We conducted a series of multivariate logistic regression models that included the aforementioned covariates.

For the outcome of continued diarrhea >3 days, we observed the protective effects of zinc supplementation when *Klebsiella* was isolated and the detrimental effects of zinc supplementation in *E. coli*-rotavirus infections were boosted by the addition of copper. We found similar results when we used other cut-points for dichotomizing the diarrheal duration like 5 days and 7 days (data not shown). Thus, the influence of zinc supplementation on the proportion of subjects with prolonged diarrhea was substantially modulated by the micro-organisms isolated. For the outcome of high stool volume we observed similar effects; however, in most instances the observations did not reach statistical significance (Table 2).

### 4. Discussion

There are four-key findings of this study. First, the beneficial effect of zinc supplementation in acute diarrhea was not equal against all organisms isolated from stools. Of special concern, however, was our observation that zinc may actually increase the risk of prolonged diarrhea in the presence of *E. coli*-rotavirus (prevalence of 12% in this study). Prescreening for rotavirus reactivity may enhance the utility of zinc supplementation by restricting it to subjects not reactive to rotavirus. It is interesting to note that two studies in young infants in four countries (Bangladesh, Ethiopia, India, and Pakistan) showed no effect of zinc supplementation on...
| Subgroup                        | N     | OR  | 95% CI    | P   | OR  | 95% CI    | P   | OR  | 95% CI    | P   |
|--------------------------------|-------|-----|-----------|-----|-----|-----------|-----|-----|-----------|-----|
| **All children**               |       |     |           |     |     |           |     |     |           |     |
| Overall                        | 798   | 1.16| 0.81–1.67 | .421| 1.05| 0.73–1.50 | .808| 1.10| 0.80–1.51 | .547|
| E. coli infection              | 449   | 1.59| 0.96–2.61 | .069| 1.42| 0.97–2.33 | .164| 1.50| 0.97–2.32 | .068|
| E. coli single infection       | 273   | 1.32| 0.68–2.57 | .413| 1.16| 0.59–2.26 | .667| 1.24| 0.69–2.22 | .477|
| Klebsiella infection           | 275   | 0.73| 0.39–1.36 | .322| 0.58| 0.31–1.09 | .092| 0.65| 0.38–1.21 | .121|
| Klebsiella single infection    | 170   | 0.46| 0.20–1.05 | .065| 0.43| 0.18–0.98 | .045| 0.44| 0.22–0.90 | .025|
| Rotavirus infection            | 169   | 2.30| 0.98–5.39 | .054| 3.00| 1.31–6.87 | .009| 2.65| 1.28–5.48 | .009|
| E. coli + rotavirus mixed infection | 100 | 2.43| 0.71–8.33 | .159| 4.54| 1.33–15.5 | .016| 3.39| 1.13–10.1 | .029|
| Klebsiella + rotavirus mixed infection | 53  | 2.29| 0.36–14.6 | .381| 1.50| 0.33–6.91 | .600| 1.72| 0.42–7.05 | .444|

| Subgroup                        | N     | OR  | 95% CI    | P   | OR  | 95% CI    | P   | OR  | 95% CI    | P   |
|--------------------------------|-------|-----|-----------|-----|-----|-----------|-----|-----|-----------|-----|
| **Children with RBCs in stool** |       |     |           |     |     |           |     |     |           |     |
| Overall                        | 334   | 1.33| 0.74–2.40 | .341| 1.04| 0.58–1.88 | .879| 1.18| 0.71–1.97 | .537|
| E. coli infection              | 193   | 2.20| 0.99–4.91 | .054| 1.81| 0.83–3.91 | .135| 1.98| 0.98–3.98 | .056|
| E. coli single infection       | 121   | 2.21| 0.69–7.10 | .183| 1.44| 0.47–4.51 | .522| 1.76| 0.62–4.91 | .282|
| Klebsiella infection           | 109   | 0.51| 0.15–1.65 | .260| 0.26| 0.08–0.91 | .035| 0.37| 0.13–1.08 | .069|
| Klebsiella single infection    | 64    | 0.37| 0.07–2.12 | .262| 0.23| 0.04–1.23 | .085| 0.28| 0.06–1.27 | .099|

*a*All the results are from multivariate logistic regression analyses which adjusted for effects of age, gender, presence of stunting (weight-for-age z-score < -2), presence of wasting (length-for-age z-score < -2), wealth index score, hand washing score, baseline plasma zinc level, baseline plasma copper level, and baseline hemoglobin level.

*b*Children receiving zinc alone or zinc and copper compared to those receiving placebo.

*c*OR: odds ratio; CI: confidence interval; N: number of subjects with complete covariate information; Zn: zinc; Zn + Cu: zinc and copper; Pl: placebo.

*d*There were only 28 children with Rotavirus isolates who had RBCs in stool. Due to this small number, analyses separately for the Rotavirus group have not been carried out.
Two other trials [20, 21]—both from India—had found that to benefit from zinc supplementation. Including this study, a randomized controlled trial [18] that has conducted a subgroup analysis based on the presence or absence of rotavirus in diarrhea concurs with our finding that the beneficial effect of zinc could not be observed in rotavirus-infected children. Diarrhea in rotavirus infection may be caused by several mechanisms, namely, enterocyte destruction, villus ischaemia, activation of enteric nervous system by release of vasoactive agents, and intestinal secretion by intracellular and extracellular action of nonstructural protein (NSP4) which stimulates Ca++ dependant cell permeability [19]. The action of zinc supplementation on these mechanisms is currently unknown. Our findings suggest that zinc supplementation has a more probable cause of diarrhea. Our findings along with those of Surjawidjaja et al. [8], Dutta et al. [20], and Sachdev et al. [21] also raise an interesting possibility that an optimum dose of zinc may be beneficial against most of the causative organisms and that a possible future direction to take would be to design studies focused on the dose-response to zinc supplementation. It should be remembered however that in vitro, ex vivo, or in vivo benefit of zinc supplementation in rotavirus diarrhea is currently unstudied and unknown.

Table 2: Influence of zinc supplementation on the outcome of high stool volume in different subgroups based on the isolated organisms.

| Subgroup                             | N       | Zn versus Pl 95% CI | P     | Zn + Cu versus Pl 95% CI | P     | Any zinc\textsuperscript{a} versus Pl 95% CI | P     |
|--------------------------------------|---------|---------------------|-------|--------------------------|-------|-----------------------------|-------|
| Overall                              | 798     | 0.88 (0.62–1.26)    | .496  | 0.88 (0.62–1.25)         | .472  | 0.88 (0.65–1.20)            | .418  |
| E. coli infection                    | 449     | 1.17 (0.72–1.89)    | .519  | 1.41 (0.87–2.26)         | .159  | 1.29 (0.85–1.95)            | .236  |
| E. coli single infection             | 273     | 0.97 (0.52–1.81)    | .922  | 1.20 (0.65–2.22)         | .560  | 1.08 (0.63–1.86)            | .776  |
| Klebsiella infection                 | 275     | 0.67 (0.36–1.24)    | .204  | 0.55 (0.30–1.03)         | .062  | 0.61 (0.36–1.04)            | .071  |
| Klebsiella single infection          | 170     | 0.70 (0.31–1.58)    | .395  | 0.48 (0.21–1.08)         | .077  | 0.58 (0.29–1.18)            | .131  |
| Rotavirus infection                  | 169     | 1.44 (0.62–3.39)    | .398  | 2.31 (0.99–5.39)         | .052  | 1.85 (0.89–3.84)            | .097  |
| E. coli + rotavirus mixed infection  | 100     | 1.19 (0.35–4.00)    | .781  | 2.44 (0.72–8.27)         | .151  | 1.74 (0.59–5.09)            | .313  |
| Klebsiella + rotavirus mixed infection| 53      | 0.92 (0.14–6.08)    | .930  | 2.09 (0.37–11.9)         | .408  | 1.51 (0.32–7.07)            | .601  |

\textsuperscript{a}All the results are from multivariate logistic regression analyses which adjusted for effects of age, gender, presence of stunting (weight-for-age z-score < −2), prevalence of wasting (length-for-age z-score < −2), wealth index score, hand washing score, baseline plasma zinc level, baseline plasma copper level, and baseline hemoglobin level.

\textsuperscript{b}Children receiving zinc alone or zinc and copper compared to those receiving placebo.

\textsuperscript{c}OR: odds ratio; CI: confidence interval; N: number of subjects with complete covariate information; Zn: zinc, Zn + Cu: zinc and copper; Pl: placebo.

duration of acute diarrhea. Rotavirus is known to be the commonest causative organism in breast fed young infants, and although the authors acknowledge that it could be the reason for failure of zinc effect, it was not assessed in any of these studies [16, 17]. The only other randomized controlled trial [18] that has conducted a subgroup analysis based on the presence or absence of rotavirus in diarrhea concurs with our finding that the beneficial effect of zinc could not be observed in rotavirus-infected children. Diarrhea in rotavirus infection may be caused by several mechanisms, namely, enterocyte destruction, villus ischaemia, activation of enteric nervous system by release of vasoactive agents, and intestinal secretion by intracellular and extracellular action of nonstructural protein (NSP4) which stimulates Ca++ dependant cell permeability [19]. The action of zinc supplementation on these mechanisms is currently unknown. Our findings suggest that zinc supplementation has a more probable cause of diarrhea. Our findings along with those of Surjawidjaja et al. [8], Dutta et al. [20], and Sachdev et al. [21] also raise an interesting possibility that an optimum dose of zinc may be beneficial against most of the causative organisms and that a possible future direction to take would be to design studies focused on the dose-response to zinc supplementation. It should be remembered however that in vitro, ex vivo, or in vivo benefit of zinc supplementation in rotavirus diarrheas is currently unstudied and unknown.

Third, although mechanistically unclear, our results suggest that Klebsiella-associated diarrheas may be more responsive to zinc supplementation. Klebsiella enterotoxins cause reduced Na absorption and net Cl− secretion in rabbit ileum by increasing cGMP. Although the addition of zinc does not affect cGMP-mediated ion secretion, zinc may still have a protective effect that is associated with its action on basal ion transport [7]. It is well recognized that the enteroaggregative patterns exhibited by Klebsiella spp and their heat-stable enterotoxins are structurally and functionally distinct from those of E. coli and chiefly induce diarrhea by chloride ion depletion [22, 23]. Since it has been shown that zinc can specifically inhibit the luminal secretion of the chloride ion [7, 24], it is likely that the Klebsiella-induced diarrhea is amenable to zinc supplementation. Considering the similarity in the mechanism of diarrhea causation it is conceivable that zinc supplementation may also be very useful in cholera—a premise that is strongly supported by in vitro and epidemiological observations [7, 25]. Finally, our results shown in Table 2 indicated that the significant interactions observed for the outcome of high stool volume may likely have been false and could have originated from the complex correlation structure among the covariates that better predicted the outcome. This reiterates the general view that zinc supplementation has a more significant effect on the diarrheal duration rather than on the volume of stool.

We believe that the limitations of this study must be recognized before a generalization of the results can be undertaken. First, we did not have the resources to serotype the organisms isolated in the stools. Consequently, it cannot...
be presumed that the isolated organisms would necessarily indicate a causal association with the diarrhea episode. Nevertheless, we could demonstrate a differential response in rotavirus infection and in the presence of different bacterial organisms in the gut regardless of the fact that some may not have been pathogenic. Second, this study only had statistical power in excess of 60% which can be considered as sufficient but not very high. Thus, replication and substantiation of our findings in larger and more statistically powerful studies is needed. Third, our findings are essentially epidemiological. Whether they readily translate to bedside management cannot be forecast in the absence of more direct mechanistic studies. This study suggests that more research is needed to understand the effect of zinc supplementation on acute diarrhea due to different causative pathogens. Therefore, we suggest that zinc efficacy trials should now include a more complete assessment of the causative organisms at baseline. Fourth, microbiological prescreening prior to zinc supplementation in resource-limited countries is unlikely to be feasible. Thus, future studies should address this important area of interventional feasibility.

5. Conclusion

There is now a growing recognition that the beneficial effects of zinc supplementation may not be universal [9, 26]. Although we did not have data on further typing of the isolates in this study, our findings suggest that the universal strategy of zinc supplementation regardless of the organism isolated in the stool may be an oversimplification. If our results are indeed pointing towards a true differential benefit of zinc supplementation by the causative organisms, then more care will be required in recommending an optimum dose of zinc that is most beneficial as it will depend on the differential LD50 values [6] and the relative prevalence of the microbes included in the causative spectrum of acute diarrhea.

References

[1] WHO/UNICEF, WHO/UNICEF Joint Statement: Clinical Management of Acute Diarrhoea, WHO/UNICEF, Geneva, Switzerland, 2004.

[2] Z. A. Bhutta, S. M. Bird, R. E. Black, et al., “Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials,” American Journal of Clinical Nutrition, vol. 72, no. 6, pp. 1516–1522, 2000.

[3] M. Lazzarini and L. Ronfani, “Oral zinc for treating diarrhoea in children,” Cochrane Database of Systematic Reviews, no. 3, Article ID CD005436, 2008.

[4] M. Lukacik, R. L. Thomas, and J. V. Aranda, “A meta-analysis of the effects of oral zinc in the treatment of acute and persistent diarrhea,” Pediatrics, vol. 121, no. 2, pp. 326–336, 2008.

[5] B. Patro, D. Golicki, and H. Szaewskia, “Meta-analysis: zinc supplementation for acute gastroenteritis in children,” Alimentary Pharmacology and Therapeutics, vol. 28, no. 6, pp. 713–723, 2008.

[6] S. K. Roy, R. H. Behrens, R. Haider, et al., “Impact of zinc supplementation on intestinal permeability in Bangladeshi children with acute diarrhea and persistent diarrhea syndrome,” Journal of Pediatric Gastroenterology and Nutrition, vol. 15, no. 3, pp. 289–296, 1992.

[7] R. B. Canani, P. Cirillo, V. Buccigrossi, et al., “Zinc inhibits cholera toxin-induced, but not Escherichia coli heat-stable enterotoxin-induced, ion secretion in human enterocytes,” Journal of Infectious Diseases, vol. 191, no. 7, pp. 1072–1077, 2005.

[8] J. E. Surjawidjaja, A. Hidayat, and M. Lesmana, “Growth inhibition of enteric pathogens by zinc sulfate: an in vitro study,” Medical Principles and Practice, vol. 13, no. 5, pp. 286–289, 2004.

[9] A. Patel, M. J. Dibley, M. Mamtani, N. Badhoniya, and H. Kulkarni, “Zinc and copper supplementation in acute diarrhea in children: a double-blind randomized controlled trial,” BMC Medicine, vol. 7, article 22, 2009.

[10] Mackie and McCartney’s Practical Medical Microbiology, Churchill Livingstone, New York, NY, USA, 14th edition, 1996.

[11] S. W. Lagakos, “The challenge of subgroup analyses—reporting without distorting,” The New England Journal of Medicine, vol. 354, no. 16, pp. 1667–1669, 2006.

[12] R. Wang, S. W. Lagakos, J. H. Ware, D. J. Hunter, and J. M. Drezner, “Statistics in medicine—reporting of subgroup analyses in clinical trials,” The New England Journal of Medicine, vol. 357, no. 21, pp. 2189–2194, 2007.

[13] L. W. Hahn, M. D. Ritchie, and J. H. Moore, “Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions,” Bioinformatics, vol. 19, no. 3, pp. 376–382, 2003.

[14] M. Gonen, “Planning for subgroup analysis: a case study of treatment-marker interaction in metastatic colorectal cancer,” Controlled Clinical Trials, vol. 24, no. 4, pp. 355–363, 2003.

[15] H. J. Cordell, “Detecting gene-gene interactions that underlie human diseases,” Nature Reviews Genetics, vol. 10, no. 6, pp. 392–404, 2009.

[16] W. A. Brooks, M. Santosham, A. Naheed, et al., “Effect of weekly zinc supplements on incidence of pneumonia and diarrhea in children younger than 2 years in an urban low-income population in Bangladesh: randomised controlled trial,” Lancet, vol. 366, no. 9490, pp. 999–1004, 2005.

[17] C. L. Fischer Walker, Z. A. Bhutta, N. Bhandari, et al., “Zinc supplementation for the treatment of diarrhea in infants in Pakistan, India and Ethiopia,” Journal of Pediatric Gastroenterology and Nutrition, vol. 43, no. 3, pp. 357–363, 2006.

[18] S. Bhatnagar, R. Bahl, P. K. Sharma, G. T. Kumar, S. K. Saxena, and M. K. Bhan, “Zinc with oral rehydration therapy reduces stool output and duration of diarrhea in hospitalized children: a randomized controlled trial,” Journal of Pediatric Gastroenterology and Nutrition, vol. 38, no. 1, pp. 34–40, 2004.

[19] M. K. Estes, G. Kang, C. Q. −Y. Zeng, S. E. Crawford, and M. Garlet, “Pathogenesis of rotavirus gastroenteritis,” Novartis Foundation Symposium, vol. 238, pp. 82–100, 2001.

[20] P. Dutta, U. Mitra, A. Datta, et al., “Impact of zinc supplementation in malnourished children with acute water diarrhea,” Journal of Tropical Pediatrics, vol. 46, no. 5, pp. 259–263, 2000.

[21] H. P. Sachdev, N. K. Mittal, S. K. Mittal, and H. S. Yadav, “A controlled trial on utility of oral zinc supplementation in acute dehydrating diarrhea in infants,” Journal of Pediatric Gastroenterology and Nutrition, vol. 7, no. 6, pp. 877–881, 1988.
[22] A. Guarino, S. Guandalini, M. Alessio, et al., “Characteristics and mechanism of action of a heat-stable enterotoxin produced by Klebsiella pneumoniae from infants with secretory diarrhea,” Pediatric Research, vol. 25, no. 5, pp. 514–518, 1989.

[23] S. K. Niyogi, A. Pal, U. Mitra, and P. Dutta, “Enteraggregative Klebsiella pneumoniae in association with childhood diarrhoea,” Indian Journal of Medical Research, vol. 112, pp. 133–134, 2000.

[24] K. M. Hoque, V. M. Rajendra, and H. J. Binder, “Zinc inhibits cAMP-stimulated Cl secretion via basolateral K-channel blockade in rat ileum,” American Journal of Physiology, vol. 288, no. 5, pp. G956–G963, 2005.

[25] S. K. Roy, M. J. Hossain, W. Khatun, et al., “Zinc supplementation in children with cholera in Bangladesh: randomised controlled trial,” BMJ, vol. 336, no. 7638, pp. 266–268, 2008.

[26] M. Garenne, H. Becher, Y. Ye, B. Kouyate, and O. Müller, “Sex-specific responses to zinc supplementation in Nouna, Burkina Faso,” Journal of Pediatric Gastroenterology and Nutrition, vol. 44, no. 5, pp. 619–628, 2007.