Study Protocol and Statistical Analysis Plan

Research Protocol Title: Next generation ORS: Randomized controlled trial comparing ORS with calcium vs standard ORS in reducing severity with acute watery diarrhea of adults

NCT05814042

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Project Summary

[The summary, within a word limit of 300, should be stand alone and be fully understandable.]

Principal Investigator: Dr. Shafiqul Alam Sarker

Research Protocol Title: Next generation ORS: Randomized controlled trial comparing ORS with calcium vs standard ORS in reducing severity with acute watery diarrhea of adults

Proposed start date: 1st September, 2022 Estimated end date: 31st December, 2023

Background (brief):

a. **Burden:** Globally, there are nearly 1.7 billion cases of childhood diarrheal illness every year, and 1.9 million children younger than 5 years die from diarrhea each year mostly in lower and middle income countries. The deaths amount 18% of all deaths and is the second leading cause of death (after pneumonia) in this age group. Diarrheal disease is leading cause of malnutrition, growth failure and long term cognitive deficit. The current recommendation of diarrhea management is to ensure proper hydration through oral rehydration solution (ORS), continuedfeeding and zinc therapy. Although rehydration therapy can replace losses of intestinal fluid and ions (Na+, K+, Cl- and $HCO3^-$), it reduces neither the severity nor duration of diarrhea, and in some cases even worsens the diarrheal symptoms. Thus, over the past several decades there have been continuous efforts to search for ways to not only rehydrate patients, but to significantly decrease or even stop diarrheal losses.

b. **Knowledge gap:**Diarrhea causes monovalent (e.g., Na^+, K^+, Cl^- and HCO_3^-) and divalent (e.g., Zn^{2+}, Ca^{2+}) ion losses. Unlike the losses of monovalent ions which are large and are therefore replaced through rehydration therapy, the losses of divalent ions are relatively small in osmoles and are often overlooked during diarrheal treatment. Relative to loss of Zn^{2+} (which is 3.4 times normal loss), the loss of Ca^{2+} to diarrheal stool is far more severe, at about 20.5 times normal loss. Studies now suggest that despite divalent ions contributing relatively few osmoles in the stool, their effects are large due to the presence of divalent ion-sensing receptors (e.g., Zn^{2+}-sensing receptor, ZnSR; Ca^{2+}-sensing receptor, CaSR) and their amplifying effects in the gut. As a result, losses of these divalent ions without replacement may affect the magnitude of, or recovery from acute diarrheal illnesses. Without replacement of Zn^{2+} and restoration of ZnSR anti-diarrheal function, diarrhea is more severe and protracted; adding back Zn^{2+} and correcting ZnSR defect reduce the severity and duration of diarrhea.

c. **Relevance:** This application seeks to improve diarrhea rehydration therapy by testing a novel antidiarrheal concept: reduce diarrhea by activation of intestinal CaSR through replacement of lost Ca^{2+} during diarrhea. This idea is **creative** because it has not previously been described and tested. This proposed study represents the first of such efforts.

Hypothesis (if any): We hypothesize that prompt replacement of lost Ca^{2+} and Zn^{2+} will significantly reduce the severity and shorten the duration of diarrheal symptom

Objectives: We have two objectives

1. To examine if ORS with Ca^{2+} is more efficacious than standard ORS in reducing stool output and diarrhea duration in severely dehydrated adults with cholera treated initially with IV fluid
2. To examine if ORS with Ca^{2+} is more efficacious than standard ORS in reducing stool output and diarrhea duration in some-dehydrated adults with acute watery diarrhea.

Methods: We will conduct randomized, doubled-blinded, placebo-controlled clinical trial (RCT) at the Dhaka Hospital of International Centre for Diarrheal Disease Research, Bangladesh (icddr,b). Over a period of 24 months, we plan to recruit 60 severely dehydrated adult patients infected with *V. cholerae* (Rapid diagnostic test (RDT+), (n=30/group x 2 groups) (**Aim 1**) and 336 some dehydrated adults with non-bloody acute diarrhea (n=168/group x 2 groups) (**Aim 2**). The Participants recruited will be randomised to receive new ORS with calcium (intervention) or standard WHO ORS (control) in hospital for 72 hours or up to the time when the patients fulfil the criteria of cessation of diarrhea, whichever is shorter.

Outcome measures/variables: Quantitative diarrhoea parameters (stool output, duration of diarrhoea, stool frequency, percentage of patients who vomit, percentage of patients who require unscheduled intravenous therapy, and intake of ORS.

Description of the Research Project

Hypothesis to be tested:

In a hypothesis testing research proposal, briefly mention the hypothesis to be tested and provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Does this research proposal involve testing of hypothesis: ☐ No ☒ Yes (describe below)

We hypothesize that prompt replacement of lost Ca^{2+} will significantly reduce the severity and shorten the duration of diarrheal symptoms.

Specific Objectives:

Describe the specific objectives of the proposed study. State the specific parameters, gender aspects, biological functions, rates, and processes that will be assessed by specific methods.

1. To examine if ORS with Ca^{2+} is more efficacious than standard ORS in reducing stool output and diarrhea duration in severely dehydrated adults with cholera.

2. To examine whether ORS with Ca^{2+} is more efficacious than standard ORS in reducing stool output and diarrhea duration in some dehydrated adults with non-cholera non-bloody acute diarrhea.

Background of the Project including Preliminary Observations:

Provide scientific validity of the hypothesis based on background information of the proposed study and discuss previous works on the research topic, including information on sex, gender and diversity (ethnicity, SES) by citing specific references. Critically analyze available knowledge and discuss the questions and gaps in the knowledge that need to be filled to achieve the proposed aims. If there is no sufficient information on the subject, indicate the need to develop new knowledge.

Treatment of infectious diarrhea remains a global challenge[1,2]. This is particularly so in infants and young children whose diarrhea is often severe and protracted. Without proper treatment, diarrhea can cause significant mortality and morbidity. According to UNICEF/WHO[3], in 2008, diarrhea was the second leading cause of death in children under the age of 5 globally. Nearly one in every five child deaths - around 1.5 million a year - was due to diarrhea, which killed more children than AIDS, malaria, and measles combined. The mortality and morbidity of diarrhea remains today. In 2015, there were 2.39 billion estimated cases of diarrhea. Of these cases, approximately 957.5 million occurred in children under age 5[4]. According to a recent article in NEJM[5], diarrheal diseases remain the third leading cause of death in children under age 5 in Africa. In the United States from 2001-2006, the cost of hospitalizations, emergency room visits and outpatient visits for diarrhea in pediatric patients was approximately $10 billion per year, not including indirect costs for the family. Notably, 77% of children visited an outpatient clinic for diarrhea by the age of five[6]. Importantly, most of the morbidity and mortality of diarrhea is not due to infection but dehydration and electrolyte imbalance. How to rehydrate diarrheal patients has become critical.

Children with infectious diarrhea who become dehydrated are generally treated with oral rehydration solution (ORS) (e.g., Pedialyte) or intravenous rehydration therapy. Although currently recommended rehydration therapy can replace losses of intestinal fluid and ions (Na^+, K^+, Cl^- and HCO_3^-), it reduces neither the severity nor duration of diarrhea[7], and in some cases even worsens the diarrheal symptoms. Consequently, ORS is currently used in only one third of cases[3,8]. Thus, over the past several decades there have been continuous efforts to search for ways to not only rehydrate patients, but to significantly reduce or even stop diarrheal losses[9-11].

Importantly, diarrhea causes not only monovalent but also divalent ion (e.g., Ca^{2+} and Zn^{2+}) losses, leading to a negative metabolic balance for these cations[12,13]. However, while losses of monovalent ions are routinely replaced through ORS, losses of divalent ions (e.g., Ca^{2+}) are often overlooked in diarrheal treatment. A deeper analysis of earlier metabolic balance data has revealed that the most dramatic ionic loss in diarrhea is actually not Na^+ (which is 5.7x normal), K^+ (which is 1.2x normal), Cl^- (3.5x), HCO_3^- (2.2x) or Zn^{2+} (3.4x). Instead, the largest loss is Ca^{2+}, at about 20.5 times normal losses[14] (**Table 1**).

Table 1: Total fluid, electrolyte and mineral losses in normal infants and those with diarrhea and their consequences

Loss	Normal	With Diarrhea	Fold increase	Consequences	
Total *	84	171	2.0		
H_2O *	81	162	2.0	Hypovolemia	↑ Mortality
Na^+ **	2.15	12.35	5.7	Hypovolemia	
Cl^- **	2.69	9.36	3.5	Hypovolemia	
K^+ **	4.15	4.92	1.2	Hypokalemia	
HCO_3^- **	3.61	7.91	2.2	Metabolic acidosis	
Ca^{2+} ***	0.32	6.54	20.5	Ca^{2+} deficit → ↓CaSR	↑ Morbidity
Zn^{2+} ***	0.00144	0.00486	3.4	Zn^{2+} deficit → ↓ZnSR	

*gram/kg/day, **mmole/kg/day, ***mEq/kg/day

Given the presence of divalent cation-sensing receptors (e.g., Ca^{2+}-sensing receptor, CaSR[15,16]) and their antidiarrheal effects in the intestine shown in recent studies[17-26], a question arises of whether divalent cations lost in diarrhea should also be promptly replaced in diarrhea treatment. Without replacement of Zn^{2+} and restoration of Zn^{2+}-sensing receptor (ZnSR) anti-diarrheal function,
diarrhea is more severe and/or more protracted[3]; adding back Zn^{2+} and correcting the intestinal ZnSR defect[27] reduce diarrhea[3]. This is well documented. As a result, Zn^{2+} replacement during acute diarrhea has been adopted as policy in all countries[3]. However, despite the recent advances in Ca^{2+}/CaSR research, limited information is available on the role and function of Ca^{2+}/CaSR in diarrhea.

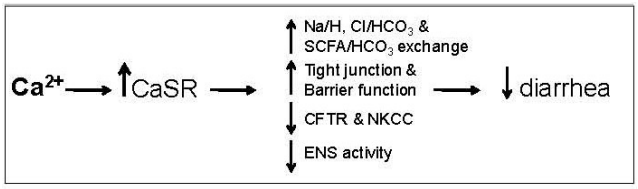


Fig 1. Hypothesized role of Ca^{2+} replacement

DR. Cheng's laboratory at the University of Florida was the first to report the presence of a potent CaSR-based antidiarrheal mechanism in the gastrointestinal tract[15,17,19]. After having demonstrated its 'inclusive' antidiarrheal action (i.e., anti-secretory, pro-absorptive, anti-inflammatory and anti-motility) in laboratory animals[17-26] (**Fig. 1**), we recently performed mechanistic studies to understand the changes of CaSR in diarrhea and the response to Ca^{2+} replacement using cultured human intestinal Caco-2 epithelium (**Fig. 2**).

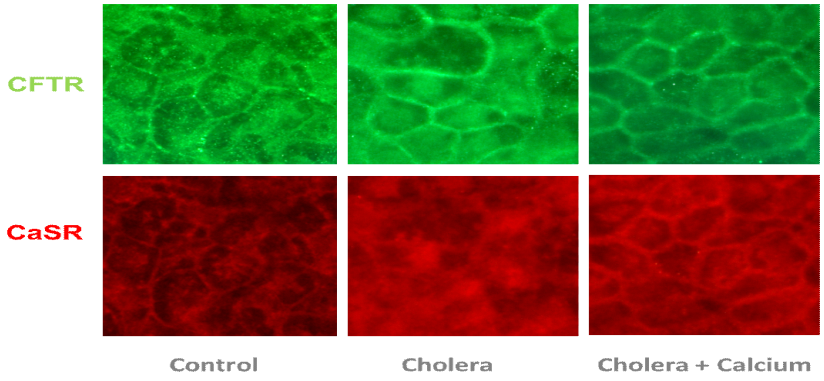


Fig 2: CFTR (green) and CaSR (red) immunofluorescence in Caco-2 cells. While 90 min treatment with cholera toxin (100 nM) induces CFTR trafficking to cell membrane, it moves CaSR away from cell membrane causing internalization. Increasing medium Ca^{2+} from 1.2 to 5 mM reverses these processes.

We found that without proper concentrations of Ca^{2+} in the extracellular environment, the enterocyte CaSR becomes internalized in response to pathogen exposure and loses its antidiarrheal function; by contrast, with proper Ca^{2+} concentrations, the CaSR returns to the plasma membrane and regains its function (**Fig. 2**). Similarly, upon exposure to pathogens animals develop more severe and protracted diarrhea when ORS contains no Ca^{2+} than when ORS contains Ca^{2+} (see **Fig. 3**).

Fig 3: Diarrhea response to Ca^{2+} replacement in C57BL/6 mice. Cholera toxin (10 µg/mouse) was gavaged into stomach to induce diarrhea. 90 min later, mice were allowed to drink ORS (Pedialyte)$\pm Ca^{2+}$, as indicated. N= 7-10. * P<0.05 vs. ORS.

This suggests that replacing lost Ca^{2+} in diarrhea could be beneficial. To further test this, we observed six diarrheic infants and children and compared their daily stool output before and after Ca^{2+} replacement[14,22] (**Fig. 4**). We found that shortly after Ca^{2+} replacement, diarrheal symptoms stopped[14,22] (**Fig. 4**).

Fig 4: Diarrhea response to Ca^{2+} replacement in a 10 year old boy s/p renal transplantation with persistent norovirus and cryptosporidium infection. Adding back of Ca^{2+} "halts" diarrhea promptly and dramatically.

The next step is to conduct clinical trials to prove the concept. Based on our case studies, we expect the effect of Ca^{2+} replacement to be dramatic. Therefore, we would suggest a relatively small RCT to start, as this would be less expensive to conduct. The purpose of this small RCT study is to obtain pilot data to further quantify the size of effect of Ca^{2+} replacement on clinical outcomes (stool output and diarrhea duration) before a more powerful and more expensive RCT is performed. We hypothesize that prompt replacement of lost Ca^{2+} will significantly reduce the severity and shorten the duration of diarrheal symptoms. University of Florida has its tradition for translational research and is home to Gatorade – a drink for athletes that provides complete replacement of all electrolytes lost in sweat. The ultimate goal of this research is to generate a new Gatorade-like inexpensive ORS for people with diarrhea.

Innovation

This application seeks to improve diarrhea rehydration therapy by testing a novel antidiarrheal concept: activation of intestinal CaSR through replacement of lost Ca^{2+} during diarrhea. This idea is creative because it has not previously been described and tested. However, it has formidable therapeutic potential because, in contrast to current therapies that target single absorptive (e.g. "oral rehydration therapy") or secretory component (e.g. Cl^- channel inhibitors Crofelemer), the Ca^{2+}/CaSR-based antidiarrheal therapy attacks multiple diarrhea-causing pathways. The latter include, but not limited to:

- Reversal of intestinal secretion evoked by cholera toxin, E. coli enterotoxin STa and other secretagogues (NSP4).
- Inhibition of secretagogue-induced intestinal bicarbonate loss.
- Inhibition of intestinal electrolyte secretion evoked by exaggerated enteric nerve activity.
- Enhancement of absorption of salt and solute (e.g., short-chain fatty acids).
- Enhancement of tight junction and barrier function, and reduction of intestinal permeability, and suppression of gut inflammation.
- Enhancement of intestinal microbial balance.
- Correction of hypocalcemia and prevention of tetany.

Thus, the therapy to be developed could potentially open up a new prevention/treatment mechanism for diarrhea overall not just childhood diarrhea. Moreover, because it exerts profound effects on diarrheal symptoms and is not just simply supportive as is the current ORS, it is expected to increase patient compliance rates and decrease not only the mortality and but also morbidity of diarrheal diseases. Finally, this innovation is appealing because it uses the naturally occurring, child-friendly mineral Ca^{2+} to treat diarrhea. Thus, if the results from the present study are promising, this simple affordable therapy will have clinical utility for billions of infants, children and adults worldwide.

Rationale:

According to the current WHO classification, dehydration is classified as "no", "some" and "severe". In patient studies, we propose to study both patients with severe dehydration (**Aim 1**), the group of diarrhea that has caused most of the diarrhea-associated deaths, and patients with some dehydration (**Aim 2**). We propose to study diarrhea with some dehydration rather than no dehydration because the former, normally requires medical treatment whereas the latter does not. We do not include diarrheal patients with no dehydration in this initial proposal also because of R21 tight budget. The reason why we propose to study both severe and some dehydration is for a maximal fund use consideration. Lastly, antibiotics used in Aim 1 will greatly limit the effect size. Including some dehydration patients that do not receive antibiotics (Aim 2) will provide a strong contingency if Aim 1 fails. The primary purpose of this proposal is to prove the concept and to determine the size of effect in order to guide the design of future RCTs. In these pilot studies, we do not plan to do etiology testing. In some respects, the etiology is not relevant. The primary interest is the diarrhea severity regardless of etiology. While it is of some interest to know whether any specific pathogen is an important variable effecting outcome, the present study is not powered for this. Nonetheless, we will do a stratified analysis by the type of infection as a secondary analysis, and correlate patients' responses with stool pathogens detected at entry and assess if a better response is associated with any type of diarrhea in terms of etiology (bacterial *vs.* viral *vs.* parasitic). The two ORS solutions are identical except that intervention ORS contains 10 mM Ca^{2+} whereas control ORS does not. Both solutions appear and taste similarly. The concentration of 10 mM Ca^{2+} is proposed because at this concentration extracellular Ca^{2+} had shown anti-secretory/anti-diarrheal effect, both *in vitro*, in perfused intestinal crypts[15,17,19] and *in vivo*, in live animals (**Fig. 3**). This intervention ORS will provide 1500-2000 mg elemental Ca^{2+}/day, which is equivalent to 1.5-2x RDA (recommended daily allowance) or 1.5-2 mEq/kg/day for a 50-kg adult assuming that he/she is rehydrated at a rate of 75ml/kg (some dehydration) or 100ml/kg (severe dehydration) per WHO guideline. This dose of Ca^{2+} also has anti-diarrheal action in patients with bacterial, viral and/or parasitic infections[14,22] (**Fig. 4**). We propose to replace Ca^{2+} for 72 hours for the consideration of the relative short duration of acute infectious diarrhea[28,29].

Anticipated results/pitfalls/alternatives:

Based on our preliminary studies, we anticipate that ORS with Ca2+ will reduce the volume of stool by approximately 30% compared to the control and shorten the mean duration of diarrhea by at least 1 day, in some or severely dehydrated patients. This is considered clinically meaningful as replacement of Zn2+, recommended by the WHO, is associated with a 25% reduction in the duration of acute diarrhea3. Resistant starch, which is also recommended by the WHO9, reduces the duration of diarrhea by 3-6 hours31. Another promising treatment adjunct that has recently received considerable attention is the use of probiotics. However, recent studies show that the use of probiotics shortens diarrhea in children only by 15 hours32 or has no effect at all33. On the other hand, Ca2+ is an ancient anti-diarrheal agent, and has been exploited by marine species to survive hyperosmotic (~1000 mOsm), high Ca2+ (~10 mM) seawater. Ca2+, through the CaSR, helps fish preserve water, avoid dehydration, and prevent occurrence of hypercalcemia34,35. Such antidiarrheal effects are preserved in mammals. In rodents, increased dietary Ca2+ intake was found
to reduce diarrhea induced by various etiologies, including infectious (e.g., *Salmonella* enterocolitis36-39), chemically induced (e.g., DSS colitis40), and immune-mediated (e.g., colitis in HLA-B27 transgenic rats41); by contrast, lowering dietary Ca2+ intake in mice was found to increase diarrhea42. The anti-diarrheal effects of Ca2+ are also well-known in humans. For example, people subsisting on high-Ca2+ diets (such as dairy and cheese) or taking high Ca2+ supplements are often constipated, as are patients with hypercalcemia. Ca2+-enriched tempeh and tofu (~3% of soybean dry weight) have long been utilized by the inhabitants of Indonesia and many other Oriental cultures to protect against diarrhea43-46. Along with Bovee-Oudenhoven, *et al*, we have recently demonstrated that supplementation of Ca2+ reduced diarrhea caused by enterotoxigenic *Escherichia coli*, *Norovirus*, and *Cryptosporidium,* with the mean duration reduced by at least 24 hours with no adverse effect other than mild reversible constipation22,47,48. Similar efficacy and safety were observed more recently by Sayyahfar et al 49, who showed that oral calcium gluconate replacement at a dose as low as 0.3 mEq/kg/day can shorten diarrhea duration by ~20%, although similar efficacy was not observed by Agustina, et al50. Agustina, et al examined effects of milk calcium phosphate intake on diarrhea occurrence and duration in healthy children aged 1-6 years and found no effect. The reason for this discrepancy is unclear; it may be related to the accuracy of data collected and the definition of diarrhea used. For example, the 'positive' study by Sayyahfar was performed in a hospital setting where diarrhea was defined using the WHO definition as ≥3 loose stools per day, whereas the 'negative' study by Agustina was performed in a

community setting, in which diarrhea was based largely upon mothers' report and was loosely defined using a broader definition of diarrhea as ≥2 loose stools per day. With this latter definition, some nonspecific noninfectious milder physiologic and osmotic forms of diarrhea that are commonly seen in the studied age group cannot be completely excluded. Indeed, in another study in which a more accurate stool measurement and a more definite infectious diarrhea were applied, the same calcium agent was found to reduce both stool output and diarrhea duration47. Our proposed clinical trials will be performed in the best equipped icddr,b, using the best validated WHO definition of diarrhea, and the most accurate method to quantitate stool output. We anticipate we are able to detect the anticipated impact of replacement of Ca2+ loss on stool output and diarrhea duration. Finally, we will do a stratified analysis by the type of infection as a secondary analysis. We will correlate patients' responses with stool pathogen results obtained at entry and assess if a better response is associated with any type of diarrhea in terms of etiology (bacterial vs. viral vs. parasitic). However, based on current available data, this proposed therapy should work equally well in all types of infections as reported in our preliminary studies22,47,48,49 (**Fig. 4**), because it targets, not pathogens, but common downstream pathways leading to diarrhea (see the above Innovation section).
As stated above, the dose of Ca2+ used is equivalent to 1.5-2x RDA, and we anticipate that the dose of Ca2+ is well tolerable and poses no health risk to diarrheal patients as demonstrated previously22,47,48 and in a recent study49. In fact, without concurrent administration of vitamin D, the bulk (~85%) of ingested Ca2+ remains unabsorbed in the lumen of the gut47,51. There, it produces local anti-diarrheal effects without causing unwanted systemic outcomes47,51. In infants, taking Ca2+ intake as high as 1750 mg/day (~6.7x RDA) was not found any health risk52.

Research Design and Methods

Describe the research design and methods and procedures to be used in achieving the specific aims of the research project. If applicable, mention the type of personal protective equipment (PPE), use of aerosol confinement, and the need for the use BSL2 or BSL3 laboratory for different part of the intended research in the methods.. Define the study population with inclusion and exclusion criteria, the sampling design, list the important outcome and exposure variables, describe the data collection methods/tools, and include any follow-up plans if applicable. Justify the scientific validity of the methodological approach (biomedical, social, gender, or environmental).

Also, discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them.

We will conduct a randomized, doubled-blinded, placebo-controlled clinical trial (RCT) at the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b). icddr,b is referred to as by many locals "cholera hospital" because it treats the world largest population of patients with diarrhea, particularly cholera. For the last 2 years, 103,243-137,519 patients annually were treated in the Dhaka Hospital. These numbers were higher before COVID-19 pandemic. 12.2% were cholera and 44% were adults age 18-60 years.

This will be a phase II-III randomized, double-blinded, placebo-controlled trial (RCT). Over a period of 18-24 months, we will recruit 60 severely dehydrated adult patients treated for cholera (n=30/group x 2 groups) (**Aim 1**) and 336 some dehydrated adults with non-bloody acute diarrhea (n=168/group x 2 groups) (**Aim 2**) to receive new ORS (intervention) or standard WHO ORS (control) in hospital for 72 hours or up to the time when the patients fulfill the criteria of cessation of diarrhea, whichever is shorter. Clinical outcomes (stool output and diarrhea duration) will be assessed and compared.

Specific Aim 1:
To examine if ORS with Ca^{2+} is more efficacious than standard ORS in reducing stool output and diarrhea duration in severely dehydrated adults with cholera.

Inclusion criteria:

i. Adult male and female of any race or ethnicity
ii. Age between 18 and 60 years
iii. History of acute watery diarrhea (defined as the passage of ≥3 loose or watery stools in the past 24 hours) for less than 24 h before admission, with signs of severe dehydration (WHO criteria)
iv. Stool positive for *Vibrio cholerae*, by rapid diagnostic test (Sayeed MA et al 2018) and successful rehydration with intravenous fluids within the first 6 h after admission (cholera group)

v. Written informed consent. Participants or legal guardians (when patients are unable to provide consent themselves due to altered consciousness) will be requested for written informed consent for participation in the study.

Exclusion criteria:

i. Pregnancy as determined by history of last menstrual period,
ii. Bloody diarrhea,
iii. History of taking antibiotic or ant diarrheal mediation
iv. Signs of systemic infection that needed intravenous antibiotics, or inability to rehydrate with intravenous fluid therapy in the first 6 h after admission.
v. Not willing to participate in the study

Prior treatments before randomization:

Initially a health worker will screen the participants from the patients attending to Short Stay Unit (SSU) of icddr,b,. A demographic information and medical history will be obtained by a nurse. A clinical examination will be performed. Vital signs will be recorded. Dehydration status will be assessed using Dhaka method. If the participant is severely dehydrated, an intravenous fluids containing polyelectrolytes (Na+ 133 mM, K+ 13 mM, Cl- 98 mM, and acetate equivalent to 48 mM bicarbonate) will be initiated immediately at a rate of 100 ml/kg over 4-6 h, in addition to replacement of ongoing stool losses. ORS is also allowed as soon as the patient is able to drink. A stool samples will be collected and tested for cholera by a rapid diagnostic test (RDT) Cholkit. (Sayeed MA et al 2018). If the rapid test for cholera is positive, the participant will be enrolled in study after taking informed consent (Specific aim 1). All patients will receive 1 gram of azithromycin and are initially rehydrated with intravenous

Randomization: For this particular aim, 60 total participants will be enrolled and will be randomized to 2 arms: Arm 1: 30 ORS and arm 2:30 ORS+Ca^{2+}. A permuted block randomization with variable block size will be used. Both types of ORSs will be packaged in an aluminum foil in the same manner)

Procedure:

Maintenance of oral therapy will begin immediately after completion of rehydration and continued until cessation of diarrhea or 72 h after randomization, whichever comes first. During maintenance therapy, patients will consume the assigned ORS according to need, with a minimum volume equal to replacement of continued losses of watery or loose stool and emesis. All patients will be offered food (bread, banana) immediately after rehydration and standard meals (rice, fish or meat, vegetables, and lentils as practice in the Dhaka Hospital of icddr,b) three times daily thereafter. Plain water will be offered as desired, usually during the meals. If signs of dehydration appear during the maintenance phase, the calculated deficit will be replaced with standard ORS or the investigational ORS over 6 h with an interim assessment at 3 h. Intravenous fluids will be administered only if signs of dehydration persist at the end of 6 h. If severe dehydration appears despite appropriate oral therapy, intravenous fluid therapy will be immediately started. Fluids will be infused according to the methods of the initial rehydration phase. Patients with serum Na^+ concentration below 125 mmol/L and with clinical signs of hyponatremia (lethargy, poor response or no response to vocal commands), and patients with serum Na^+ concentration below 120 mmol/L regardless of clinical signs of hyponatremia will be treated with 12 mL/kg bodyweight hypertonic (3% NaCl) saline solution. Patients will be removed from the study if they withdraw their informed consent, are transferred to Longer Stay Unit of the hospital for treatment of a severe underlying illness, continue with diarrhea for more than 72 h, or consume more than 30 L of the assigned ORS. Data collected on patients withdrawn from the study will be included in the analysis up to the time of withdrawal.

Specific Aim 2:
To examine if ORS with Ca^{2+} is more efficacious than standard ORS in reducing stool output and diarrhea duration in some-dehydrated adults with acute watery diarrhea.

Inclusion criteria:

i. Adult male and female of any race or ethnicity
ii. Age between 18 and 60 years
iii. History of acute watery diarrhea (defined as the passage of ≥3 loose or watery stools in the past 24 hours) for less than 24 h before admission, with signs of SOME dehydration (WHO criteria)
iv. Stool negative for *Vibrio cholerae*, by rapid diagnostic test (Sayeed MA et al 2018)
v. Written informed consent. Participants or legal guardians (when patients are unable to provide consent themselves due to altered consciousness) will be requested for written informed consent for participation in the study

Exclusion criteria: Same as in Aim 1.
i. Pregnancy as determined by history of last menstrual period,
ii. Bloody diarrhea,
iii. History of taking antibiotic or ant diarrheal mediation
iv. Signs of systemic infection that needed intravenous antibiotics, or inability to rehydrate with intravenous fluid therapy in the first 4- 6 h after admission.
v. Not willing to participate in the study

Prior treatments before randomization:

As outlined above (Aim 1) CHRW will screen the participants for the study. Dehydration status will be assessed using Dhaka method. If the participant has some sign of dehydration, he/she will be provisionally selected and a stool sample will have tested for cholera by RDT. The participant will be rehydrated initially with standard WHO ORS at a rate of 75 ml/kg over 4-6 hours in addition to replacement of ongoing stool losses. If the stool is negative, for cholera by RDT, the participant will be will be randomized.

Randomization:
Arm 1: 168 ORS and arm 2 168 ORS+ Ca^{2+}. A permuted block randomization with variable block size will be used. Assigned ORS is allowed to drink and will be continued until cessation of diarrhea or 72 h after randomization, whichever comes first

Procedures:
After initial rehydration and randomization, all cholera patients will receive antimicrobial therapy for cholera while all non-cholera patients will not. During maintenance therapy, patients will consume the assigned ORS according to standard of care WHO/icddr,b guidelines, with a minimum volume equal to replacement of continued losses of watery or loose stool and emesis. All patients will be offered food (bread, banana) immediately after rehydration and standard meals (rice, fish or meat, vegetables, and lentils as practice in the Dhaka Hospital of icddr,b) three times daily thereafter. Plain water will be offered as desired, usually during the meals. If signs of dehydration appear during the maintenance phase, the calculated deficit will be replaced with standard ORS or the investigational ORS over 6 h with an interim assessment at 3 h. Intravenous fluids will be administered only if signs of dehydration persist at the end of 6 h. If severe dehydration appears despite appropriate standard or interventional ORS, intravenous fluid therapy will be immediately started. Fluids will be infused according to the methods of the initial rehydration phase. Patients with serum sodium concentration below 125 mmol/L and with clinical signs of hyponatremia (lethargy, poor response or no response to vocal commands), and patients with serum sodium concentration below 120 mmol/L regardless of clinical signs of hyponatremia will be treated with 12 mL/kg bodyweight hypertonic (3% NaCl) saline solution. Patients will be removed from the study if they withdraw their informed consent, are transferred to Longer Stay Unit of the hospital for treatment of a severe underlying illness, continue with diarrhea for more than 72 h, or consume more than 30 L of the assigned ORS. Data collected on patients withdrawn from the study will be included in the analysis up to the time of withdrawal.

Patients will be put onto a cholera cot and stool will be collected in a bucket, and weight measured on an electronic scale (Sartorius, Germany) that has a precision of 1 gram. Urine will be separated in a urine collector and volume measured with a calibrated cylinder. The volume of vomit will be measured by collection in a pre-weighted bowl. Intake of ORS and free water will be measured by calibrated cylinder. Bodyweight in light clothing is measured on admission, at randomization, and every day thereafter until discharge, with an electronic

scale (Sartorius) that has a precision of 1 gram. Scales are calibrated daily with standard weights. Patient will be discharged when diarrhea ceased.

Cessation of diarrhea will be defined as the last watery or loose stool before passage of two consecutive soft or formed stools, or the beginning of 8 consecutive hours without any stool.

Duration of diarrhea is the time in hours from randomization until cessation of diarrhea.

Stool output is defined as the weight of stool in g per kg bodyweight after intravenous hydration and is to be expressed per 24 h or for the entire duration of diarrhea after randomization.

At day 7, a cell phone call will be made to all participants to know the status of their disease.

Clinical outcomes:

Stool output will serve as the **primary outcomes** and will be assessed and compared.

Secondary outcomes will include 1) Duration of diarrhea, defined as the time in hours from randomization until cessation of diarrhea, 2) stool frequency, 3) percentage of patients who vomit, 4) percentage of patients who require unscheduled intravenous therapy, and 5) intake of ORS. High calcium intake may occasionally cause local gastrointestinal irritation/vomiting[30].

Potential Risks:

These include 1) physical discomfort during baseline and follow up phlebotomies or bruising or infection at the site of blood draw; 2) psychological stress from interruption of daily routine, blood withdrawals or providing personal health information to investigators. None of these is deemed significant risks to subject health. Blood withdrawal will be performed by skilled, research-oriented nursing staff under antiseptic conditions. Blood withdrawal sites will be carefully inspected for signs of infiltration or inflammation throughout each procedure. To minimize psychological stress, subjects will be seated comfortably in a reclining chair during blood withdrawal. They will be able to return to their regular activities or hospital care during the study days and have free access to food and water allowed by his/her medical condition.

Researchers will take appropriate steps to protect any personal health information they collect. However, there is a slight risk that information about a subject could be revealed inappropriately or accidentally. Depending on the nature of the information, such a release could upset or embarrass that person, or possibly affect his/her insurability or employability. Calcium is a key active ingredient in the new ORS and is a basic nutrient. This new ORS is estimated to provide patients within the recommended ~1-2x RDA doses and is therefore not anticipated detrimental. This is particularly true in diarrheic patients in whom the metabolic balance for Ca^{2+} is known to be negative due to its significant losses from diarrhea. To ensure the safety of our study subjects, however, we will still monitor serum and urine Ca^{2+}. If hypercalcemia or hypercalciuria occurs, such subjects will be called in and be monitored carefully until the problem resolves.

We describe herein the general strategy to be employed in responding to adverse events (AEs), serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs). Operative definitions for this trial that are acceptable to the FDA, Health Canada and EU countries are:

• **Adverse Event (AE):** Any untoward medical occurrence in a clinical trial subject administered an investigational product that does not necessarily have causal relationships with the treatment. This may include an unfavorable or unintended sign, symptom or disease temporarily associated with the use of the investigational drug, whether or not considered related to the drug product.

• **Serious Adverse Event (SAE):** Any AE that results in death, is life-threatening, requires hospitalization or prolongation of hospitalization or results in persistent or significant disability.

• **Suspected Unexpected Serious Adverse Reaction (SUSAR)**: Any SAE that is possibly related to the study treatment and is unexpected. These events are reported to the University of Florida within 24 hours. Mild, asymptomatic increases or decreases in serum electrolytes and specific or nonspecific gastrointestinal symptoms are potential AEs, not SAEs or SUSARs, because they are expected and not even considered serious, unless the symptom was debilitating or required hospitalization. Moreover, these are also known complications of the underlying disease. In contrast, an SUSAR might be a serious arrhythmia or seizure, the onset of which occurred proximate to administration of the study product; both would be unanticipated to occur in response to ORS.
Researchers will take appropriate steps to protect any personal health information they collect. Each staff member will be trained in how to protect PHI, which includes keeping records locked, discussions should be held in a private setting, using password protected devices, and not sharing data with non-study staff. However, there is a slight risk that information about a subject could be revealed inappropriately or accidentally. Depending on the nature of the information, such a release could upset or embarrass that person, or possibly affect his/her insurability or employability.

Adequacy of Protection against Risks

The study will be conducted in compliance with the procedures outlined in the protocol, the International Conference on Harmonization's Good Clinical Practice (ICH_GP), and in accordance with the ethical guidelines and local regulatory requirements for the trial. The protocol will be submitted to local Independent Institutional Review Board (IRB) comprising Research Review Committee (RRC) and Ethical Review Committee (ERC) for approval, as well as the University of Florida IRB.

a. *Informed Consent*

Written, informed consent will be obtained from all eligible participants prior enrollment into the study after explanation of the aims, procedures, risks, benefits, and rights for participation and refusal to the study, and assurances regarding confidentiality of personal and medical information. If any questions arise, study physician or investigator will answer those questions. Signature (or thumbprint, if illiterate) of each of the participants will be obtained before their enrollment in the study and prior to any study-related activity. A witness and study personnel who obtains the consent must sign and date in the informed consent form. The participants will be provided with a copy of the signed consent form for their retention. All consent forms will be collected and compiled in hard copy and will be stored in a safe and locked cabinet.

b. *Protections Against Risk*

As stated above, we anticipate a low risk of study. However, if a subject is harmed as a direct result of his/her participation in this study, the sponsor will pay for all reasonable and necessary medical expenses required to treat the injury, as long as:

1) The injury occurs during participation in the study;
2) The injury results directly from the study product or study-related procedures that the person would not have received as part of his/her routine medical care as determined by the DSMB (see below).

We will maintain the confidentiality that includes documentation, laboratory data, clinical and other related information. The privacy, anonymity and confidentiality of the information provided by participants will strictly be maintained. Information will be kept confidential and will not be used for any other purpose than the study. Maintenance of confidentiality of the data will be strictly practiced and restrictions on access to data forms will be enforced. Original source documents, data files, study records, and reports will be maintained by the Principal Investigator. Information derived from the participants will be used for research purposes only and would not be shared anywhere by the name of the participants

Protection of human subjects, AE and DSMB:

The dose of Ca^{2+} used is equivalent to 1.5-2x RDA, and we anticipate no health risk, although high Ca^{2+} intake may occasionally cause some local gastrointestinal adverse event (AE) such as vomiting[30]. Though the reported vomiting was mild and reversible[30], we will document and monitor this event and compare it. We will request IRB in Gainesville and Dhaka to constitute a DSMB to oversee data relating to safety. The initial responsibility of the DSMB will be to approve the initiation of this clinical trial. After this approval, the DSMB will review the data of primary and secondary endpoints, data for early termination of trial (stopping rules), or AE. The board will meet twice per calendar year, after the interim analysis, and or whenever there is an issue or concern. The DSMB will recommend changes in the study, including discontinuation, if significant trends in safety are identified.

Availability of Investigational Product (IP) and Investigational New Drug (IND)/Investigational Device Exemption (IDE) Status - Patient Study

Provide a summary describing the availability of study agents and support for the acquisition and administration of the study agent(s):

In this proposed clinical study, the investigational agent Next Generation ORS (oral rehydration solution) will be tested against the current standard control ORS in diarrheic patients with cholera (aim1) and non-cholera infections (aim2) in order to assess its safety and efficacy in treating diarrhea. Both study agents are available. These same investigational agents have been tested in animals in our Phase I STTR.

We have contacted the FDA - no IND/IDE approval is required for this study.

Potential Benefits of the Proposed Research to Research Participants and Others

Patients who enroll into the study will receive at least three days' hospital care of their disease free of charge. The benefits to others include advancing knowledge about Next Generation ORS and developing a novel therapy for diarrheal diseases. The risks associated with this research are limited to providing personal health information and minimal blood withdrawal and are therefore considered very small in relation to the anticipated benefits accrued by the participant and to the society.

Blinding and Code Breaking Procedure

Since the test and comparator ORS will identical in package and dose, this study will be randomized, double blind study. Study investigators along with the study staff involved in the clinical care, IP administration, safety evaluation and laboratory analyses will be blinded regarding the treatment assignment to the participants. The code will not be revealed until the end of the trial, data entry and data cleaning is completed. If the intervention assignment unblended, all collaborator or/and sponsor of the study will be notified.

Participant withdrawal from the study

The participants are allowed to discontinue or withdraw from the study anytime during the study period. There will be no replacement for such withdrawals. The investigator(s) might also withdraw the participants from the study, if in his/her clinical opinion, that is in the best interest of participant concerned or if it is considered to be non-compliant to the protocol. A participant might also be withdrawn due to protocol violation, death, or early termination of the study by the Sponsor.

Wherever possible, the tests and evaluations at termination visit will be carried out. The withdrawal will not be included in the analyses.

Materials

Health data and blood, stool and urine specimens, will be obtained. The local hospital clinical lab will proceed and analyze the blood, urine and stool specimens collected from patients by the trained research team. Interviews and examinations will be conducted by the trained research staff physicians/nurses at icddr,b at Dhaka.

Protected health information may be collected, used, and shared with others to determine if a subject can participate in the study, and then as part of his/her participation in the study. This information can be gathered from the subject or his/her past, current or future health records, from procedures such as physical examinations, x-rays, blood or urine tests or from other procedures or tests. Dr. Li, whose role is lead statistician, will supervise data management.

Sample Size Calculation and Outcome (Primary and Secondary) Variable(s)

Clearly mention your assumptions. List the power and precision desired. Describe the optimal conditions to attain the sample size. Justify the sample size that is deemed sufficient to achieve the specific aims.

In Aim 1, we plan to enroll 60 patients (30/group x 2 treatment groups). The sample size will yield an 80% statistical power to detect clinical outcome difference of the stool volume between intervention ORS and WHO ORS groups, with the use of the planned t-test at 0.05 level, assuming an effect size of 68 g/kg, which is ~30% reduction from control group (mean total stool output in control group=212 g/kg) and considering a 15% attrition rate (i.e., complete data for 52 patients).

In Aim 2, we plan to enroll 336 patients (168/group x 2 treatment groups). The sample size will yield an 80% statistical power to detect clinical outcome difference of the stool volume between intervention ORS and WHO ORS groups, with the use of the planned t-test at 0.05 level, assuming an effect size of 15 g/kg, which is 30% reduction from control group (mean total stool output in control group=50 g/kg) (S.A. Sarker, unpublished observation) and considering a 15% attrition rate (i.e., complete data for 286 patients).

Data Analysis

Describe plans for data analysis, including stratification by sex, gender and diversity. Indicate whether data will be analysed by the investigators themselves or by other professionals. Specify what statistical software packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to determine further course of the study.

Intent-to-treat analysis will be considered throughout the analysis stage. Standard summary statistics including mean and standard deviations (SD) for continuous variables and frequency tables for categorical variables will be provided by treatment groups for outcome measures. Appropriate transformations such as natural-log will be used as necessary on variables to comply with normality. Figures such as scatter plots will be constructed to visualize the data. The primary study outcome measure stool output will be compared between the two groups with a two-sample t-test or a nonparametric Wilcoxon rank sum test at two-sided significance level of 0.05. Regression models will be employed as well if potential confounders such as age, gender, type of infection, and severity of dehydration need to be adjusted in the analysis. Secondary study outcome measures will be analyzed in a similar fashion. For binary outcomes including “stool frequency”, “vomit” and “require unscheduled intravenous therapy”, 2 by 2 tables with chi-square tests will be conducted to compare the two groups. Logistic regression models will be used to compare the binary outcomes as well when potential confounders need to be adjusted. Missing data (if any) will be handled with multiple imputation assuming missing at random. If it is not missing at random, we will consider a sensitivity analysis to study the impact of missing data on the analysis results.

Data Safety Monitoring Plan (DSMP)

All clinical investigations (research protocols testing biomedical and/or behavioural intervention(s)) should include the Data and Safety Monitoring Plan (DSMP). The purpose of DSMP is to provide a framework for appropriate oversight and monitoring of the conduct of clinical trials to ensure the safety of participants and the validity and integrity of the data. It involves involvement of all investigators in periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of trial sites, and other factors that can affect study outcome.

In Dhaka, the local Ethical Review Committee (ERC) will be requested to constitute a local DSMB to oversee at interval the progress of the trial and safety of the investigational product in Dhaka. The DSMB will comprise of

3-5 members including biostatistician, medicine specialist, a pharmacologist, and a clinical researcher from icddr,b or other institute in Bangladesh. The initial responsibility of the DSMB will be to approve the initiation of this clinical trial. After this approval, the DSMB will review the data of primary and secondary endpoints (safety and efficacy), data for early termination of trial (stopping rules), or adverse events. The board will meet twice per calendar year, after the interim analysis, and whenever there is an issue or concern. The DSMB will recommend changes in the study, including discontinuation, if significant trends in safety or efficacy are identified earlier than expected. None of DSMB members will be involved in the study in any way.

The board will meet twice per calendar year, after the interim analysis, and whenever there is an issue or concern. The DSMB will recommend changes in the study, including discontinuation, if significant trends in safety or efficacy are identified earlier than expected.

To minimize risk of data leak, Dr. Li, whose role is lead statistician, will supervise data management. We will use secure on-line data entry that directly stores the data on protected and dedicated servers. This process facilitates data entry and collection, and offers optimal security for collecting research-related information. The proposed Data Management will be housed within the Data Coordinating Center at the UF Clinical and Translational Science Institute (CTSI). A remote data-entry platform will be constructed for study coordinators in both study sites (UF and icddrb) to view and/or enter data according to a defined set of read/write permissions. This data entry platform will include real-time error checking based on either range or relational checks. The database will monitor access down to the data-field level and track the user, time
and date for each successful data submission. Additionally, the data management group will provide a public website, including an overview of study information, background and responsibilities of key personnel, news items, conference information and announcements, information for potential study subjects; and a secure website including access to the Manual of Procedures, reports to the steering committee and funding agency, minutes of conference calls with study investigators, reports and updates on recruitment status.

Ethical Assurance for Protection of Human rights

Describe the justifications for conducting this research in human participants. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how participants' rights will be protected, and if there would be benefit or risk to each participants of the study. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Discuss procedures safeguarding participants from injuries resulting from study procedures and/or interventions, whether physical, financial or social in nature. [Please see Guidelines]

The study will be conducted in compliance with the procedures outlined in the protocol, the International Conference on Harmonization's Good Clinical Practice (ICH_GP) and in accordance with the ethical guidelines and local regulatory requirements for the trial. The protocol will be submitted to local Independent Institutional Review Board (IRB) comprising Research Review Committee (RRC) and Ethical Review Committee (ERC) for approval, as well as the University of Florida IRB.

We anticipate this is a low risk of study. However, if a subject is harmed as a direct result of his/her participation in this study, the sponsor will pay for all reasonable and necessary medical expenses required to treat the injury, as long as:
1) The injury occurs during participation in the study;
2) The injury results directly from the study product or study-related procedures that the person would not have received as part of his/her routine medical care as determined by the DSMB.

Use of Animals

Describe if and the type and species of animals to be used in the study. Justify with reasons the use of particular animal species in the research and the compliance of the animal ethical guidelines for conducting the proposed procedures.

No animals will be used in the study.

Collaborative Arrangements

Describe if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization.

The study is being conducted under a Sponsored Research Agreement with national Institute of Health (NIH), USA in collaboration with (Dr. Cheng X Sam, Associate Professor and Pediatric Gastroenterologist, Department of Pediatrics at the University of Florida College of Medicine, Clinical & Translational Science Institute (CTSI), University of Florida (UF), Dr. Eric Nelson, Assistant Professor, Department of Pediatrics, and Emerging Pathogens Institute (EMI), University of Florida and George J , Fuchs, Pediatric Gastroenterologist and Professor; Vice Chair of Clinical Affairs for the Department of Pediatrics. Kentucky University, USA. Dr. Cheng will be the lead PI of the study.

The University of Florida is among the top public research university in the nation, has its tradition for translational research and is home to Gatorade, whereas the icddr,b is the international diarrheal study center that has played a major role in the discovery and implementation of ORS for the treatment of diarrhea, including cholera. Both institutions have adequate support for clinical research services including CTSI. The PI Dr. Cheng is a physician-scientist and provides a novel anti-diarrheal concept, while the co-I Drs. Sarker, Fuchs and Nelson have actively been conducting clinical studies on diarrhea in icddr,b and can help bringing this discovery into a bedside therapy. This combination is wellsuited to complete the proposed studies. Dr. Li is a biostatistician @ UF CTSI.

Facilities Available

Describe the availability of physical facilities at site of conduction of the study. If applicable, describe the use of Biosafety Level 2 and/or 3 laboratory facilities. For clinical and laboratory-based studies, indicate the provision of hospital and other types of adequate patient care and laboratory support services. Identify the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications plus field management plans specifying gender considerations for community and for research team members.

Institutional Overview
icddr,b

icddr,b is a unique global resource integrating research, humanitarian care and teaching to improve the health of billions of people living in poverty across the globe and in its host country of Bangladesh. icddr,b's current strategic plan reinforces its legacy of focusing not just on discovery, but also on strengthening the organization's ability to shape policy at the national, regional and global level, as well as advocating for the use of highly effective products and evidence-based discoveries. Integral to its research, icddr,b provides treatment through its urban and rural hospitals and clinics in Bangladesh, which ensures that evidence-based solutions are implementable - world oriented while also providing high quality care, free of charge, to over 200,000 Bangladeshis annually. Amazingly, icddr,b's case fatality rate for diarrhea, which represents the vast majority of its case load is virtually zero percent in the absence of co-morbidity.

Research Strength and Capabilities

icddr,b has a longstanding track record in the interdisciplinary research which underpins the Hub, and in maximizing its impact for the benefit of our population in Bangladesh as well as in other low and middle income countries. This is evidenced by the fact that we led the drafting of the National Nutrition Policy of Bangladesh and reviewed the Nutrition background paper that informed the country's Seventh Five Year Plan.

Comprising one of the largest, multi-disciplinary cohorts of scientists in the developing world, icddr,b collaborates with dozens of international academic, research, and development partners to develop and share global lifesaving solutions. icddr,b addresses some of the most critical health challenges facing the world today, including neonatal survival, maternal and child health, chronic diseases, enteric diseases, malnutrition, communicable and non-communicable diseases, emerging infectious diseases, neglected tropical diseases, poverty and equity and gender issues, and population and climate change with innovative, evidence-based and cost-effective public health solutions.

The proposed study will be conducted in Dhaka Hospital of Nutrition & Clinical Services Division (NCSD) of icddr,b; that provides cost free care and treatment to more than 140 000 patients each year. The patient population comprised of 40% adults and 60% children. The Dhaka Hospital is equipped with Short Stay Unit for uncomplicated diarrhea patients, Longer Stay Unit for diarrheal patients with associated co-morbidities and Intensive Care Unit. Additionally, the Dhaka Hospital is equipped with a Study Ward to support clinical trial.

The Laboratory Sciences and Services Division (LSDD) of icddr,b hosts some of the finest research laboratories and its clinical diagnostic laboratories serve as reference laboratories for clinical diagnostic analysis of human disease cohorts and control subjects. The diagnostic laboratories are the only accredited labs under ISO15189 (quality) and ISO15190 (safety) in Bangladesh for as many as 160 different tests and parameters. A state of art animal house and a bacteriological media facility also cater to the requirements of the researchers from within icddr,b and outside collaborators. The LSSD has always carried out and supported field based and clinical research programs and trials through its laboratory based solutions and platforms besides its flagship diagnostic laboratories.

Team's Strength

The Nutrition & Clinical Services Division (NCSD) of icddr,b; which carries out most of the clinical research, research in nutrition and food security has a highly skilled multidisciplinary research team experienced in conducting studies spanning the full spectrum of nutrition and food security, randomized control trial to community-based intervention trials and informing policy.

Facilities and Resources – University of Florida

The University of Florida (UF) is one of only 17 public, land-grant universities that belong to the prestigious group of 61 academic research institutions that comprise the Association of American Universities. With more than 50,000 students, including approximately 15,000 graduate and professional degree students, the University is the fifth largest in the nation. UF is a major teaching and research institution with more than 200 educational programs and external research grants and contracts of over $600 million in the past year. Nationally, UF ranks among the top 15 public universities in research funding.

As a land grant university, UF, in collaboration with the U.S. Department of Agriculture and local county governments, administers the Florida Cooperative Extension Service (CES). With offices in all 67 counties of the state of Florida, the CES is dedicated to making knowledge available that will enhance and sustain quality of life. In addition to traditional services in agriculture and the natural sciences, the CES offers major programs in health and nutrition education throughout the state of Florida. Coordination with the CES offers an ideal environment for dissemination and implementation of health intervention programs.

The UF Clinical Research Center (CRC) is the CTSA-funded Clinical and Translational Science Institute (CTSI) and is available to Dr. Cheng when needed. The CRC serves as the University's principal venue for state of the art patient-oriented investigations into the causes, prevention and treatment of human disease. It includes a 10 bed inpatient ward, a discrete outpatient area, and dedicated space for exercise physiology. Other resources

include a Nutrition Research Unit/Metabolic Kitchen, a Data Services Laboratory with work stations and bio statistical support and a Core Laboratory.

The study will be conducted in Study ward or Short stay unit of Dhaka Hospital of icddr, b.

Dhaka Hospital was established by icddr,b in 1962 primarily to meet the urgent need to treat patients, particularly children, with severe diarrheal disease. It has since grown into a nationally important centre treating more than 140,000 patients a year. It acts as a beacon illustrating what can be achieved by a hospital facility even within a developing world setting. Dhaka Hospital provides an infrastructure for a wide range of clinical research projects. It also plays a critical role in areas such as disease surveillance, monitoring for antimicrobial resistance and clinical training.

Dhaka Hospital offers a full range of services, particularly for diarrheal and respiratory disease, with infants and children representing a high proportion of patients. It delivers outstanding care, saving an estimated 40,000 lives that might otherwise be lost every year.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however, exercise judgment in assessing the "standard" length.

1. Black, R. *et al.* Global, regional, and national causes of child mortality in 2008: a systematic analysis. *The Lancet* **375**, 1969-1987 (2008).
2. Moore, S., Lima, A. A. M. & Guerrant, R. Infection: Preventing 5 million child deaths from diarrhea in the next 5 years. *Nature Review Gastroenterology Hepatology* **8**, 363-364, doi:10.1038/nrgastro.2011.103. (2011).
3. UNICEF/WHO. Diarrhoea: Why children are still dying and what can be done? Geneva. (2009).
4. Collaborators, G. B. D. D. D. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet. Infectious diseases* **17**, 909-948, doi:10.1016/S1473-3099(17)30276-1 (2017).
5. Reiner, R. C., Jr. *et al.* Variation in Childhood Diarrheal Morbidity and Mortality in Africa, 2000-2015. *The New England journal of medicine* **379**, 1128-1138, doi:10.1056/NEJMoa1716766 (2018).
6. Cortes, J. E., Curns, A. T., Tate, J. E. & Parashar, U. D. Trends in healthcare utilization for diarrhea and rotavirus disease in privately insured US children <5 years of age, 2001-2006. *The Pediatric infectious disease journal* **28**, 874-878, doi:10.1097/INF.0b013e3181a653cd (2009).
7. Harrell, J. E. & Cheng, S. X. Inability to reduce morbidity of diarrhea by ORS: can we design a better therapy? *Pediatric research*, doi:10.1038/pr.2017.295 (2018).
8. Sreerama reddy, C. T., Low, Y. P. & Forsberg, B. C. Slow progress in diarrhea case management in low and middle income countries: evidence from cross-sectional national surveys, 1985-2012. *BMC Pediatr* **17**, 83, doi:10.1186/s12887-017-0836-6 (2017).
9. Binder, H. J., Brown, I., Ramakrishna, B. S. & Young, G. P. Oral rehydration therapy in the second decade of the twenty-first century. *Current gastroenterology reports* **16**, 376, doi:10.1007/s11894-014-0376-2 (2014).
10. Donowitz, M. *et al.* Translational approaches for pharmacotherapy development for acute diarrhea. *Gastroenterology* **142**, e1-9, doi:10.1053/j. gastro.2012.01.014 (2012).
11. Farthing, M. J. Antisecretory drugs for diarrheal disease. *Digestive Diseases* **24**, 47-58 (2006).
12. Lewis, L. D. & Phillips, R. W. Water and electrolyte losses in neonatal calves with acute diarrhea. A complete balance study. *Cornell Vet* **62**, 596-607 (1972).
13. Castillo-Duran, C., Vial, P. & Uauy, R. Trace mineral balance during acute diarrhea in infants. *The Journal of pediatrics* **113**, 452-457 (1988).

14. Fraebel, J. *et al.* Extracellular Calcium Dictates Onset, Severity, and Recovery of Diarrhea in a Child with Immune-Mediated Enteropathy. *Frontiers in pediatrics* **6**, 7, doi:10.3389/fped.2018.00007 (2018).
15. Cheng, S. X. Calcium-sensing receptor: A new target for therapy of diarrhea. *World J Gastroenterol* **22**, 2711-2724, doi:10.3748/wjg. v22.i9.2711 (2016).
16. Tang, L. *et al.* The Extracellular Calcium-Sensing Receptor in the Intestine: Evidence for Regulation of Colonic Absorption, Secretion, Motility, and Immunity. *Frontiers in physiology* **7**, 245, doi:doi: 10.3389/fphys.2016.00245 (2016).
17. Cheng, S. X. Calcium-sensing receptor inhibits secretagogue-induced electrolyte secretion by intestine via the enteric nervous system. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **303**, G60-G70, doi:10.1152/ajpgi.00425.2011 (2012).
18. Cheng, S. X., Geibel, J. & Hebert, S. Extracellular polyamines regulate fluid secretion in rat colonic crypts via the extracellular calcium-sensing receptor. *Gastroenterology* **126**, 148-158 (2004).
19. Cheng, S. X., Okuda, M., Hall, A., Geibel, J. P. & Hebert, S. C. Expression of calcium-sensing receptor in rat colonic epithelium: evidence for modulation of fluid secretion. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **283**, G240-250 (2002).
20. Geibel, J. *et al.* Calcium-sensing receptor abrogates secretagogue-induced increases in intestinal net fluid secretion by enhancing cyclic nucleotide destruction. *The Proceedings of the National Academy of Sciences of the United States of America* **103**, 9390-9397 (2006).
21. Cheng, S. X. *et al.* Epithelial CaSR Deficiency Alters Intestinal Integrity and Promotes Proinflammatory Immune Responses. *FEBS Lett* **588**, 4158-4166, doi: 10.1016/j.febslet.2014.05.007 (2014).
22. Cheng, S. X., Bai, H. X., Gonzalez-Peralta, R., Mistry, P. K. & Gorelick, F. S. Calcium ameliorates diarrhea in immunocompromised children. *Journal of pediatric gastroenterology and nutrition* **56**, 641-644, doi:10.1097/MPG.0b013e3182868946 (2013).
23. Tang, L. *et al.* Calcium-Sensing Receptor Stimulates Cl-- and SCFA-dependent but Inhibits cAMP-dependent HCO3- Secretion in Colon. *The American journal of physiology* **308**, G874-883, doi:10.1152/ajpgi.00341.2014 (2015).
24. Sun, X., Tang, L., Winesett, S., Chang, W. & Cheng, S. X. Calcimimetic R568 inhibits tetrodotoxin-sensitive colonic electrolyte secretion and reduces c-fos expression in myenteric neurons. *Life sciences*, doi: 10.1016/j.lfs.2017.12.019 (2017).
25. Tang, L., Jiang, L., McIntyre, M. E., Petrova, E. & Cheng, S. X. Calcimimetic acts on enteric neuronal CaSR to reverse cholera toxin-induced intestinal electrolyte secretion. *Scientific reports* **8**, 7851, doi:10.1038/s41598-018- 26171-4 (2018).
26. Tang, L. *et al.* Bumetanide increases Cl--dependent short-circuit current in late distal colon: Evidence for the presence of active electrogenic Cl- absorption. *PloS one* **12**, e0171045, doi: 10.1371/journal.pone.0171045 (2017).
27. Sunuwar, L., Asraf, H., Donowitz, M., Sekler, I. & Hershfinkel, M. The Zn2+-sensing receptor, ZnR/GPR39, upregulates colonocytic Cl- absorption, via basolateral KCC1, and reduces fluid loss. *Biochimica et biophysica acta* **1863**, 947-960, doi: 10.1016/j.bbadis.2017.01.009 (2017).
28. Fleisher, G. R. *Approach to diarrhea in children in resource-rich countries.*, <https://www.uptodate.com/contents/approach-to-diarrhea-in-children-in-resource-richcountries?source=search_result&search=acute diarrhea pediatrics & selected Title=2~150. > (
29. Alam, N. H., Majumder, R. N. & Fuchs, G. J. Efficacy and safety of oral rehydration solution with reduced osmolarity in adults with cholera: a randomised double-blind clinical trial. CHOICE study group. *Lancet* **354**, 296- 299 (1999).
30. Cheng, S. X., Bai, H. X., Gonzalez-Peralta, R., Mistry, P. K. & Gorelick, F. S. Calcium ameliorates infectious diarrhea in immune compromised children. *J Pediatr Gastroenterol Nutr* (in press).
31. Raghupathy, P. *et al.* Amylase-resistant starch as adjunct to oral rehydration therapy in children with diarrhea. *Journal of pediatric gastroenterology and nutrition* **42**, 362-368 (2006).

32. Dinleyici, E. C. *et al.* Lactobacillus reuteri DSM 17938 shortens acute infectious diarrhea in a pediatric outpatient setting. *Jornal de pediatria* **91**, 392-396, doi: 10.1016/j.jped.2014.10.009 (2015).
33. Schnadower, D. *et al.* Lactobacillus rhamnosus GG versus Placebo for Acute Gastroenteritis in Children. *The New England journal of medicine* **379**, 2002-2014, doi:10.1056/NEJMoa1802598 (2018).
34. Nearing, J. *et al.* Polyvalent cation receptor proteins (CaRs) are salinity sensors in fish. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 9231-9236 (2002).
35. Whittamore, J. M., Cooper, C. A. & Wilson, R. W. HCO (3) (-) secretion and CaCO3 precipitation play major roles in intestinal water absorption in marine teleost fish in vivo. *The American journal of physiology* **298**, R877-886, doi:10.1152/ajpregu.00545.2009 (2010).
36. Bovee Oudenhoven, I. M., Termont, D. S., Heidt, P. J. & Van der Meer, R. Increasing the intestinal resistance of rats to the invasive pathogen Salmonella enteritidis: additive effects of dietary lactulose and calcium. *Gut* **40**, 497- 504 (1997).
37. Bovee Oudenhoven, I. M., Wissink, M. L., Wouters, J. T. & Van der Meer, R. Dietary calcium phosphate stimulates intestinal lactobacilli and decreases the severity of a salmonella infection in rats. *The Journal of nutrition* **129**, 607- 612 (1999).
38. Bovee-Oudenhoven, I. M., Termont, D. S., Weerkamp, A. H., Faassen-Peters, M. A. & Van der Meer, R. Dietary calcium inhibits the intestinal colonization and translocation of Salmonella in rats. *Gastroenterology* **113**, 550-557 (1997).
39. Bovee-Oudenhoven, I., Termont, D., Dekker, R. & Van der Meer, R. Calcium in milk and fermentation by yoghurt bacteria increase the resistance of rats to Salmonella infection. *Gut* **38**, 59-65 (1996).
40. Cheng, S. X. Calcium-sensing receptor in the gut: evidence for its role in mediating known nutritional therapy for inflammatory bowel disease. *JPGN* **55**, E70 (2012).
41. Schepens, M. A. *et al.* Supplemental calcium attenuates the colitis-related increase in diarrhea, intestinal permeability, and extracellular matrix breakdown in HLA-B27 transgenic rats. *J Nutr.* **139**, 1525-1533, doi:10.3945/jn.109.105205 (2009).
42. Pele, L. C. *et al.* Low dietary calcium levels modulate mucosal caspase expression and increase disease activity in mice with dextran sulfate sodium induced colitis. *J Nutr.* **137**, 2475-2480 (2007).
43. *Tofu may offer protection against diarrhea.* http://thesoyfoodscouncil.com/tofu-may-offer-protection-againstdiarrhea/, <http://thesoyfoodscouncil.com/tofu-may-offer-protection-against-diarrhea/> (
44. Karyadi, D. & Lukito, W. Beneficial effects of tempeh in disease prevention and treatment. *Nutrition reviews* **54**, S94-98 (1996).
45. Karyadi, D. & Lukito, W. Functional food and contemporary nutrition-health paradigm: tempeh and its potential beneficial effects in disease prevention and treatment. *Nutrition* **16**, 697 (2000).
46. Soenarto, T., Sudigbia, I., Hermana, Karmini, M. & Karyadi, D. Antidiarrheal characteristics of tempe produced traditionally and industrially in children aged 6-24 months with acute diarrhea., 174-186 (Indonesian Tempe Foundation, 1997).
47. Bovee-Oudenhoven, I. M. J., Lettink-Wissink, M. L. G., Van Doesburg, W., Witteman, B. J. M. & Van Der Meer, R. Diarrhea caused by enterotoxigenic Escherichia coli infection of humans is inhibited by dietary calcium. *Gastroenterology* **125**, 469-476 (2003).
48. Tang, L. & Cheng, S. X. Does activation of CaSR represent a new method to reduce secretory and inflammatory diarrhea? *Journal of pediatric gastroenterology and nutrition* **60**, 17 (2015).
49. Sayyahfar S., Sadeghian. M. & Amrolalaei M. The effect of calcium on the duration of acute gastroenteritis in children: A randomized clinical trial. *Med J Islam Repub Iran* 35:83 (2021).
50. Agustina R, et al. Randomized trial of probiotics and calcium on diarrhea and respiratory tract infections in Indonesian children. *Pediatrics* 129(5): e1155-64 (2012)
51. *Gueguen, L. & Pointillart, A. Th*e bioavailability of dietary calcium. *J Am Coll Nutr* **19**, 119S-136S (2000).

52. Sargent, J. D., Dalton, M. A., O'Connor, G. T., Olmstead, E. M. & Klein, R. Z. Randomized trial of calcium glycerophosphate-supplemented infant formula to prevent lead absorption. *The American journal of clinical nutrition* **69**, 1224-1230 (1999).

Budget: Approved budget attached at the end.

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item, including the use of human resources, major equipment, and laboratory services.

International Centre for Diarrhoeal Disease Research, Bangladesh

Personnel

icddr,b salaries as listed are inclusive of all benefits, and are approved by the icddr,b finance office. Given the potential for substantial inflation in Bangladesh, icddr,b expects annual increases in the salaries of approximately 5-7% for International Staff and 10% for local staff. Annual FTE calculations for staff are based on the period in which study activities will be implemented.

Dr. Shafiqul Alam Sarker is based full-time in Dhaka and will be local PI of the study and will give effort 10% of his time to lead the study team in implementing the study as per SOP following the approved protocol throughout the study period. He will be responsible for the design and conceptual framework of the study and provide strategic guidance to the team, support ethical approval process, response to external reviewers, data analysis, report writing and dissemination. He will also guide quality about the study activities.

Dr. Shamima Sultana Co- Investigator/ functional PI is full time based in Dhaka. She is medical graduate with public health background. She will coordinate all the related activities of the project by supervising overall study activities according to SOP. She will be involved in hiring and training process of all the study personnel. Besides she will be responsible to supervise the clinical activities by the nurses and Project Research Physician. She will provide 10% of her time through the whole study period.

Senior Budget Coordinator: Allocated 5% of time for budget planning and financial analyst for 16 months. He will support on budget preparation, review of budget, financial tracking and reporting as per project need.

Project Research Physician (PRP): Two PRP will be Involved. One of them will provide 100% of time for 16 months and another one will involve100% of time for 12 months. They will be responsible for screening and enrolling study participants, clinical case management and tracking discharge of the participant

Administrative/Finance Officer: We will need 20% time of an Administrative/Finance Officer who will be involved directly in hiring process, signing agreement, logistic management etc.

Study Nurse (3): Prescreening of patients from registration desk, assist PRP in consenting procedure, enrollment. Will be responsible for hospital care and management of participants.

Health worker (3): Help study nurse in selection of eligible patients from triage, inward day to day Pt care and provide full time.

Data Management Assistant: One DMA PRP will be involved and will provide 100% of time 12 months for data record and analysis.

S**tudy Expenses:** Funds totaling ~$12,500 per year are request to pay for all non-salary costs to complete the study at the icddr,b. These funds will cover Hospitalization Cost, Lab Test, IRB, Consumable Supplies, etc.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration.

No additional financial support has been sought for this project.

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name:** Dr Shafiqul Alam Sarker

2. **Present Position:** Senior Scientist

3. **Educational background**: (last degree and diploma & training relevant to the present research proposal)

	Institution	Year
Degree	MBBS (Rajshahi University, Bangladesh)	1977
	MD (University of Basel, Switzerland)	1991
	PhD (Karolinska Institute, Sweden)	2006
	FRCP (Royal College of Physician, Edinburgh)	2017
Training	Fellowship in Nutrition and Clinical Research	1979-1981
Training	Upper GI endoscopy and Ultrasound	1996

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	2560152	2022

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
PR-15089	PI	08.06.2016	31.12.2020	65%
ACT-01013	PI	07.11.2018	31.12.2020	30%
PR-17083	Co-I	01.10.2017	30.04.2019	5%

6. **Publications**

Types of publications	Numbers
a. Original scientific papers in peer-review journals	110
b. Peer reviewed articles and book chapters	4
c. Papers in conference proceedings	-
d. Letters, editorials, annotations, and abstracts in peer-reviewed journals	35
e. Working papers	3
f. Monographs	-

7. Five recent publications including publications relevant to the present research protocol

1. Sarker SA, Sultana S, Reuteler G, Moine D, Descombes P, Charton F, Bourdin G, McCallin S, Ngom-Bru C, Neville T, Akter M, Hoq S, Qadri F, Talukdar K, Kassam M, Delley M, Loiseau C, Deng Y, El Aidy S, Berger B, Brüssow H**.** Oral Phage Therapy of Acute Bacterial Diarrhea With Two Coliphage Preparations: A Randomized Trial in Children From Bangladesh. EBioMedicine. 2016 Jan 5;4:124-37
2. Sarker SA**,** Ahmed T, Brussow H**.** Persistent diarrhea: A persistent infection with enteropathogens or a gut commensal dysbiosis? Environmental Microbiology; 2017 19(10):3789-3801.
3. Kieser S, Sarker SA, Berger B, Sultana S, Chisti MJ, Islam SB, Foata F, Porta N, Betr B, Fournier C, Descombes P, Mercenier A, Sakwinska O, Brüssow H. Antibitic Treatment Leads to Fecal *Escherichia coli* and coliphage expansion in Severely Malnourished diarrheal Patients. Cell Mol Gastroenterol Hepatol. 2017 Dec 13;5(3):458-460.e6.
4. Kieser S, Sarker SA, Sakwinska O, Foata F, Sultana S, Khan Z, Islam S. Porta N, Combremont S, Betrisey B, Fournier C, Charpagne A, Descombes P, Mercenier A, Berger B, Brüssow H. Bangladeshi Children with Acute Diarrhea Shows Fecal microbiome with Increased Streptococcal Abundanc irrespective of diarrhea etiology. Environ Microbiol. 2018 May 22. doi: 10.1111/1462-2920.14274.
5. Kienesberger S, Perez-Perez GI, Olivares AZ, Bardhan P, Sarker SA, Hasan KZ, Sack RB, Blaser MJ. When Helicobacter pylori is acquired in populations in developing countries; A birth-cohort study in Bangladesh? Gut Microbes. 2018 Mar 1:1-12.

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Shafiqul Sarker** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 11/13/2017.

Certification Number: 2560152.

1. **Name:** Dr Tahmeed Ahmed

2. **Present Position:** Senior Director, Nutrition and Clinical Services Division, icddr,b and Professor of Public Health Nutrition, James P. Grant School of Public Health, BRAC University

3. **Educational background**: (last degree and diploma & training relevant to the present research proposal)

	Institution	Year
Degree	PhD, University of Tsukuba, Japan	1996
Degree	MBBS, University of Dhaka	1983
Training	Clinical training in Pediatrics, University of Tsukuba Hospital	1990-1992
Training	Residential training in Pediatrics, Dhaka Shishu Hospital	1989-1990

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	1933611	Issued on 12 August 2015

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. List of ongoing research protocols/ activities

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
Childhood acute illness and nutrition (CHAIN) study	Co-I	01.11.2016	31.08.2019	10%
Antibiotics for childhood diarrhea (ABCD) trial	Co-PI	01.02.2017	30.04.2020	20%
Bangladesh Environmental Enteric Dysfunction (BEED) Study	PI	01.11.2015	30.04.2020	15%
Evaluation of the largest stunting control program - *Suchana*	Co-PI	01.06.2016	31.12.2020	20%
Microbiota directed complementary food clinical trials (Primary MAM and Post SAM-MAM)	PI	01.11.2018	27.02.2021	20%

6. Publications

Types of publications	Numbers
a. Original scientific papers in peer-review journals	286
b. Peer reviewed articles and book chapters	18
c. Papers in conference proceedings	25
d. Letters, editorials, annotations, and abstracts in peer-reviewed journals	5
e. Working papers	14
f. Monographs	1

7. Five recent publications including publications relevant to the present research protocol

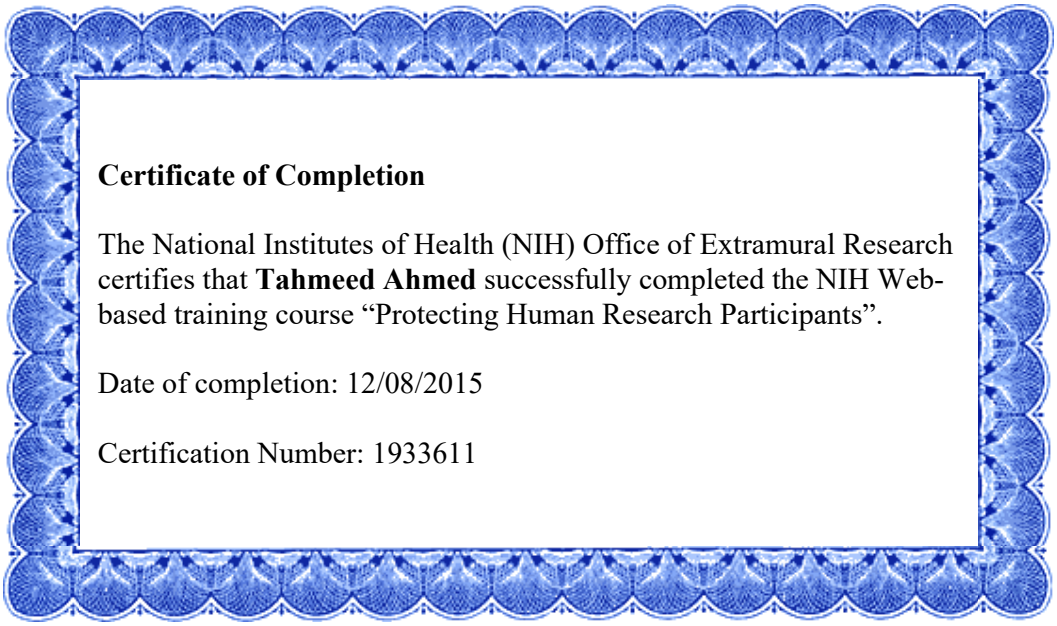
7.1. Nahar B, Hossain M, Ickes SB, Naila NN, Mahfuz M, Hossain D, Denno DM, Walson J, Ahmed T. Development and validation of a tool to assess appetite of children in low income settings. *Appetite* 2018 Dec 21. pii: S0195-6663(18)30468-9.

7.2. Ahmed T, Choudhury N, Hossain I, Tangsuphoom N, Islam MM, de Pee S, Steiger G, Fuli R, Sarker SA, Parveen M, West KP, Christian P. Development and acceptability testing of ready-to-use supplementary food made from locally available food ingredients in Bangladesh. *BMC Pediatr* 2014 Jun 27; 14:164.

7.3. Subramanian S, Huq S, Yatsunenko T, Haque R, Mahfuz M, Alam MA, Benezra A, DeStefano J, Meier MF, Muegge BD, Barratt MJ, VanArendonk LG, Zhang Q, Province MA, Petri WA Jr, Ahmed T, Gordon JI. Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature* 2014 doi:10.1038/nature13421.

7.4. Khatun H, Comins CA, Shah R, Islam MM, Choudhury N, Ahmed T. Uncovering the barriers to exclusive breastfeeding for mothers living in Dhaka's slums: a mixed method study. *Int Breastfeed J* 2018 Sep 26; 13:44.

7.5. Ahmed T, Auble D, Berkley JA, Black R, Ahern PP, Hossain M, Hsieh A, Ireen S, Arabi M, Gordon JI. An evolving perspective about the origins of childhood undernutrition and nutritional interventions that includes the gut microbiome. *Ann N Y Acad Sci* 2014 Aug 12.



Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Tahmeed Ahmed** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 12/08/2015

Certification Number: 1933611

1. **Name:** Dr. Shamima Sultana

2. **Present Position:** Senior Programme Manager, NCSD, icddr,b

3. **Educational background**: (last degree and diploma & training relevant to the present research proposal)

	Institution	Year
Degree	MBBS (University of Dhaka, Bangladesh)	1994
Degree	MPH ((NIPSOM, University of Dhaka, Bangladesh)	2006
Training	Fellowship (Swiss Federal Institute of Technology Zürich (ETH), Switzerland	2007
Training	Australian Leadership and Management (Fellowship course in Griffith University, Brisbane, Queensland, Australia)	2014

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	2983111	Issued 21 September, 2022

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

Already received training certificate on **International Conference on Harmonization (ICH) and Good Clinical Practice (GCP)** from Macro Care Clinical Research Ltd and Max-Neeman Int, India.

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
PR-15089	Co-PI	08.06.2016	31.12.2020	0%
PR-19045	Co-I	07.11.2018	31.08.2022	100 %

6. **Publications**

Types of publications	Numbers
g. Original scientific papers in peer-review journals	17
h. Peer reviewed articles and book chapters	1
i. Letters, editorials, annotations, and abstracts in peer-reviewed journals	-
j. Working papers	3

7. **Five recent publications including publications relevant to the present research protocol**

7.1. Rahman MM, Ghoshal UC, Sultana S, Kibria MG, Sultana N, Khan ZA, Ahmed F, Hasan M, Ahmed T, Sarker SA. Long-Term Gastrointestinal Consequences are Frequent Following Sporadic Acute Infectious Diarrhea in a Tropical Country: A Prospective Cohort Study *The American Journal of Gastroenterology* volume 113, pages1363–1375 (2018)

7.2. Shawna McCallin, Shafiqul A. Sarker, Shamima Sultana, Frank Oechslin and Harald Brüssow. Metagenome analysis of Russian and Georgian Pyophage cocktails and a placebo-controlled safety trial of single phage versus phage cocktail in healthy Staphylococcus aureus carriers Environmental Microbiology (2018) 20(9), 3278–3293

7.3. Shamima Sultana, Shafiqul A. Sarker and Harald Brussow. What happened to Koch's postulates in diarrhoea Environmental Microbiology (2017) 19(8), 2926–2934

7.4. Kieser S, Sarker SA, Berger B, Sultana S, Chisti MJ, Islam SB, Foata F, Porta N, Betr B, Fournier C, Descombes P, Mercenier A, Sakwinska O, Brüssow H. Antibitic Treatment Leads to Fecal *Escherichia coli* and coliphage expansion in Severely Malnourished diarrheal Patients. Cell Mol Gastroenterol Hepatol. 2017 Dec 13;5(3):458-460.e6.

7.5. Shafiqul Alam Sarker, Shamima Sultana, Gloria Reuteler, Deborah Moine, Patrick Descombes,[c] Florence Charton, Gilles Bourdin, Shawna McCallin and Harald Brüssow. Oral Phage Therapy of Acute Bacterial Diarrhea with Two Coliphage Preparations: A Randomized Trial in Children From Bangladesh. EBioMedicine. 2016 Feb; 4: 124–137.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Cheng, Sam Xianjun

eRA COMMONS USER NAME (credential, e.g., agency login): SAMCHENGMD

POSITION TITLE: Associate Professor of Pediatric Gastroenterology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Tongji Medical University, Wuhan, China	MD	08/84	Medicine
Tongji Medical University, Wuhan, China	MSc	07/87	Clinical Investigation
Karolinska Institute, Stockholm, Sweden	PhD	04/98	Cell & Molecular Physiology
SUNY Downstate, NY	Residency	06/09	Pediatrics
Yale University, CT	Fellow	06/12	Pediatric Gastroenterology

A. Personal Statement

This project proposes to test efficacy of calcium-ORS in reducing acute infectious diarrhea. It builds logically on my prior work and expertise. It is the extension of the work proposed in my K08 and R41 awards and NASPGHAN foundation grant. When completed, this research may help develop a new oral rehydrating therapy for diarrhea in children. I have the skills and knowledge necessary to successfully carry out the proposed work. I am board-certified pediatrician and pediatric specialist in gastroenterology, hepatology and nutrition. I also have a broad background in physiology, with specific training and expertise in ion transport physiology. I used to be a renal physiologist. In 2000, I "transplanted" the knowledge and skills from renal into GI and initiated the current intestinal CaSR (calcium-sensing receptor)-diarrhea project (Cheng et al, AJP'02; Cheng et al, Gastroenterology'04; Geibel et al, PNAS'06). In 2012, I moved to UF and identified this unusual 'all-inclusive' antidiarrheal mechanism in the gut (Cheng, World J Gastro'16; Owen et al, Semin Cell Dev Biol'16; Tang et al, Front Physiol'16; Tang et al, Sci Rep'18). As a physician scientist, I wish to continue to transform result from bench into bedside. This current proposal represents one such effort. As a matter of fact, I have started in 2009 the conception of the Ca/CaSR-based antidiarrheal ORS with Dr. George Fuchs @ College of Public Health, University of Kentucky and Dr. Shafiqul Sarker in ICDDRB, Dhaka, Bangladesh. Thus, following the completion of proof of concept preclinical studies in vitro and in animal models, we decided to move on and do clinical trials on this in human patients with diarrhea in ICDDRB. ICDDRB is selected because it is the international diarrheal study center, the center that has played a major role in the discovery and implementation of ORS for the treatment of diarrhea. This combination of expertise will ensure the success of the project. University of Florida has its tradition for translational research and is home to Gatorade; the ultimate goal of this research is to generate a new Gatorade-like inexpensive but therapeutically active drink for people with diarrhea.

B. Positions and Honors

Positions and Employment

1989-1991: Pediatrician, Tongji Hospital, Tongji Medical University, China
1991-1992: W.H.O. Fellow, Pediatrics, Great Ormond Street Hospital for Children, London, UK
1992-1993: Visiting Physician Scholar, St. Gorans Childrens Hospital, Karolinska Institute, Sweden.
1999-2000: Postdoc Research Fellow, Vanderbilt University Medical Center, Nashville, TN
2000-2007: Associate Research Scientist, Department of Physiology, Yale University, New Haven, CT.
2012-2020: Assistant Professor, Department of Pediatrics, University of Florida, Gainesville, FL
2020-present: Associate Professor, Department of Pediatrics, University of Florida, Gainesville, FL

Other Experience and Professional Memberships

1999	Ad Hoc Reviewer, Pediatric Research
2000	Ad Hoc Reviewer, American Journal of Physiology-Renal Physiology
2001-	Member, American Gastroenterology Association
2006	Ad Hoc Reviewer, Renal Failure
2012-	Reviewer, Journal of Pediatric Gastroenterology and Nutrition
2013-	Reviewer, World Journal of Gastroenterology
2013-	Reviewer, Inflammatory Bowel Disease
2013-	Member, Editorial Board of World Journal of Experimental Medicine
2007-	Member of American Academy of Pediatrics
2009-	Member, North America Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN)
2011-	Member, Research Committee, NASPGHAN
2013-	Member, American College of Gastroenterology
2014-	Reviewer, Cell Communication and Signaling
2015-	Reviewer, PLOS One, BMC Med Genet, FESEB J
2018-	Member, Editorial Board of World Journal of Gastroenterology
2019-	Member, Editorial Board of Physiological Report

Honors

1991	World Health Organization Award, London, UK
1992	Wenner-Gren Scholar, Stockholm, Sweden
1997	Sven & Ruth Nydahls Award, Stockholm, Sweden
1997	Ruth Lundells Award, Stockholm, Sweden
1997	Invited Lectureship, 14th International Congress of Nephrology, Sydney, Australia
2011	Fellow to Faculty Transition Award in Inflammatory Bowel Disease, the North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition Foundation
2011	Travel Award to the Single Topic Symposium "Effective Pediatric Clinical Trials in Gastrointestinal, Hepatobiliary and Pancreatic Disease and Nutrition", New Orleans, 2011, NASPGHAN Foundation
2012	Travel Award to the Single Topic Symposium "Discovering the Future of Pediatric Inflammatory Bowel Disease", Salt Lake City, 2012, NASPGHAN Foundation
2012	Travel Award for the World Congress of Pediatric, Gastroenterology, Hepatology and Nutrition in Taiwan, Taipei, Taiwan, 2012, NASPGHAN Foundation

2012	Distinguished Presentation Award, the 4th World Congress of Pediatric, Gastroenterology, Hepatology and Nutrition in Taiwan, Taipei, Taiwan, 2012, FISPGHAN
2012	Invited Lectureship, 4th World Congress of Pediatric, Gastroenterology, Hepatology and Nutrition in Taiwan, Taipei
2013	Invited Lectureship, NASPGHAN annual Meeting, Chicago
2014	NIH K08 Clinical Scientist Career Development Award, National Institutes of Health NICHD
2015	Invited Plenary Lectureship, 2nd International CaSR Symposium, American Society for Bone and Mineral Research, San Diego, CA
2016	Travel Award and Invited Lectureship, 5th World Congress of Pediatric, Gastroenterology, Hepatology and Nutrition in Montreal, Canada, FISPGHAN
2017	Invited Plenary Lectureship, 3rd International CaSR Symposium, Florence, Italy
2020	Invited Plenary Lectureship, 4th International CaSR Symposium, San Francisco, CA
2021	Invited Lectureship, American Society of Nephrology, San Diego, CA

C. Contributions to Science

1. Molecular model for bi-directional control of Na,K-ATPase activity by hormones in kidney

- *Fisone G, *Cheng SX-J, Nairn AC, Czernik AJ, Hemmings HC Jr, Höög J-O, Bertorello AM, Kaiser R, Bergman T, Jörnvall H, Aperia A & Greengard P (1994). Identification of the phosphorylation site for cAMP-dependent protein kinase on Na+,K+-ATPase and effects of site-directed mutagenesis. (*Shared 1st authorship) *J Biol Chem* 269: 9368-9373.
- Cheng XJ, Fisone G, Aizman O, Aizman R, Levenson R, Greengard P & Aperia A (1997). PKA mediated phosphorylation and inhibition of Na+,K+-ATPase in response to beta-adrenergic hormone. *Am J Physiol* 42: C893-901.
- Cheng X-J, Höög J-O, Nairn AC, Greengard P & Aperia A (1997). Regulation of rat Na+,K+-ATPase activity by PKC is modulated by state of phosphorylation of Ser943 by PKA. *Am J Physiol* 273: C1981-C1986.
- Nowicki S, Chen SL, Aizman O, Cheng X-J, Li D, Nowicki C, Nairn AC, Greengard P & Aperia A (1997). 20-Hydroxyeicosa-tetraenoic acid (20 HETE) activates protein kinase C. Role in regulation of rat renal Na+,K+-ATPase. *J Clin Invest* 99: 1224-1230.

2. Ion transport and salt-sensing by renal proximal tubules

- Cheng SXJ, Aizman O, Nairn A, Greengard P & Aperia A (1999). [Ca2+]i determines the effects of protein kinases A and C on activity of rat renal Na+,K+-ATPase. *J Physiol* 518: 37-46.
- Ibarra F, Cheng SX-J, Agren M, Svensson L-B & Aperia A (2002). Intracellular sodium can act as a signal for Na/K-ATPase phosphorylation / dephosphorylation in renal epithelial cells. *Acta Physiol Scand* 175 (2): 165-171.
- Bertuccio CA, Cheng SX, Arrizurieta EE, Martín RS & Ibarra FR (2003). Mechanisms of Na-K-ATPase phosphorylation by PKC in the medullary thick ascending limb of Henle in the rat. *Pfluger's Arch*. 447(1):87-96.

3. Discovery of a new receptor mechanism that controls intestinal fluid movement

- Cheng SX, Okuda M, Hall AE, Geibel JP & Hebert SC (2002). Expression of calcium/nutrient-sensing receptor in rat colonic epithelium: evidence for modulation of fluid secretion. *Am J Physiol* 283: G240-G250
- Cheng SX, Geibel JP & Hebert SC (2004). Extracellular polyamines regulate fluid secretion in rat colonic crypts via the extracellular calcium-sensing receptor. *Gastroenterology* 126(1):148-58.
- Geibel J, Sritharan K, Geibel R, Geibel P, Persing JS, Seeger A, Roepke TK, Deichstetter M, Prinz C, Cheng SX, Martin D & Hebert SC (2006). Calcium-sensing receptor abrogates secretagogue-induced increases in intestinal net fluid secretion by enhancing cyclic nucleotide destruction. *PNAS USA*, 103: 9390-7.
- Ring A*, Cheng SX*, Kahle KT, Leng Q, Rinehart J, Lalioti MD, Volkman HM, Wilson FH, Hebert SC & Lifton RP (2007). WNK4 regulates activity of the epithelial Na channel in vitro and in vivo. *PNAS USA*, 104:4020-4. * shared 1st authorship

4. **Identification of CaSR as the 1st inclusive antidiarrheal mechanism in the gut**

- Cheng SX (2012). Calcium-sensing receptor inhibits secretagogue-induced electrolyte secretion by intestine via the enteric nervous system. *Am J Physiol* 303: G60-70
- Cheng SX, Lightfoot YL, Yang T, Zadeh M, Tang L, Sahay B, Wang GP, Owen JL & Mohamadzadeh M (2014). Epithelial CaSR Deficiency Alters Intestinal Integrity and Promotes Proinflammatory Immune Responses. *FEBS Lett*. 588(22):4158-66.
- Tang L, Peng M, Liu L, Chang W, Binder HJ & Cheng SX (2015). Calcium-Sensing Receptor Stimulates Cl^-- and SCFA-dependent but Inhibits cAMP-dependent HCO3- Secretion in Colon. *Am J Physiol.* 308(10):G874-83
- Owen JL, Cheng SX, Sahay B and Mohamadzadeh M (2016). The Role of the Calcium-Sensing Receptor in Gastrointestinal Inflammation. *Semin Cell Dev Biol* 49:44-51.
- Cheng SX (2016). Calcium-sensing receptor: a new target for therapy of diarrhea. *World J Gastro* 22(9):2711-24.
- Tang L, Cheng CY, Sun X, Pedicone AJ, Mohamadzadeth M and Cheng SX (2016). The Extracellular Calcium-Sensing Receptor in the Intestine: Evidence for Regulation of Colonic Absorption, Secretion, Motility, and Immunity. *Front. Physiol.* 7:245. doi: 10.3389/fphys.2016.00245
- Tang L, Fang X, Winesett SP, Cheng CY, Binder HJ, Rivkees SA & Cheng SX (2017). Bumetanide increases Cl--dependent short-circuit current in late distal colon: evidence for the presence of active electrogenic Cl- absorption. *PLOS ONE* 12(2):e0171045
- Sun X, Tang L, Winesett S, Chang W & Cheng SX (2018). Calcimimetic R568 inhibits tetrodotoxin-sensitive colonic electrolyte secretion and reduces c-fos expression in myenteric neurons. *Life Sci* 194:49-58. doi: 10.1016/j.lfs.2017.12.019
- Tang L, Jiang L, McIntyre ME, Petrova E & Cheng SX (2018). Calcimimetic acts on enteric neuronal CaSR to reverse cholera toxin-induced intestinal electrolyte secretion. *Sci Rep* 8(1):7851. doi: 10.1038/s41598-018-26171-4.

D. Research Support

Ongoing research Support

None

Completed Research Support

Research Grant Cheng (PI) 07/01/2012-06/30/2013
NASPGHAN foundation
The calcium-sensing receptor and nutritional therapy of IBD in children
The specific aims of this proposal are designed to assess the role of the CaSR as a mechanism in mediating known nutritional therapy of inflammatory bowel disease (IBD) in children and to identify CaSR as a potential new target for pharmaco-nutritional therapy for IBD.
Role: PI

K08 HD079674 Cheng (PI) 04/10/2014-03/31/2020
NIH/NICHD
Calcium receptor action in childhood diarrhea
The specific aims of this proposal are designed to understand the molecular and cellular mechanisms by which CaSR acts as an anti-diarrheal in the gut.
Role: PI

R41 HD092133 Cheng (PI) 04/01/2017-03/31/2019
NIH/NICHD
Development of CaSR nutrient therapy for childhood diarrhea
The goal of this proposal is to use animal models of infectious diarrhea to test anti-diarrheal efficacy and safety of child-friendly, CaSR-activating nutrients.
Role: PI

CURRICULUM VITAE

(September 2022)

GEORGE J. FUCHS, III, M.D.

Personal Information:

Office Address:	University of Kentucky College of Medicine Department of Pediatrics 138 Leader Ave, Office 209 Lexington, KY 40506 Tel: (859) 218-1676 Fax: (859) 257-7799 or (413) 793-1376 E-mail: gjfuchs@usa.net; george.fuchs@uky.edu

Education:

1970-1974	University of Missouri-Columbia, Columbia, Missouri 65212 Degree: BA Zoology
1975-1976	Universidad Autónoma de Guadalajara, School of Medicine Guadalajara, Mexico
1977-1980	University of Missouri-Columbia, School of Medicine Columbia, Missouri 65212, Degree: M.D.

Clinical Training:

Internship

July - December, 1980	Internal Medicine, Department of Internal Medicine Good Samaritan Hospital, Phoenix, Arizona 85062
January - June, 1981	Pediatrics, Department of Pediatrics Phoenix Hospitals Affiliated Pediatric Program, Arizona 85062,

Residency

July, 1981 - June, 1983	Pediatrics, Tufts-New England Medical Center, Boston Floating Hospital for Infants and Children,Boston, Massachusetts 02111

Clinical/Research Fellowships:

July, 1983 - June, 1984	Divisions of Geographic Medicine and Pediatric Gastroenterology/Nutrition, Tufts-New England Medical Center Boston, Massachusetts 02111
July, 1984 - June, 1985	Pediatric Infectious Diseases, University of Texas Health Science Center at Houston,Houston, Texas 77225
July, 1985 - June, 1986	Pediatric Gastroenterology/Nutrition, University of Texas Health Science, Center at Houston, Houston, Texas 77225

Professional Background: **Academic Appointments**

2015 – Present: Professor of Pediatrics (with tenure), University of Kentucky College of Medicine
2016 – Present: Professor, Department of Epidemiology, University of Kentucky College of Public Health
2016 – Present: Professor, Department of Preventive Medicine, and Environmental Health, University of Kentucky College of Public Health
2002 – 2015: Professor of Health Policy and Management, Faye W. Boozman College of Public Health, University of Arkansas for Medical Sciences

2001 – 2015: Professor of Pediatrics (with tenure), College of Medicine, University of Arkansas for Medical Sciences

1998 – 2001: Professor of Pediatrics (with tenure), Louisiana State University, School of Medicine in New Orleans

1992 - 1998: Associate Professor of Pediatrics (with tenure), Louisiana State University School of Medicine in New Orleans

1986 - 1992 Assistant Professor of Pediatrics, Louisiana State University School of Medicine in New Orleans

Other Professional Positions (last 7 years):

2019 – 2022 Senior Ambulatory Medical Director for Pediatrics, UK HealthCare, Kentucky Children's Hospital

2017 - 2022 Vice Chair for Clinical Affairs, Dept. of Pediatrics, University of Kentucky College of Medicine

2015 - 2022 Chief, Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics University of Kentucky College of Medicine

2017 – Present: Member, Primary Care Provider Association Advisory Board, Academy of Nutrition and Dietetics

2017 – Present: Member, Committee on Nutrition, American Academy of Pediatrics

2013 - Present : Adjunct Scientist, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b)

2013 – 2018: Member, Gastroenterology & GI Surgery Working Group, (annually through 2018) US News and World Report Best Children's Hospitals project

2013 – present: Editor, Nutrition and Metabolism Section of the Journal of Translational Medicine (JTM)

2011 – 2020: Editorial Board, Journal of Pediatric Gastroenterology and Nutrition

2015 – 2016: Member, Expert Committee, NIAID Food Allergy Clinical Guidelines update. National Institutes of Health (NIH)

Society Memberships:

- North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition 1986 - Present
- Infectious Disease Society of America 1986 - Present
- American Academy of Pediatrics 1987 - Present
- American Gastroenterological Association 1989 - Present
- American Society for Nutrition 1994 – Present
- Global Nutrition Council 1994 - Present

Grants and Contracts (last 5 years):

1. Funding entity: Unicef
   Title of project: Effectiveness trial of day-care versus usual care management of severe pneumonia and malnutrition in children using a health system approach".
   Dates: 2015-2018
   Amount: $1,500,000
   Role: Co-P.I. (External P.I.)

2. Funding entity: Unicef
   Title of project: Effectiveness trial of day-care versus usual care management of severe pneumonia with or without malnutrition in children using the health system of Bangladesh". Expansion proposal

Dates: 2017-2019
Amount: $897,499
Role: Co-P.I. (External P.I.)

3. Funding entity: Botnar Foundation
   Title of project: Effectiveness trial of day-care versus usual care management of severe pneumonia with or without malnutrition in children using the health system of Bangladesh".
   Dates: 2017-2019
   Amount: $602,192
   Role: Co-P.I. (External P.I.)

4. Funding entity: Biomarin
   Title of project: An Open-label Extension Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase (> 40 mg/day Dose) in Adults with Phenylketonuria".
   Dates: 2018-2021
   Amount: $98,976
   Role: Site P.I.

5. Funding entity: UBS Optimus Foundation
   Title of project: Proposal for Expansion Phase II "Analysis and Dissemination", Effectiveness trial of day-care versus usual care management of severe pneumonia and malnutrition in children using a health system approach".
   Dates: 2019
   Amount: $142,000
   Role: Co-P.I. (External P.I.)

6. Funding entity: ModernaTx, Inc.
   Title of project: A Phase 2/3, Two-Part, Open-Label, Dose Escalation, Age De-escalation and Randomized, Observer-Blind, Placebo-Controlled Expansion Study to Evaluate the Safety, Tolerability, Reactogenicity, and Effectiveness of mRNA-1273 SARS-CoV-2 Vaccine in Healthy Children 6 months to < 12 Years of Age.
   Dates: 2021-23
   Amount: $2,255,362
   Role: Site P.I.

7. Funding entity: ModernaTx, Inc.
   Title of project: COVID-19: ROVER-An Open-Label, Phase 3 Study to Evaluate the Safety and Immunogenicity of the mRNA-1273.214 Vaccine for SARS-CoV-2 Variants of Concern in Participants Aged 6 Months to < 6 Years
   Dates: 2022-24
   Amount: $330,332
   Role: Site P.I.

Bibliography (last 5 years):

1. Togias A, Cooper SF, Acebal M, Assa'ad A, Baker JR, Beck LA, Block J, Byrd-Bredbenner C, Chan ES, Eichenfield LF, Fleischer DM, **Fuchs GJ**, Furuta GT, Greenhawt MJ, Gupta RS, Habich M, Jone SM, Keaton, K, Muraro A, Plaut MS, Rosenwasser, LJ, Rotrosen D, Sampson HA, Schneider LC, Sicherer SH, Sidbury R, Spergal J, Stukus DR, Venter C, Boyce JA. Addendum Guidelines for the Prevention of Peanut Allergy in the United States: Report of the National Institute of Allergy and Infectious Diseases-Sponsored Expert Panel. *J Allergy Clin Immunol*. 2017;139:29-44.

- Togias A, Cooper SF, Acebal M, Assa'ad A, Baker JR, Beck LA, Block J, Byrd-Bredbenner C, Chan ES, Eichenfield LF, Fleischer DM, **Fuchs GJ**, Furuta GT, Greenhawt MJ, Gupta RS, Habich M, Jone SM, Keaton, K, Muraro A, Plaut MS, Rosenwasser, LJ, Rotrosen D, Sampson HA, Schneider LC, Sicherer SH, Sidbury R, Spergal J, Stukus DR, Venter C, Boyce JA. Addendum Guidelines for the Prevention of Peanut Allergy in the United States: Report of the National Institute of Allergy and Infectious Diseases-Sponsored Expert Panel. *J Pediatr Nurs* 2017;32:91-98.

- Togias A, Cooper SF, Acebal M, Assa'ad A, Baker JR, Beck LA, Block J, Byrd-Bredbenner C, Chan ES, Eichenfield LF, Fleischer DM, **Fuchs GJ**, Furuta GT, Greenhawt MJ, Gupta RS, Habich M, Jone SM, Keaton, K, Muraro A, Plaut MS, Rosenwasser, LJ, Rotrosen D, Sampson HA, Schneider LC, Sicherer SH, Sidbury R, Spergal J, Stukus DR, Venter C, Boyce JA. Addendum Guidelines for the Prevention of Peanut Allergy in the United States: Report of the National Institute of Allergy and Infectious Diseases-Sponsored Expert Panel. *Pediatr Dermatol.* 2017;34(1):e1-e21.

- Togias A, Cooper SF, Acebal M, Assa'ad A, Baker JR, Beck LA, Block J, Byrd-Bredbenner C, Chan ES, Eichenfield LF, Fleischer DM, **Fuchs GJ**, Furuta GT, Greenhawt MJ, Gupta RS, Habich M, Jone SM, Keaton, K, Muraro A, Plaut MS, Rosenwasser, LJ, Rotrosen D, Sampson HA, Schneider LC, Sicherer SH, Sidbury R, Spergal J, Stukus DR, Venter C, Boyce JA. Addendum Guidelines for the Prevention of Peanut Allergy in the United States: Report of the National Institute of Allergy and Infectious Diseases-Sponsored Expert Panel. *Ann Allergy Asthma Immunol* 2017;118:166-73.

- Togias A, Cooper SF, Acebal M, Assa'ad A, Baker JR, Beck LA, Block J, Byrd-Bredbenner C, Chan ES, Eichenfield LF, Fleischer DM, **Fuchs GJ**, Furuta GT, Greenhawt MJ, Gupta RS, Habich M, Jone SM, Keaton, K, Muraro A, Plaut MS, Rosenwasser, LJ, Rotrosen D, Sampson HA, Schneider LC, Sicherer SH, Sidbury R, Spergal J, Stukus DR, Venter C, Boyce JA. Addendum Guidelines for the Prevention of Peanut Allergy in the United States: Report of the National Institute of Allergy and Infectious Diseases-Sponsored Expert Panel. *J Acad Nutr Diet* 2017;117:788-93.

- Togias A, Cooper SF, Acebal M, Assa'ad A, Baker JR, Beck LA, Block J, Byrd-Bredbenner C, Chan ES, Eichenfield LF, Fleischer DM, **Fuchs GJ**, Furuta GT, Greenhawt MJ, Gupta RS, Habich M, Jone SM, Keaton, K, Muraro A, Plaut MS, Rosenwasser, LJ, Rotrosen D, Sampson HA, Schneider LC, Sicherer SH, Sidbury R, Spergal J, Stukus DR, Venter C, Boyce JA. Addendum Guidelines for the Prevention of Peanut Allergy in the United States: Report of the National Institute of Allergy and Infectious Diseases-Sponsored Expert Panel. *JAAPA* 2017;30:1-5.

2. Das SK, Chisti MJ, Sarker MHR, Das J, Ahmed S, Shahunja KM, Nahar S, Gibbons N, Ahmed T, Faruque ASG, Rahman M, **Fuchs GJ**, Mamun AA, Baker PJ. Long-term impact of changing childhood malnutrition on rotavirus diarrhoea: two decades of adjusted association with climate and socio-demographic factors from urban Bangladesh. *PlOS ONE* 2017;12:e0179418.

3. **Fuchs GJ**. The future of the discipline of infectious diseases. (letter) *Clin Infect Dis* 2017;65:1597-98.

4. **Fuchs GJ**. Imported infant formula not reviewed by FDA may pose health risks. *AAP News* 2018;39:12.

5. **Fuchs GJ.** Efficacy of reslizumab for eosinophilic esophagitis. (letter) *JPGN* 2018;67:E86.

6. Ofei SY, **Fuchs GJ**: Constipation burden in children with autism spectrum disorder: emergency department and healthcare use. *J Pediatr* 2018;202:12-13.

7. Ashraf H, Alam NH, Sultana M, Jahan SA, Begum N, Farzana S, Chisti MJ, Kamal M, Shamsuzzaman A, Ahmed T, Khan JAM, **Fuchs GJ**, Duke T, Gyr N. Day clinic vs. hospital care of pneumonia and severe malnutrition in children under five: a randomised trial. *Trop Med Int Health.* 2019;24:922-31. doi: 10.1111/tmi.13242

8. Ofei SY, **Fuchs GJ**. Gastroenterology. In: *Handbook of Interdisciplinary Treatments for Autism Spectrum Disorder*. R. D. Rieske (ed.). Springer Nature Switzerland AG 2019. Pp297-307.

9. Greer FR, Sicherer SH, Burks AW; **Committee on Nutrition**; Section on Allergy and Immunology. The Effects of Early Nutritional Interventions on the Development of Atopic Disease in Infants and Children: The Role of Maternal Dietary Restriction, Breastfeeding, Hydrolyzed Formulas, and Timing of Introduction of Allergenic Complementary Foods. *Pediatrics* 2019 Apr;143(4):e20190281. doi: 10.1542/peds.2019-0281. Epub 2019 Mar 18. PMID: 30886111.

10. Muth ND, Dietz WH, Magge SN, Johnson RK, American Academy of Pediatrics; Section on Obesity; **Committee on Nutrition**; American Heart Association. Public Policies to Reduce Sugary Drink Consumption in Children and Adolescents. *Pediatrics*. 2019 Apr;143(4). pii: e20190282. PMID: 30910915.

11. Bernstein AS, Oken E, de Ferranti S, AAP COUNCIL ON ENVIRONMENTAL HEALTH, AAP **COMMITTEE ON NUTRITION**. Fish, Shellfish, and Children's Health: An Assessment of Benefits, Risks, and Sustainability. Pediatrics. 2019;143(6): e20190999.

12. Bray AE, Ahmed S, Das SK, Khan SH, Christi MJ, Faruque ASG, Ahmed T, **Fuchs GJ**. Viral pathogen-specific clinical and demographic characteristics of children with moderate-to-severe diarrhea in rural Bangladesh. *Am J Trop Med Hyg* 2019;101:304-9.

13. Baker-Smith CM, de Ferranti SD, Cochran WJ; **Committee on Nutrition**; Section on Gastroenterology, Hepatology, and Nutrition. The Use of Nonnutritive Sweeteners in Children. *Pediatrics* 2019 Nov;144(5). pii: e20192765. doi: 10.1542/peds.2019-2765.

14. American Academy of Pediatrics **Committee on Nutrition**. *Pediatric Nutrition* ("Yellow Book"). Kleinman RE and Greer FR (eds). 8th ed. Itasca, IL: American Academy of Pediatrics; 2019.

15. **Fuchs GJ**, contributor, *Pediatric Nutrition* ("Yellow Book"). Kleinman RE and Greer FR (eds). 8th ed. Itasca, IL: American Academy of Pediatrics; 2019.

16. Ofei SY, **Fuchs GJ**. Principles and Practice of Oral Rehydration. *Curr Gastroenterol Rep*. 2019;7;21:67-72. doi: 10.1007/s11894-019-0734-1.

17. **Fuchs GJ**. Is it OK to buy imported formulas online? AAP, healthychildren.org, https://www.healthychildren.org/English/tips-tools/ask-the-pediatrician/Pages/Is-it-OK-to-buy-imported-formulas-online.aspx, Feb 26, 2020.

18. Preliminary Technical Report: The Martin County Kentucky Community-Engaged Drinking Water Health Pilot Study. Unrine, JM, Sanderson WT, Christian WJ, Pennell K, Ormsbee L, Hoover A, **Fuchs GJ**. 2020.

19. Coauthor. Referrals to a Registered Dietitian Nutritionist Primary Care Provider Toolkit. Academy of Nutrition and Dietetics, 2020. https://www.eatrightpro.org/payment/getting-started/referrals-and-primary-care-partnership/referrals-to-a-registered-dietitian-nutritionist-primary-care-provider-toolkit

20. Chowdhury F, Bin Shahid ASMS, Tabassum M, Parvin I, Ghosh PK, Hossain MI, Alalm NH, Faruque ASG, Huq S, Shahrin L, Homaira N, Hassan Z, Akhtar Z, Mah-E-Muneer S, **Fuchs GJ**, Ahmed T, Chisti MJ. Vitamin D supplementation among Bangladeshi children under-five years of age hospitalised for severe pneumonia: a randomised placebo controlled trial. *PLoS One* 2021;16:e0246460. doi: 10.1371/journal.pone.0246460.

21. Sultana M, Alam NH, Faruque ASG, **Fuchs GJ**, Gyr N, Chisti MJ, Duke T, Ahmed T, Gold L. Household economic burden of childhood severe pneumonia in Bangladesh: A cost-of-illness study. *Arch Dis Child* 2021;106:539-546.

22. Parker MG, Stellwagen LM, Noble L, et al; AAP Section on Breastfeeding, **Committee on Nutrition**, Committee on Fetus and Newborn. Promoting Human Milk and Breastfeeding for the Very Low Birth Weight Infant. *Pediatrics*. 2021;148(5):e2021054272.

23. Kamal I. A. Chowdhury KIA, Jabeen I, Rahman M, Faruque ASG, Alam NH, Ahmed T, **Fuchs GJ**, Duke T, Gyr N, Sarma H. Barriers to seeking timely treatment for severe childhood pneumonia in rural Bangladesh. Arch Dis Child 2022;107:436-440.

24. Radulescu A, Dugan AJ, Killian M, Attia S, Mouzaki M, **Fuchs G**, Kohli R, Bada H, Kern P, Softic S. Stratification by obesity class rather than age, can identify a higher percent of children at risk for non-alcoholic fatty liver disease and metabolic dysfunction. *Pediatr Obes* 2022;17:e12862. https://doi.org/10.1111/ijpo.12862.

25. Ofei SY and **Fuchs GJ**. Fluid and electrolytes. In: *Pediatric Nutrition in Practice*. Koletzko B, Bhutta ZA, Dhansay MA, Makrides M, Orsi M, Cai W, Duggan CP (eds): Pediatric Nutrition in Practice. 3rd Edition. Ofei SY, Fuchs GJ. 1.3.6 Fluid and Electrolytes. World Rev Nutr Diet. 2022;124:75-80. doi: 10.1159/000516731. Epub 2022 Mar 3. PMID: 35240630.

26. Brar S, Haugh C, Robertson N, Owuor PM, Waterman C, **Fuchs GJ**, Attia SL. The impact of Moringa oleifera leaf supplementation on human and animal nutrition, growth, and milk production: A systematic review. *Phytotherapy Research* 2022;1600–15.

27. Nasrin S, Tariqujjaman Md, Sultana M, Zaman RA, Shahjahan A, Chisti MJ, Faruque ASG, Ahmed T, **Fuchs GJ**, Gyr N, Alam NH. Factors associated with community acquired severe pneumonia in under five children in Dhaka, Bangladesh: a case control analysis. *PLOS One* 2022 Mar 23;17(3):e0265871. doi: 10.1371/journal.pone.0265871. PMID: 35320317.

28. Hand I, Noble L, Abrams SA; AAP Committee on Fetus and Newborn, Section on Breastfeeding, **Committee on Nutrition**. Vitamin K and the Newborn Infant. *Pediatrics* 2022; 149: 58-61. 2022;149(3):e2021056036

29. Creech CB, Anderson E, Berthaud V, Yildirim I, Atz AM, Melendez Baez I, Finkelstein D, Pickrell P, Kirstein J, Yut C, Blair R, Clifford RA, Dunn M, Campbell JD, Montefiori DC, Tomassini JE, Zhao X, Deng W, Zhou H,

Ramirez Schrempp D, Hautzinger K, Girard B, Slobod K, McPhee R, Pajon R, Das R, Miller JM, Schnyder Ghamloush S; **KidCOVE Study Group**. Evaluation of mRNA-1273 Covid-19 Vaccine in Children 6 to 11 Years of Age. *N Engl J Med*. 2022;386:2011-23.

- Largest pediatric mRNA vaccine trial conducted to date (as of June 2022).
- Basis for emergency use authorization for mRNA-1273 (COVID-19 vaccine) in children age 6 months to 11 years.

30. Murphy MO, Lee TM, Ryzowicz TA, **Fuchs GJ**. Restricted Diets. In: *Pediatric Nutrition for Dietitians*. Goday PS, Walia CLS (eds). CRC Press, Boca Raton 2022. ISBN 9780367705046

31. Mogaka JN, Owuor PM, Odhiambo Silvia, Waterman C, McGuire MK, **Fuchs GJ**, Attia SL. Investigating the Impact of Moringa oleifera Supplemented to Kenyan Breastfeeding Mothers on Maternal and Infant Health: A Cluster Randomized Single-Blinded Controlled Pilot Trial Protocol. *JPGN Reports* (2022) 3:3(e237).

32. Anderson EJ, Creech B, Berthaud V, Piramzadian A, Johnson KA, Zervos M, Garner F, Griffin C, Palanpurwala K, Turner M, Gerber J, Bennett RL, Ali K, Ampajwala M, Berman G, NayakJ, Chronis C, Rizzardi B, Muller W, SmithCA, **Fuchs G**, Hsia D, Tomassini JE, DeLucia D, Reuter C, Kuter B, Zhao X, Deng W, Zhou H, Ramirez Schrempp D, Hautzinger K, Girard B, Slobod K, McPhee R, Pajon R, Aunins A, Das R, Miller JM, Schnyder Ghamloush S, on behalf of the **KidCOVE Study Group**. Evaluation of mRNA-1273 Vaccine in Children 6 Months through 5 Years of Age. *New England Journal of Medicine* (in press).

Submitted/in preparation:

1. Sultana M, Alam NH, Faruque ASG, **Fuchs GJ**, Gyr N, Chisti MJ, Duke T, Ahmed T, Gold L. Economic evaluation alongside the cluster-randomised controlled trial of Day care versus Existing care management of severe childhood pneumonia with or without malnutrition in Bangladesh: study protocol. *Submitted*

2. Ali, S, Tariquzzaman M, Sultana M, Nasrin S, Chisti MJ, Alam NH, Ahmed T, Faruque ASG, Gyr N, **Fuchs GJ**. Incidence, case fatality, mortality, and healthcare-seeking behavior of children with severe pneumonia in rural Bangladesh. (in preparation).

3. Alam NH, Faruque ASG, Ashraf H, Chisti MJ, Ahmed T, Sultana M, Zaman K, Ali S, Shahnawaz, Nasrin S, Tariqujjaman, Ehsan, Amin R, Mollah AH, Kabir L, Shahidullah, Khanam W, Islam K, Kim M, Duke T, Gyr N, **Fuchs GJ**. Effectiveness, safety and economic viability of daycare versus usual care management of severe pneumonia with or without malnutrition in children within the existing health system of Bangladesh: a cluster randomized controlled trial. (Submitted)

4. AAP Clinical Report: Older Infant- Young Child Formulas. **Fuchs GJ**, Abrams S, Amevor A. (in preparation)

5. Haugh C, Attia S, Brar S, Robertson J, DiTommaso L, **Fuchs G**, Schadler A, Radulescu A, Attia SL. Cardiometabolic Risk Factors in South American Children: A Systematic Review and Meta-Analysis. (submitted)

6. Sultana M, Watts JJ, Alam NH, Faruque ASG, **Fuchs GJ**, Gyr N, Ali N, Chisti MJ, Ahmed T, Abimanyi-Ochom J, Gold L. Cost of childhood severe pneumonia management in selected health facilities in Bangladesh: a provider perspective. (submitted)

BIOGRAPHICAL SKETCH

NAME: Zhigang Li

eRA COMMONS USER NAME (credential, e.g., agency login): ZHIGANGL

POSITION TITLE: Associate Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Nankai University, Tianjin, China	BS	06/2001	Mathematics
Nankai University, Tianjin, China	MS	06/2004	Probability theory
Auburn University, Auburn, AL	MPS	08/2006	Statistics
Columbia University, New York, NY	PHD	10/2010	Biostatistics

A. Personal Statement

I am a tenured associated professor in the Department of Biostatistics at the University of Florida. My research interests include microbiome data modeling, mediation data analysis, high-dimensional data analysis, generalized estimation equations, mixed-effect models and structural equation modeling (SEM). I am currently the PI of an NIH-funded R01 grant (R01GM123014) to study human microbiome mediating disease-leading causal pathways in children. I am also the PI of the Data Science Core of a P01 grant (P01AA029543) to evaluate interventions including a probiotic intervention to improve alcohol-related comorbidities along the gut-brain axis in persons with HIV infection. I was also a project PI of the molecular epidemiology center (P20GM104416) to investigate mediation effects of high-dimensional mediators. I was the PI of an R03 grant (2014-2016) from NIH to develop modeling approaches for jointly analyzing survival data and longitudinal quality of life (QOL) data in palliative care clinical trials. Prior to that, I was the contact-PI of a two-year pilot grant (2012-2014) from the NCI-designated Norris Cotton Cancer Center to develop joint analysis models for survival data and longitudinal QOL data. Ongoing and recently completed projects that I would like to highlight are provided below:

R01GM123014 (Li) 09/12/17-07/31/22 NCE
NIGMS
Mediation Analysis Methods to Model Human Microbiome Mediating Disease-Leading Causal Pathways in Children
Role: PI

P01AA029543 (Cook) 9/10/2021-8/31/2026
NIAAA
Interventions to improve alcohol-related comorbidities along the gut-brain axis in persons with HIV infection
Role: PI of the Data Science Core

U24AA029959 (Wu, Cook) 9/22/2021-8/31/2026
NIAAA
Southern HIV and Alcohol Research Consortium Biomedical Data Repository
Role: co-I

R01AG068128 (Armstrong) 7/1/2020-6/30/2025
NIDA
Identifying Factors Predicting ACcurately End-of-Life in Dementia with Lewy Bodies and Promoting Quality End-of-Life Experiences: the PACE-DLB Study
Role: Co-I

R01AG071729 (Wang) 2/15/2022-11/30/2026
NIA
Real-Time and Long-Term Effects of Medical Marijuana on Older Adults: A Prospective Cohort Study
Role: co-I

(El-Jawahri) 7/1/2018-6/30/2023
American Cancer Society – Research Scholar Grant
Randomized Trial of a multimodal sexual dysfunction intervention for hematopoietic stem cell transplant survivors
Role: UF Subaward PI

UG3DE030433 (Fillingim, Ribeiro-Dasilva) 7/1/2022- 6/30/2028
NIDCR
Photobiomodulation for the Management of Temporomandibular Disorder Pain
Role: Co-I

T32NS082168 (Bowers) 7/1/2020-6/30/2025
NINDS
Interdisciplinary Training in Movement Disorders and Neurorestoration
Role: Co-I

R21CA264927 (Jobin, El Haddad) 5/10/2022- 2/29/2024
NCI
Bacteriophage-mediated microbiota modification to prevent colorectal cancer development
Role: Co-I

Selected Completed Research Support
R01DA042069 (Cook) 04/01/2019- 3/31/2021
NIDA
Alzheimer's-gut-microbiome grant supplement for MAPLE
Role: co-I

B. Positions and Honors

Positions and Employment

08/2018-present	Associate Professor (University Preeminence hire), Department of Biostatistics, College of Public Health & Health Professions, University of Florida, Gainesville, FL
08/2018-present	Associate Member, UF Health Cancer Center, University of Florida, Gainesville, FL
08/2018-present	Adjunct Associate Professor, Department of Epidemiology, Geisel School of Medicine at Dartmouth, Hanover, NH
02/2018-7/2018	Associate Professor, Department of Biomedical Data Science, Department of Epidemiology, Department of Community and Family Medicine, Geisel School of Medicine at Dartmouth, Hanover, NH
2011-01/2018	Member, Norris Cotton Cancer Center, NH
2010-01/2018	Assistant Professor, Department of Community and Family Medicine, Department of Biomedical Data Science (2015-2018), Department of Epidemiology (2017-2018), Geisel School of Medicine at Dartmouth College, Hanover, NH
2007-2010	Biostatistician, Columbia Center for Children's Environmental Health, Columbia University, New York, NY

Other Experience and Professional Memberships

2022	Educational Advisory Committee, ENAR2023 conference
07/2022	Reviewer in NIH Special Emphasis Panel ZRG1 PSE-F(02)
03/2022	Reviewer in NIH IRAP Study Section: Infectious Diseases, Reproductive Health, Asthma and Pulmonary Conditions
07/2021	Reviewer in NIH Study Section: NIH Special Emphasis Panel ZHL1 CSR-A (O2)

01/2021	Department of Defense DMDRP grant review panel
07/2020	Reviewer in NIH Study Section: Special Emphasis Panel ZDK1 GRB-G (O4)
02/2020	Department of Defense PHP-2 grant review panel
2019	Department of Defense PSAAD grant review panel, DC
2019	Hitchcock foundation grant review, NH
2019	UF Health Cancer Center CPS_CTHR Collaborative Pilot Program, University of Florida, FL
11/2018	Reviewer in NIH Study Section: Special Emphasis Panel ZHL1 CSR-R (F3)
02/2018	NIH ECHO Opportunities and Infrastructure Fund review panel
04/2017	The PRESTIGE post-doc fellowship program reviewer, Campus France
05/2016,17	Laura and John Arnold Foundation external reviewer, Houston, Texas
08/2015,16	Cancer Center Developmental Funds Review Panel, Norris Cotton Cancer Center, NH
02/2019-present	Associate editor: *Surgery*
2013-2020	Associate editor: *Frontiers in Public Health*
2013- present	Associate editor: *BioData Mining*
2011- 2017	Clinical Cancer Review Committee, Norris Cotton Cancer Center, NH
2017- present	Member, Association for the Advancement of Artificial Intelligence
2017- present	Member, American Public Health Association
2011- present	Member, International Chinese Statistical Association
2010- present	Member, Eastern North American Region/International Biometric Society
2009- present	Member, American Statistical Associations

Honors

2022	Outstanding Student Support Award, Dept of Biostatistics, University of Florida, Gainesville, FL
2019	Outstanding Teaching Award, Dept of Biostatistics, University of Florida, Gainesville, FL
2018-present	University Preeminence Faculty Hire, University of Florida, Gainesville, FL
2006-2010	PhD student fellowship, Columbia University
1996	First Prize, Chinese National Mathematical Olympiad

C. Contribution to Science (*corresponding author, #co-first author)

1. **Microbiome analysis.**

- Wu Q, O'Malley J, Datta S, GharaiBeh RZ, Jobin C, Coker M, Hoen AG, Christensen BC, Madan JC, Karagas MR, Li Z* (2022) MarZIC: A Marginal mediation model for Zero-Inflated Compositional mediators with applications to microbiome data. *Genes* Vol 13 Issue 6
- Li Z*, Tian L, O'Malley J, Karagas MR, Hoen AG, Christensen BC, Madan JC, Wu Q, Gharaibeh RZ, Jobin C, Li H (2021) IFAA: Robust association identification and Inference For Absolute Abundance in microbiome analyses. *Journal of the American Statistical Association*
- Jie Zhou, Weston D. Viles, Boran Lu, Zhigang Li, Juliette C. Madan, Margaret R. Karagas, Jiang Gui, Anne G. Hoen (2020) Identification of microbial interaction network: zero-inflated latent Ising model based approach. *BioData Mining*
- Li Z*, Lee K, Karagas M, Madan J, Hoen A, O'Malley AJ and Li H (2018) Conditional regression based on a multivariate zero-inflated logistic normal model for modeling microbiome data. *Statistics in Biosciences* 10(3):587-608. PMCID: PMC6432796
- Modupe O. Coker, Hannah E. Laue, Anne G. Hoen, Margaret Hilliard, Erika Dade, Zhigang Li, Thomas Palys, Hilary G. Morrison, Emily Baker, Margaret R. Karagas and Juliette C. Madan (2021) Infant Feeding Alters the Longitudinal Impact of Birth Mode on the Development of the Gut Microbiota in the First Year of Life. Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2021.642197
- Coker MO, Hoen AG, Dade EF, Lundgren S, Li Z, Wong A, Zens MS, Palys T, Morrison HG, Sogin ML, Baker E, Karagas MR, Madan JC (2020) Specific class of intrapartum antibiotics relate to maturation of the infant gut microbiome: a prospective cohort study. BJOG: An International Journal of Obstetrics and Gynaecology 127(2):217-227 PMCID: PMC6803026

- Yaohua Yang, Wei Zheng, Qiuyin Cai, Martha J. Shrubsole, Zhiheng Pei, Robert Brucker, Jennifer Sonderman, Mark Steinwandel, Seth R. Bordenstein, Zhigang Li, William J. Blot, Xiao-Ou Shu, Jirong Long (2019) Cigarette Smoking and Oral Microbiome in Low-income and African American Populations. *Journal of Epidemiology & Community Health*
- Hoen A, Madan J, Li Z, Coker M, Lundgren S, Morrison H, Palys T, Jackson B, Sogin M, Cottingham K, Karagas M (2018) Sex-specific gut microbiome impacts of infant arsenic exposure in a US population. *Scientific Reports* 8

2. **Developing modeling approaches for analyzing survival data and longitudinal data**.

- Li Z* and McKeague IW (2013) Power and sample size calculations for generalized estimating equations via local asymptotics. *Statistica Sinica* 23, 231-250 PMCID: PMC3903421
- Li Z*, Tosteson TD and Bakitas M (2013) Joint modeling quality of life and survival using a terminal decline model in palliative care studies. *Statistics in Medicine* 32(8):1394-406 PMCID: PMC3623280
- Li Z*, McKeague IW and Lumey LH (2014) Optimal design strategies for sibling studies with binary exposures. *The International Journal of Biostatistics* 10: 185-96 PMCID: PMC4412035
- Li Z*, H. R. Frost, Tor Tosteson, Lihui Zhao, Lei Liu, Kathleen Lyons, Huaihou Chen, Bernard Cole, David Currow, Marie Bakitas (2017) A Semiparametric Joint Model for Terminal Trend of Quality of Life and Survival in Palliative Care Research. *Statistics in Medicine* 1 36(29):4692-4704 PMCID: PMC5698117

3. **Mediation analysis in children's environmental health research**

- Gilbert-Diamond D, Li Z, Perry A, Spencer S, Gandolfi AJ and Karagas MR (2013) A population-based case–control study of urinary arsenic species and squamous cell carcinoma in New Hampshire, USA. *Environmental Health Perspectives.* 121:1154-1260 PMCID: PMC3801199
- Fei DL, Koestler DC, Li Z, Giambelli C, Sanchez-Mejias A, Gosse JA, Marsit CJ, Karagas MR and Robbins DJ (2013) Association between *In Utero* arsenic exposure, placental gene expression, and infant birth weight: a US birth cohort study. *Environmental Health* July 16; 12:58 PMCID: PMC3733767
- Davis MA, Li Z, Gilbert-Diamond D, MacKenzie T, Cottingham KL, Jackson BP, Lee JS, Baker ER, Marsit CJ and Karagas MR (2014) Infant toenails as a biomarker of *in utero* arsenic exposure. *Environmental Health* 24(5): 467–473 PMCID: PMC4141012
- Lesseur C, Armstrong DA, Paquette AG, Li Z, Padbury JF and Marsit CJ (2014) Maternal obesity and gestational diabetes are associated with placental leptin DNA methylation. *The American Journal of Obstetrics & Gynecology* 211(6):654. e1-9 PMCID: PMC4254188

a)**Mediation analysis in psychology and behavioral science.**

- Gibbons F, Roberts M, Gerrard M, Li Z, Beach S, Simons R, Weng C-Y and Philibert R (2012) The impact of stress on the life history strategies of African American adolescents: cognitions, genetic moderation, and the role of discrimination. *Developmental psychology* 48: 722-739. PMCID: PMC4324554
- O'Hara RE, Gibbons F, Gerrard M, Li Z and Sargent J (2012) Greater exposure to sexual content in popular movies predicts earlier sexual debut and increased sexual risk taking. *Psychological Science* 23(9):984-93 PMCID: PMC3779897
- O'Hara RE, Gibbons FX, Li Z, Gerrard M & Sargent J (2013). Specificity of early movie effects on adolescent sexual behavior and alcohol use. *Social Science & Medicine* 96: 200-207 PMCID: PMC3804111
- Emond J, Bernhardt A, Gilbert-Diamond D, Li Z and Sargent J (2016) Commercial TV exposure, fast-food toy collecting, and family visits to fast food restaurants among families living in rural communities. *The Journal of Pediatrics* 168:158-163 PMCID: PMC4698028

Informed Consent Form
Next generation ORS: ORS with calcium

icddr,b protocol # PR-22091	Version No. 1.0	Version Date: October 19, 2022

Protocol Title
Next generation ORS: Randomized controlled trial comparing ORS with calcium vs standard ORS in reducing severity with acute watery diarrhea of adults.

Investigators' names
Dr. Shafiqul A Sarker (Local PI)
Dr. Dr. Sam Cheng (External PI)
[List of other investigators is available on request]

Funder
The funder of this research is the National Institute of Health, USA.

Organizations
International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,
University of Florida, Gainesville, *USA*
University of Kentucky, Lexington, USA

Conflicts of interest
The Principal Investigators, Dr. Sam and Dr. Sarker, and the other research team members, have no conflicts of interest to declare.

Since you have diarrhoea, and admitted in the Short Stay Unit or Longer Stay Unit, you are being asked to provide informed consent. Unless otherwise specified, the words "you" and "your" refer to you (i.e., the person participating in the research). The words "we" or "us" refer to study workers and investigators.

Purpose of the research
We invite you to take part in a clinical trial. Clinical trials are research studies that test if new ways to detect, prevent, or treat diseases work well, are safe, and whether they lead to any unexpected outcomes.

Treatment of infectious diarrhea as remains a global challenge. Oral Saline and normal feeding are the most important parts of treatment of diarrhoeal diseases. These simple and inexpensive treatment saves millions of lives each year, but they do not shorten the duration of illness. Diarrhea causes losses of several ions from the body (one kind of salt like Na, K, Hco3 and minerals like Zn & Ca). The losses of salts are replaced through rehydration therapy and adding zinc but information on the role of Ca in diarrhea is limited. Recently preliminary studies in humans show that, with replacement of Ca^{++}, diarrhea was both promptly and dramatically reduced in both animals and humans at the University of Florida. However, so far, no formal randomized controlled trials (RCTs) on Ca^{++} replacement in diarrheal patients have been performed. At icddr,b (Cholera Hospital) we are carrying out a research study to examine if treatment with ORS and Ca reduce the severity and/or duration of diarrhoea in Bangladeshi population.
Up to 396 participants will be asked to join this study.

Invitation to participate
You are being invited to join this study because:

- Adult male
- Non-pregnant adult female
- Age between 18 and 60 years
- History of acute watery diarrhea with signs of some OR severe dehydration
- No bloody diarrhea

- No signs of systemic infection that needed intravenous antibiotics

Methods and procedures
If you agree to our proposal of enrolling you, we will ask some questions about your health condition related to the current illness and before this illness; perform a thorough physical examination. Measure your weight, height, count pulse and respiratory rates, body temperature and record findings.

A fresh stool specimen will be collected for detection of cholera. After initial rehydration and randomization, all cholera patients will receive antimicrobial therapy for cholera while all non-cholera patients will not. Participants will consume the assigned ORS (standard ORS or the investigational ORS) according to standard of care WHO/icddr,b guidelines, immediately after completion of rehydration and continued until cessation of diarrhea or 72 h after randomization, whichever comes first. Which of the products you will receive will depend on a process called "Randomization" (like flipping a coin) that will give you an equal chance to receive either of them. Neither any one of us nor you know which of the product you will have received. It will be disclosed only after analysis of the results.

Two and half millilitre (half of a teaspoon) of venous blood will be collected only on enrolment for various laboratory tests that will allow us to assess disease condition and test results may help the doctors to treat you. A repeat sample will be drawn only when clinically indicated. A urine sample also will be collected for laboratory asses.

If diarrhoea ceased, you will be discharged. Continuation of diarrhoea beyond 72 hours or consume more than 30 L of the assigned ORS will constitute treatment failure and such cases you will be withdrawn from the study and will be treated according to the standard protocols of the hospital

Risks and Potential benefits
We do not expect any problem from ingesting the test product. You will be monitored closely for signs of any injury to health and referred for lab testing and medical care if necessary in the same manner as you would ordinarily obtain any other medical treatment. At the time of collection of blood, you will feel a momentary pain due to needle prick. There also is a rare chance of bluish discolouration (bruish) surrounding the prick site, due to mild leaking of blood in the skin, which may result in a small bruise or swelling of the skin, and there is a very low chance of bleeding or infection. However, we will take required precaution including the use of disposable syringes and needles to prevent these problems and only well-trained personnel will collect this sample. In the event you may develop any of these problems, we will provide the best possible treatment at the Dhaka hospital of icddr,b at our own cost.

You may benefit from the medical care that will be provided by the study up to next 7 days. The medical care provided to you during this period will be free of charge. If you require treatment for any injuries or illness related to your participation in the study, you should contact the investigators or icddr,b immediately. The information we learn may help your community and in similar places around the world by providing important information on whether the this ORS with Ca is effective to reduce diarrhoea severity and duration.

Privacy, anonymity and confidentiality
We will respect your privacy. No identifying information about you will be given to anyone or be published without your permission, unless the law requires us to do this. If you join this study, we will collect only the information we need for this study including some personal information that identifies you (name, address, phone number) for the purposes of contacting you. Data and samples related to the study will only be accessed by the research team or by people or institutions authorized by the Principal Investigators. Data Safety Monitoring Board (DSMB) that will particularly look at the reliability of data and safety of the test products, and the Ethical Review Committee (ERC) of icddr,b will have access to those information, which will be kept in a secured place under the supervision of the principal investigator of the study. However, we would also like to inform you that disclosure of such information is subject to the laws of Bangladesh. At the time of publishing the results of this study, we will not use your name or identity.
Signing this consent form does not give up your legal rights and does not change the legal and professional responsibilities of the study doctor(s) or involved institutions.

Future use of information
Your data collected during this study will be kept by the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), according to institutional policies for at least five years after the main study report is published. Data will only be used for scientific purposes to benefit society.

Right not to participate and withdraw
Participation is voluntary, meaning it is your choice to take part or not in this study. You can change your mind at any time. You will also be able to withdraw consent of participation at any time during the study without paying any penalty or effect on your/your family member treatment at the Dhaka hospital in future. If you decide to leave the study, you can contact the Principal Investigator or a member of the study team to let them know. The investigators will also have the right to withdraw you from the study if it is deemed to be in your best interest.

Principle of compensation
We will reimburse you up to a maximum of 400 BDT per visit for transportation costs between your home and icddr,b for wage loss.

Communication
If you have any question you can ask me right now or at any time later on. If you want to know anything about rights and benefits for participation in this study you can ask Mr M A Salam Khan, IRB Coordinator. The addresses are as follows:
The addresses are as follows:

Purpose of contact	Name and address	Address for communication
For any question related to the study, or any problem	Local PI :Shafiqul Alam Sarker	Address: Nutrition and Clinical Services Division, icddr,b, 68 Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka 1212 Mobile No.+8801713039813 (to be open 7/24 hours)
To know the rights or benefits or to log any complaint or dissatisfaction	M A Salam Khan (IRB Coordinator)	IRB Secretariat, Research Administration, icddr,b, Mohakhali, Dhaka-1212 Phone: (+88-02) 9827084 or Mobile: 01711428989

I am giving my consent to the investigator to join this study ☐ Yes ☐ No

I am giving my consent to the investigator to obtain photos and videos ☐ Yes ☐ No

I am giving my consent to give my provided information to other researchers on condition that my identifiable information cannot be disclosed or shared with anybody. ☐ Yes ☐ No

I am giving consent to be contacted about additional research opportunities in the future. ☐ Yes ☐ No

Participant's name	Participant's signature or thumbprint	Date (DD/MMM/YYYY)

Name of witness to the consent discussion	Signature of witness	Date (DD/MMM/YYYY)

Name of person who explained consent (representative of the PI)	Signature of person who explained consent	Date (DD/MMM/YYYY)

Check-list for Submission of Research Protocol
For Consideration of the Research Review Committee (RRC)
[Please check all appropriate boxes]

1. Has the proposal been reviewed, discussed and cleared by all listed investigators?

   ☐ Yes ☐ No

   If the response is No, please clarify the reasons:

2. Has the proposal been peer-reviewed externally?

   ☐ Yes ☐ No ☐ External Review Exempted

   If the response is 'No' or "External Review Exempted", please explain the reasons:

   If the response is "Yes", please indicate if all of their comments have been addressed?

   ☐ Yes (please attach)

   ☐ No (please indicate reason(s)):

3. Has the budget been reviewed and approved by icddr,b's Finance Office?
   ☐ Yes ☐ No (reason): _____

4. Has the **Valid** Ethics Certificate(s) been attached with the Protocol?

   ☐ Yes ☐ No
   If the answer is 'No', please explain the reasons:

5. Has the Bangla & English Information Sheets and Consent Forms been attached with the protocol?

   ☐ Yes ☐ No ☐ Not applicable

6. Has the Bangla & English Tools/Questionnaire been attached with the protocol?

   ☐ Yes ☐ No ☐ Not applicable

7. Has the list of abbreviations been attached with the Protocol?

   ☐ Yes ☐ No
   If the answer is 'No', please explain the reasons:

_____ _____
Signature of the Principal Investigator Date

College of Medicine
Department of Pediatrics

1600 SW Archer Rd
PO Box 100296

Gainesville, FL 32610-0296
(352) 392-3337
(352) 392-0481 fax

September 21, 2022

Dr. Md. Shafiqul Alam Sarker
ICDDR, B
Bangladesh

RE: Next Generation ORS: Randomized controlled trial comparing ORS with calcium vs standard ORS in reducing severity of adults with acute watery diarrhea

Impact score: 20

Dear Dr. Sarker,

I am so excited for the favorite result by the reviewers on the aforementioned NIH grant clinical trial and anticipate to work with you on this project if funded

Sincerely,

Sam X Cheng, MD, MSc, PhD
Associate Professor
Pediatrics, Gastroenterology, Hepatology, and Nutrition

Department of Pediatrics

PO Box 100296
Gainesville, FL 32610-0296
(352) 392-3337
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