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Evaluation of the Therapeutic Management of Childhood Diarrhea

Ana F. Diallo PhD

University of Connecticut, 2016

Therapeutic management of childhood diarrhea had led to a significant decrease in diarrhea related morbidity and mortality. However, diarrhea diseases remain one of the leading cause of preventable deaths worldwide, especially in children under 5 years. The work presented in this dissertation intended to evaluate the current the management of childhood diarrhea, to identify the major gaps in the management and introduce a novel approach to address these gaps. First, an assessment of oral rehydration therapies (ORT) coverage in the management of childhood diarrhea is performed to evaluate healthcare providers' prescriptive practices of ORT in South Asia. Next, we assess the effect of breastfeeding cessation in reported episodes of diarrhea using a national database. The study highlighted suboptimal breastfeeding practices among American mothers who participated in the study and its impact on reported onset of diarrhea episodes in infants during their first year of life. The results show that breastfeeding cessation before the first 6 months is a significant predictor of diarrhea between 7 and 12 months. Lastly, sensitivity of a diarrheagenic pathogen identification tool was tested in two laboratory simulated environments. Despite the limitation of the experiments, the study offers insight of the tool's properties under specific environment, guiding future optimization. The manuscripts comprised in this dissertation provide evidence for the need to re-evaluate the use of therapies known to be effective and to conduct future research in innovative approach to the management of childhood diarrhea.

Evaluation of the Therapeutic Management of Childhood Diarrhea

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APPROVAL PAGE

Doctor of Philosophy Dissertation

Evaluation of the Therapeutic Management of Childhood Diarrhea

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2016

Allow yourself to dream,
And when you do dream big

Allow yourself to learn
And when you do learn all you can

Allow yourself to laugh
And when you do share your laughter

Allow yourself to set goals
And when you do reward yourself as you move forward

Allow yourself to be determined
And when you do you will find you will succeed

Allow yourself to believe in yourself
And when you do you will find self confidence

Allow yourself to lend a helping hand
And when you do a hand will help you.

Allow yourself relaxation
And when you do you will find new ideas.

Allow yourself love
And when you do you will find love in return

Allow yourself to be happy
And when you do you will influence others around you.

Allow yourself to be positive
And when you do life will get easier.

Catherine Pulsifer

Accomplishing the highest degree of education was the reason why I came to the United Sates. Now that I am at the final stage of my doctoral degree, words are not enough to express my gratitude to the people who helped me throughout this journey. Dr Jackie McGrath, an extraordinary mentor, my scientific mother, with a golden heart. Seven years ago, you saw in me a potential that I never believed I had. You immediately acknowledged and respected my cultural values and attachment to my motherland. With that in mind, you have always encouraged me to stay true to myself and supported me to find the resources I needed to accomplish the work. I could have not done it without your support and guidance.

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This work would have never been done without the support of my siblings, the unconditional love, support and sacrifice of by parents. You are not perfect, but I would have never asked for better role models. I could only die in peace if I can accomplish a third of the goodness you have spread around you through your hard work, generosity and kindred spirit. Lastly, I am thankful to my wonderful husband. My soulmate, your presence, patience and ontineous support helped me become a better person.

To Ibrahim and Idriss…

TABLE OF CONTENT

CHAPTER ONE

1.1 Childhood diarrhea: etiology and burden

Accounting for 9% of all death worldwide, approximately 578,000 children under the age of 5 die from infectious diarrhea each year (Liu et al., 2015). Childhood diarrhea is chiefly manifested as a symptom of intestinal tract infections. There are three major factors that must be considered when defining diarrhea: the frequency of stool output, stool consistency and stool weight (Pawlowski, Warren, & Guerrant, 2009). According to the World Health Organization (WHO), diarrheal diseases is defined as followed:

> "The passage of three or more loose or liquid stools per day, or more frequently than is normal for the individual."(World Health Organization, 2013)

Over the past 30 years, the therapeutic management of childhood diarrhea has led to a significant decrease in under-five mortality. It has been estimated that mortality rates related to diarrheal infections decreased by more than two fold, from 1.2 million in 2000 to half a million in 2015 (United Nations Children's Funds, 2016).[4] Even though this is a significant decrease, many of these deaths are preventable with appropriate management strategies.

While child mortality has significantly decreased, diarrheal infections remain one of the leading cause of death in children under the age of 5. Approximately 1.7 million episodes of childhood diarrhea are reported each year, with 1,600 deaths occurring daily (Liu et al., 2015). The majority of the burden caused by diarrheal diseases occur in low and middle income countries where pathogen transmission occurs mainly through overcrowding, poor sanitation, limited access to clean water and inadequate food hygiene (Leung, Chisti, & Pavia, 2016). South Asia and Sub Saharan Africa alone account for 90% of the total childhood mortality rate due to diarrhea (Liu et al., 2015).

Diarrhea is caused by a number of pathogens including bacteria, viruses, protozoa and helminths (Larson, Henning, Luby, & Faruque, 2009). A multiple sites birth cohort study (MAL-ED) was conducted in eight different countries in South America, Sub-Saharan Africa and South Asia between 2009 and 2014 to estimate the pathogen-specific burdens of diarrhea in children between 0 to 2 years of age (Platts-Mills et al., 2015). The researchers reported that cases of acute to persistent diarrhea in children under 2, were mainly attributed to norovirus, rotavirus, *Campylobacter spp,* astrovirus and *Shigella spp* (Platts-Mills et al., 2015). Factors such as age, location and climatic variability were also reported to have a significant impact on the incidence of diarrhea. The authors (2015) concluded that significant reduction in the incidence of childhood diarrhea, at the community level, could only be achieved by diversifying the diarrhea control programs rather than focusing on a single-pathogen strategies.

1.2 Classification of diarrhea

Due to financial and technical constraints, diarrheal infections are often managed on the basis of clinical symptoms rather than the identification of etiologic pathogens (Larson et al., 2009) Diarrheal diseases can be classified in three major categories based on the clinical presentations. They are: 1) acute watery diarrhea, 2) persistent diarrhea and 3) bloody diarrhea. Each category is physiologically different, hence requiring specific diagnosis and management.

Acute watery diarrhea, also described as secretory diarrhea, is characterized by symptoms lasting for less than 2 weeks (Pawlowski et al., 2009). It is mainly associated with small bowel infections resulting in a decrease sodium absorption and an increase mucosal permeability as well as chloride secretion (Pawlowski et al., 2009). The inflammatory process causes severe, acute dehydration and electrolyte imbalance.

Persistent diarrhea is diagnosed when an episode lasts for 2 to 4 weeks (Pawlowski et al., 2009). Commonly caused by the inflammation of the small bowel, this type of diarrhea is associated with nutritional malabsorption and chronic malnutrition. Children with persistent diarrhea accompanied with chronic malnutrition can suffer from stunting, vitamin A deficiency, other systemic infections and more severe diarrhea; setting them up in a viscious cycle (Keusch et al., 2006). That is the reason why persistent diarrhea significantly increases the case- fatality rates and have long-lasting impact on the quality of life (Keusch, Walker, Das, Horton, & Habte, 2016)

Bloody diarrhea, interchangeably called dysentery in the clinical setting, is commonly associated with dehydration and fever (Keusch et al., 2006) This type of diarrhea is characterized by traces of blood in the stool and is indicative of intestinal damage secondary to an inflammation of the colon or distal part of the small intestine (Keusch et al., 2006; Keusch et al., 2016; Pawlowski et al., 2009).

Based on these classifications, clinical management of childhood diarrhea has focused primarily on the prevention and reduction of dehydration with oral rehydration therapy (ORT), zinc supplementation and the maintenance of oral feeding, especially breastfeeding when possible (World Health Organization, 2005). These preventive measures have been proven to decrease stool output, frequency and duration of diarrhea episode. ORT and breastfeeding, mainly, are described as the major contributors to the significant drop in childhood diarrhea worldwide. The subsequent sections present, in more details, the properties and roles of ORT and breastfeeding in the management of childhood diarrhea.

1.3 Preventive and therapeutic management of childhood diarrhea

1.3.1 Oral rehydration therapy

The updated WHO guidelines on the clinical management of childhood diarrhea suggest the use of low osmolality ORT containing decreased glucose and sodium concentrations (World Health Organization, 2005). Studies demonstrated that the reduced osmolarity of oral rehydration was safer than the original formulation of ORT and has been reported to decrease stool output by 20% (Hahn, Kim, & Garner, 2002). Recommendations for healthcare providers include prescribing the solution volume based on the child's weight and presenting signs of dehydration.

Oral rehydration works through several different mechanisms of the GI track. Intestinal absorption of fluids and electrolytes results from ion transport processes across the trans-epithelial cell membrane (absorptive and secretory) and osmosis. Osmotic gradient is dictated by sodium (Na^+) transport (Figure 1) (Suh, Hahn, & Cho, 2010). Absorption of the organic solutes (sugar, oligopeptide and amino acid) is accompanied with the absorption of Na^+, which results in water absorption via osmosis (Field, 2003).

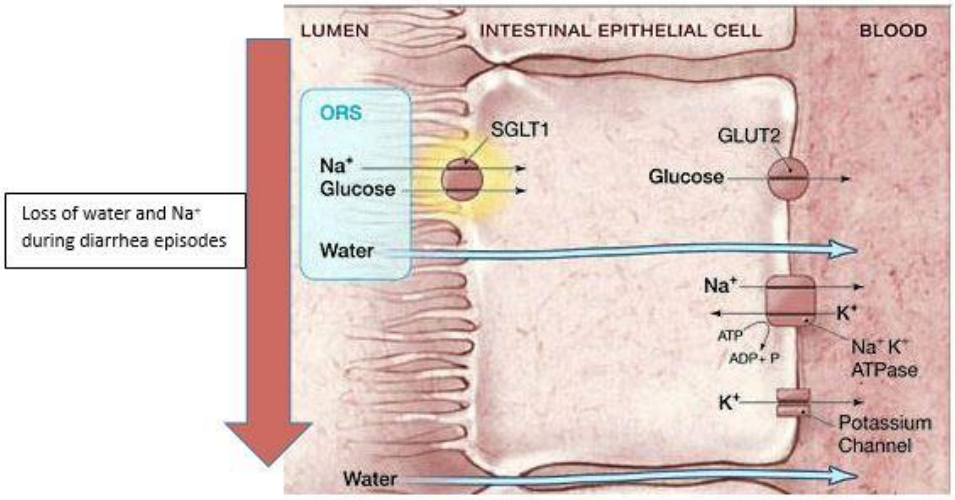

Figure 1. ORT mechanism. During an episode of diarrhea, there is massive loss of water and electrolytes mainly, sodium ions (Na^+). Coupled transport of sodium and glucose is preserved. Glucose facilitates Na^+ reabsorption (water follows) back into the cell on a 1:1 molar basis against concentration gradient through the glucose symporter, sodium-glucose transporter type 1 (SGLT1). Na^+ present in the epithelial cells is then pumped into the blood via adenosine triphosphate (Na^+K^+ATPase). Transport of glucose into the blood is mediated by the glucose transporter 2 (GLUT2). (Duggan, Fontaine, Pierce, & et al., 2004).

During an episode of diarrheal infection, there is disruption of these mechanisms, with the exception of the sodium absorption coupled with sugars and amino acids process (Suh et al., 2010). The integrity of the latter mechanism in diarrheal diseases was discovered in the 1960's and has been the theoretical foundation of oral rehydration therapy (ORT) (Hoque, Chakraborty, Sheikh, & Woodward, 2012) Considered the most important medical advance of the 20th century, ORT has been the cornerstone in the prevention and management of childhood diarrhea (Santosham et al., 2010).

Despite the success of the early diarrhea-control programs and the updated World Health Organization guidelines, many children under the age of 5 still do not receive adequate treatment during an episode of diarrhea. Recent reports indicated that only 40% of children suffering from diarrhea worldwide, received oral rehydration or increased fluid intake with continued feeding as part of their management (United Nation Children's Funds, 2016). It is important to note, that this degree of treatment is only 10% greater (approximately) than the 1995 global percentage of children under 5 years who received oral rehydration as treatment for their diarrhea episode (Fontaine et al., 2009). Even though ORT has been proven to be effective it is still not used routinely by all health professionals worldwide in the management of childhood diarrhea.

1.3.2 Breastfeeding

Since 1984, breastfeeding has been identified as one of the most important and cost-effective intervention, significantly preventing and reducing the incidence of infectious diseases in children, especially diarrhea and pneumonia (Field, 2003; Hoque et al., 2012; Suh et al., 2010). Human breastmilk contains bioactive molecules, mostly secretory antibodies, lactoferrin and glycans promoting protection and maturation of the gastrointestinal track and immune system during infancy and early childhood (Brandtzaeg, 2010; Lamberti, Fischer Walker, Noiman, Victora, & Black, 2011; Newburg, 2000; Zivkovic, German, Lebrilla, & Mills, 2011).

Exclusive breastfeeding during the first 6 months of life has been reported to offer the best immune protection against diarrheal diseases. In a systematic review of the literature, Lamberti and colleagues (2011) assessed the protective effect of optimal versus suboptimal breastfeeding practices on diarrhea associated childhood morbidity, mortality and hospitalization in low income countries. The study findings indicated that among infants between 0 and 5 months, those who were partially breastfed had 1.68 times (95% Confidence interval [1.03-2.76]) greater risk of having diarrhea compared to those who were exclusively breastfed (Lamberti et al., 2011). The risk of hospitalization from diarrhea was 6.05 times greater in infants between 6 and 11months who were not breastfed compared to those who were breastfed (Lamberti et al., 2011).

While the protective effect of breast milk is undeniable, significant gaps still exist in the promotion and practice of early breastfeeding initiation and continuation for the first 6 months of life, even in countries like the US. Non-exclusive or early interruption of breastfeeding, also known as suboptimal breastfeeding, was ranked in 2010 by the Global Burden of Disease Study as the second leading risk factor of disease burden in children under the age of 5 worldwide

regardless of whether the child was in a developed or under-developed country (Gaffney, Kitsantas, & Cheema, 2012).

1.4 Overall goal of the dissertation

Even with these known findings and recommendations about how to prevent and manage childhood diarrhea, a gap in the use of evidence-based practice strategies remains. The limited use of oral rehydration solutions and exclusive breastfeeding practices for more than three decades has been linked to the diversion of international funding towards malaria and AIDS after the incorporation of diarrhea-control programs into the integrated management of childhood illness (IMCI) approach (Fontaine et al., 2009). This incorporation of the diarrhea-control programs into the integrated management of childhood illness (IMCI) has led to inconsistencies in healthcare professionals training specific to diarrhea management (Fontaine et al., 2009).

Also lacking in the management of diarrhea in limited recourses settings is the diagnosis based on laboratory testing. Current clinical management of diarrheal episodes are mostly symptoms based (Kotloff et al., 2013). The diagnosis and treatment based only on clinical symptoms can be inaccurate and result in the unnecessary use of broad spectrum antibiotics (Pathak, Pathak, Marrone, Diwan, & Lundborg, 2011; Reddington, Tuite, Minogue, & Barry, 2014). There is, therefore, a critical need to develop a point-of-care pathogen identification tool enabling fast and accurate identification of diarrheal pathogens.

The overall goal of this dissertation is to address these gaps and introduce a novel approach in the management of diarrhea by healthcare professionals. The two studies reported in chapter two and three examine the limited use of interventions such as ORT and breastfeeding in the management of childhood diarrhea. The focus was only on these interventions because of

their scientifically proven efficacy, their low to no cost value and their known success for more than 30 years.

The two studies shed some lights on the limitations of the current diarrhea-control programs. A systematic review of the literature has been performed in chapter two, to assess the reported management of childhood diarrhea by trained physicians and pharmacists in South Asia; where coverage of ORT increased by only 5% from 2000 to 2015 (United Nation Children's Funds., 2016).

The study presented in chapter three explores the protective effect of breastfeeding on diarrhea outcomes during the first year of life, using a large cohort of infants in the United States. The findings of the secondary data analysis suggest statistically strong evidence for the protective effect of breastfeeding on the incidence of diarrheal episodes. The results also show that suboptimal breastfeeding remains a public health issue even in developed countries like the US.

Finally. The third study reports on the laboratory testing of a paper-based diarrheagenic pathogens identification tool. The aim of this pilot study is to evaluate the effectiveness of a new pathogen identification tool by determining it's efficacy in identifying a common diarrheagenic pathogen, E coli, under high temperature range; simulating the climate in the geographical areas with the highest morbidity and mortality rates due to diarrhea. Such a tool has the potential to help formulate accurate diagnosis and effective treatment of children suffering from diarrhea in a timely manner, thus reducing the impact of diarrhea on childhood morbidity and mortality.

1.5 Specific aims and hypotheses

Specific aim for study #1: To describe the use of ORT in the management of childhood diarrhea by physicians and pharmacists as reported in observational studies performed in South Asia. Hypothesis: In cases of childhood diarrhea, physicians and pharmacists will prescribe ORT in limited proportions. Healthcare professionals (mainly physicians, pharmacists, midwives and nurses) at the public and private levels play an important role in the management of childhood diarrhea. Recent studies performed in South India and Sub-Saharan Africa have shown that, regardless of receiving formal diarrhea management training, healthcare professionals treating children with diarrhea tended to prescribe more antibiotics, injections and anti-diarrheal medications than oral rehydration solutions and zinc (Pathak et al., 2011; Sood & Wagner, 2014).

Specific aim for study #2: To evaluate the impact of breastfeeding duration during the first 6 months after birth, on the maternal reports of diarrhea episodes in infants during their first year. Hypothesis: Compared to infants who discontinued breastfeeding between 0 and 6 months, the infants who were breastfed up to 6 months or longer will have a lower count of diarrheal episodes, reported by mothers within 2 weeks prior to completion of questionnaires at 7, 9, 10, and 12 months. Multiple studies have shown that breastfeeding, the recommended source of nutrition for at least the first six months of life, is essential for the nutritional, immunologic, neurobehavioral and social development of the infant and young child (Group, 2016; Gupta, Dadhich, & Suri, 2013).

Specific aim for study #3: To evaluate the efficacy of a pathogen identification tool in detecting the presence of E. coli under simulated high temperature conditions similar to limited resources settings with the highest morbidity and mortality rates due to diarrhea. Hypothesis: The

pathogen identification tool will accurately detect diarrheagenic pathogens under environmental conditions that are specific to the region of the worlds most affected by diarrheal diseases. This hypothesis is based on results of preliminary laboratory evaluation of the pathogen identification tool.

To address the growing interest to develop diagnostic technologies targeting low-resource settings, the WHO has outlined in seven points the characteristics point-of-care tests should have (Kettler, White, & Hawkes, 2004; Mabey, Peeling, Ustianowski, & Perkins, 2004). Corresponding to the acronym "ASSURED", these tests should be: 1) affordable, 2) sensitive, 3) specific, 4) user-friendly, 5) rapid and robust, 6) equipment-free, and 7) deliverable to end users (Kettler et al., 2004). The successful completion of this study is in line with these criteria and is expected to further optimize a point-of-care diarrhea pathogen identification tool appropriate to resource-limited sites.

1.6 Conclusion

Inappropriate management of diarrhea episodes contribute to the slow progress made in the last four decades to reduce mortality and morbidity due to diarrheal diseases. The findings of each study presented in this dissertation will contribute to the understanding of the most current management of childhood diarrhea and identify the gaps justifying the need to develop alternative approach to continue reducing the impact of diarrhea in children. The development of point-of-care diagnostics at the service of global health issues is critical to address the gap in using adequate management measures, hence improving child survival and reducing morbidity and mortality rates caused by diarrheal diseases.

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CHAPTER TWO

Prescription Rates of Oral Rehydration Therapy by Health Providers in South Asia:

A Systematic Review

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This publication is intended to be submitted at Paediatrics International Child Health

Keywords: childhood diarrhea, oral rehydration solutions, oral rehydration therapy, medical practitioners, pharmacists, South Asia

Abstract

Background: Oral rehydration therapies (ORT) has been the cornerstone of diarrheal control programs and is associated with a significant drop in diarrhea related childhood mortality. Despite the proven evidence and low cost, routine use of ORT remains limited.

Objectives: A systematic review of the literature was performed to evaluate healthcare providers (medical doctors and pharmacists) ORT prescriptive practices during management of childhood diarrhea.

Method: A meta-analysis of observational studies reporting on medical doctors and pharmacists prescribing behaviors during management of childhood (ages 0 to 15 years) diarrhea in South Asia was the initial intent of this work. Statistically significant evidence of study heterogeneity was achieved, not allow for combining data across studies. Therefore, an integrative review was completed following PRISMA guidelines. An electronic search was performed using PubMed, Embase, Ovid Global Health and the World Health Organization (WHO) Global Health Library. Studies reporting on healthcare providers' ORT prescriptive practices during management of diarrhea South Asia were included.

Results: Eighteen studies were included in the review. The lack of robust evidence and inability to combine the findings in a meta-analysis were due to variations between studies' design, method, population and geographical settings. Overall study findings demonstrated considerable variability in ORT coverage that was consistent regardless of publication before or after the 2004 WHO guidelines for the management of childhood diarrhea. Pharmacists and/or pharmacy personnel were reported to prescribe lower rates of ORT compared to medical doctors and/or health facilities workers.

Conclusions: Available evidence demonstrates limited use of ORT during management of childhood diarrhea by health providers in South Asia and that it is inconsistent with the 2004 WHO guideline. Variability in the study methodologies, designs, settings and reporting was significant. Findings highlight the need for stronger study methodologies specifically related to sampling, measurement and reporting of ORT in management of childhood diarrhea.

2.1 Introduction

Worldwide, childhood mortality due to diarrheal diseases has dramatically decreased from 4.6 million in 1980 to 526, 000 in 2015.[1] This significant drop is primarily due to the improved therapeutic management of diarrheal diseases which mainly includes: oral rehydration therapy (ORT), zinc supplementation, and antibiotics for dysentery. Additional supportive interventions include routine use of the rotavirus vaccine, vitamin A supplementation, initiation and maintenance of breastfeeding, access to clean water, and hand washing with soap and water.[2] Use of ORT alone as the first line treatment has the potential to reduce diarrhea mortality rates by approximately 93 percent.[3]

Since the 1970's, the World Health Organization (WHO) has continuously recommended ORT for the prevention and treatment of dehydration caused by diarrhea.[4] Over time, the formulation of ORT has been extensively studied and refined to optimize reduction of stool output while at the same time minimizing vomiting and other side effects such as hypernatremia.[4] Three decades of research have provided strong evidence to support the 2004 WHO recommendation for the use of a low osmolality ORT from 311mOsm/l (90mEq/L of sodium) to 245mOsm/l (75mEq/l of sodium).[5] The new formulation has been proven to reduce stool output, vomiting, and the need for intravenous rehydration.[3] Even with the existing recommendations, many children suffering from diarrheal diseases do not receive ORT, the appropriate recommended treatment.[6]

Regardless of the proven efficacy, low cost, and ease of use, the rate of ORT prescription in the management of diarrheal episodes in the clinic setting or in the home remains stagnant. It is estimated that worldwide only about 40% of children under the age of 5 suffering from diarrhea receive ORT for the treatment of childhood diarrhea.[6] For example, between 2000 and

2015 the use of ORT increased by only 4% (from 48% to 52%) in South Asia. As a consequence, about 4.7 million episodes of childhood diarrhea are reported each year and approximately 1,600 deaths associated with diarrhea occurring daily in South Asia.[1]

We performed a meta-analysis and integrative review to evaluate medical doctors and pharmacists' prescription of ORT in the management of childhood diarrhea in South Asia. We chose South Asia as it is one of the regions of the world with the highest rate of childhood mortality due to diarrhea.[6] The study focuses only on observational study designs published between 1988 and July 2016 to allow exploration of medical doctors and pharmacists' ORT prescription practices, in their natural setting, without an alteration of behaviors and patterns.[8-10] We used Lipsey and Wilson method of meta-analysis (2001) to answer the following research question:

What are the medical doctors and pharmacists' ORT prescription behaviors as reported in published observational design studies?

2.2 Method

2.2.1 Study retrieval

After problem statement formulation and eligibility criteria were confirmed, a literature search was performed according to the Lipsey and Wilson method.[10] Initial eligibility criteria included research with an observational study design, published in English language and research respondents being children under the age of 15 suffering from diarrhea or any healthcare professionals providing treatment for childhood diarrhea.

The literature search was completed using five different databases including Pubmed, Scopus, WHO Global Health Library as well as PsychInfo and Sociological abstract. The search

also included screening of references in related review articles and eligible studies. The keywords and mesh terms used in combination were *oral rehydration therapy, fluid therapy, oral rehydration salts, oral rehydration solutions, sugar and salt solutions, diarrhoea, diarrhea, chronic diarrhea, acute diarrhea, children, childhood, infant, toddler and pediatric.* To ensure that each study was comparable to the others and provided a consistent effect size statistic, critical to producing strong analytical results, the literature search was further narrowed to the health professional groups that were the most frequently reported in the studies to choose ORT for diarrheal treatment; medical doctors and pharmacists.

2.2.2 Selecting, computing and coding the effect size statistics

Eligible studies were independently reviewed by two authors who then met to further discuss inclusion of each study. The final selection was completed after the third reviewer adjudicated on any uncertainty and disagreement about inclusion and exclusion. The encoding process was then used for data extraction and to further organize each study's findings. The extracted data included information about: geographical location for study recruitment; the study population; design; study objective; interventions; outcomes; and reported effect size statistics.[10] Proportions of prescribed ORT during the management of childhood diarrhea by medical doctors and/ or pharmacists were extracted and organized in a Microsoft (MS) Excel spreadsheet. The standardized mean difference effect sizes, correlation coefficients, odds ratio and homogeneity were computed using Excel and Statistical Package for Social Sciences (SPSS, version) Macros programs.[10]

Despite the extensive literature retrieved related to diarrheal management, a high degree of variability was noted between studies in terms of methodologies and designs, intervention and study population. Statistically significant heterogeneity was detected ($Q = 87.70$, $p = 6.83 \times 10^{-}$

[19]); which does not allow for combining of the studies' findings for further analyses. As illustrated in Table 1, the nature of reported outcomes differed from one publication to the other. Therefore, the authors moved on to completing an integrative review of the literature following the PRISMA guideline to further delineate the study findings.[11] The review focused on describing the results of observational studies describing the prescription of ORT in the management of childhood diarrhea in children under 15 years old by health professionals with formal and informal health training in South Asia.

2.3 Results

2.3.1 Study characteristics

The literature search yielded 4442 articles from the different databases and reference lists. Of those articles, 2917 were excluded after removing duplicates and articles published in non- English language outside of the study period. Titles and abstracts of 1525 articles were screened for the initial eligibility criteria. Limiting the review to South Asia and study design and methodology, 1433 articles were excluded and 92 articles were identified for full article review by two co-authors. Based on the review and discussion, 74 did not meet the additional eligibility criteria mainly because they did not separate prescription practices based on the healthcare providers' groups. In total, 18 articles were included in the analysis after limiting the eligibility criteria to medical doctor and/or pharmacists. See PRISMA Diagram for details of study selection process (Figure 1).

Of the 18 studies, seven were published before 2004 and 50% were studies performed in India (n = 9). In six articles, the study's method and design was not specified while nine were surveys. Respondents in the majority of the studies (n = 10) were medical doctors and/or

pharmacists and their prescribing behaviors while the remaining studies focus on children suffering of diarrhea and their caregivers' reports. The age of the children varied with majority referencing children under the age of 5 years. The definition and lengths of reported episodes of diarrhea also differed within the sample. The reported period of diarrhea episodes varied from diarrhea within the past 24 hours to the last diarrhea episode.

2.3.2 ORT prescriptions during management of childhood diarrhea

Before publication of 2004 WHO Guidelines. A majority of the findings of studies published before 2004 (5 out of 7) reported healthcare providers' prescriptions of any ORT tended to be higher than 50% .[12-17] Depending on the sample sizes of these studies, percentages of any ORT prescribed to children ranged between 60 % to 94.6% .[15,18] Rarely did the studies report the prescription of ORT alone. The highest percentage of ORT prescriptions were reported when prescribed in combination with other medications, mainly antibiotics. Only three studies mentioned ORT formulation recommended by the WHO.

Medical doctors were reported to prescribe higher rates of any ORT compared to pharmacists.[12-14,17] For example, Raghu and colleagues (1995) evaluated the awareness and attitude of medical doctors and pharmacists in India toward oral rehydration therapy for the treatment of diarrhea in children within a survey study and found that 77% (n = 53) of medical doctors reported prescribing ORT compared to 59% of pharmacists who declared prescribing ORT.[14] The authors also reported that only one pharmacist in the study prescribed ORT using the WHO recommended formulation compared to 48% of pharmacists who prescribed ORT formulations that were not compliant with the WHO recommendation.[14]

After publication of the 2004 WHO guideline. More variability in ORT prescription rates was noted in studies published after the 2004 WHO guideline on the management of childhood diarrhea.[19-22] Depending on the study design, sample population and sample size, ORT prescription rates varied between 2.4 % and 100%.[22,23] In a prospective observational study auditing the prescriptions rates of doctors treating children between the age of 6 months and 5 years in an Indian tertiary care hospital, ORT and zinc were reported to be prescribed in accordance with the 2004 WHO protocol in all cases of children suffering from diarrhea.[23] However, the authors also reported that among the 402 children who were prescribed additional medications for the treatment of diarrhea, deviation from the WHO protocol was noted in 78.4% of the cases.[23] In other words, more than two thirds of the children who were treated by medical doctors, were prescribed additional medications not recommended by the WHO guideline during the management of the child's diarrheal episode.

Similar to the studies published before 2004, studies examining the practices of pharmacists with formal and informal training tended to report lower rates of ORT prescriptions then what was recommended by WHO guidelines.[14,22] Diwan and colleagues used a simulated client survey to assess the treatment of childhood diarrhea in private pharmacies in the province of Madhya Pradesh, India.[22] Of the 164 private pharmacists surveyed using a questionnaire with a simulation of a visit with a child suffering from diarrhea and their caregiver, only four prescribed ORT.[22] Antibiotics, however, were prescribed by 40.24% (n = 66) pharmacies and anti-motility drugs were prescribed by 31.1% (n = 51) of pharmacists.[22] It was also noted that ORT is cheaper and thus less, profitable for pharmacists to prescribe.

Several other studies also reported on the significant number of prescriptions of other medications mainly antibiotics, anti-motility and probiotics drugs for the treatment of diarrheal

episodes. The WHO guideline has been published for more than a decade, yet, findings from the most recent study included in this review (published in 2016) indicate that antibiotics were unnecessarily prescribed in 12.2% of the cases and probiotics were prescribed in 78.1% of the cases of children with diarrhea (n = ,406).[23]

2.4 Discussion

The findings of the present study indicate that numerous observational studies have reported on healthcare providers' management of childhood diarrhea. However, prescription rates varied significantly across the selected studies based on the population, study's method and design as well as the type of data collected.

A systematic review of the selected studies suggest that ORT was mainly prescribed in conjunction with other drugs. ORT was seldom prescribe alone and was not the first-line of intervention. In addition, the findings also indicated more variability in ORT prescription practices in the management of childhood diarrhea in the studies published after 2004 compared to those published before the 2004 WHO guideline. The overall limited rate of ORT prescription and wide disparity in ORT coverage was associated with healthcare professional training. Consistently pharmacists and/or pharmacy personnel were reported to prescribe lower rates of ORT compared to medical doctors and/or health facilities workers.

These findings are consistent with a previous review on childhood diarrhea treatment coverage at the global and regional level.[24] These authors stated that following the success of diarrhea management focusing on ORT usage in the 1990's, the adherence to the 2004 WHO guidelines on the management of childhood diarrhea has been "extremely slow" and even "stagnant".[24] Low use of ORT has been associated with certain factors such as healthcare

providers' knowledge and perception of the severity of the diarrhea episode, parents' expectation and the financial profit to sell more expensive therapies.[25] Each of the factors has been reported to influence the use of ORT and increase prescription of antibiotics and other drugs. Efforts must be made to improve national policies encouraging healthcare providers' education, increasing community awareness, large scale manufacturing, and increase awareness and demand for ORT in the treatment of childhood diarrhea.

2.4.1 Limitations

Given the variability of the study designs, methodology, and population, findings from the studies could not be combined and extrapolated. This is indicative of the lack of consistency in research methodology of published studies. While mentioned in three studies, the review did not include research evaluating the care provided by traditional healers or community health workers, who play significant roles within communities in this region of the world. However, a systematic review reveals trends in ORT prescription that are consistent with previously published reviews and with the United Nation's current published data.

2.4.2 Implication for research

A significant amount of evidence exists on the cost-effectiveness of ORT in diarrhea control programs, the findings suggest the need for more studies evaluating the actual prescribing behaviors of health providers during management of childhood diarrhea. This type of evaluation will offer important insight on the effectiveness and sustainability of already existing programs as well as identifying local and regional issues and barriers hindering the prescription of ORT by health providers in South Asia. This integrative review uncovers the need for observational

studies using more rigorous and standardized methodology to evaluate the overall prescription rates of ORT in the management of childhood diarrhea in South Asia.

While the WHO guideline has been used to formulate national policies, the studies suggest the need to evaluate healthcare providers' education and adherence to the WHO guideline and impact of the unnecessary use of antibiotics. Since prescription of the known strategies have been stagnant for the past 40 years, it is evident that the current training programs should incorporate a more sustainable message. These health education strategies must address the cultural norms of a given community and include ongoing re-assessment and education sessions.

2.5 Conclusion

With one of the highest rates of childhood diarrhea worldwide, South Asia remains the region of the world with the second lowest usage of ORT. While the evidence from a meta-analysis cannot be combined, a systematic review of the selected articles reveals a trend that is consistent with already existing data in other regions of the world. Despite the publication of the 2004 WHO guideline and the strong published research evidence on the cost effectiveness of ORT, observational study findings indicate that ORT is prescribed in low rates and inconsistently by medical doctors and pharmacists with formal and informal training. Allocating more investment in studies with stronger methodology will allow a more accurate evaluation of the medical doctors and pharmacists prescriptions of ORT in the management of childhood diarrhea.

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Figure 1. Studies Selection Process

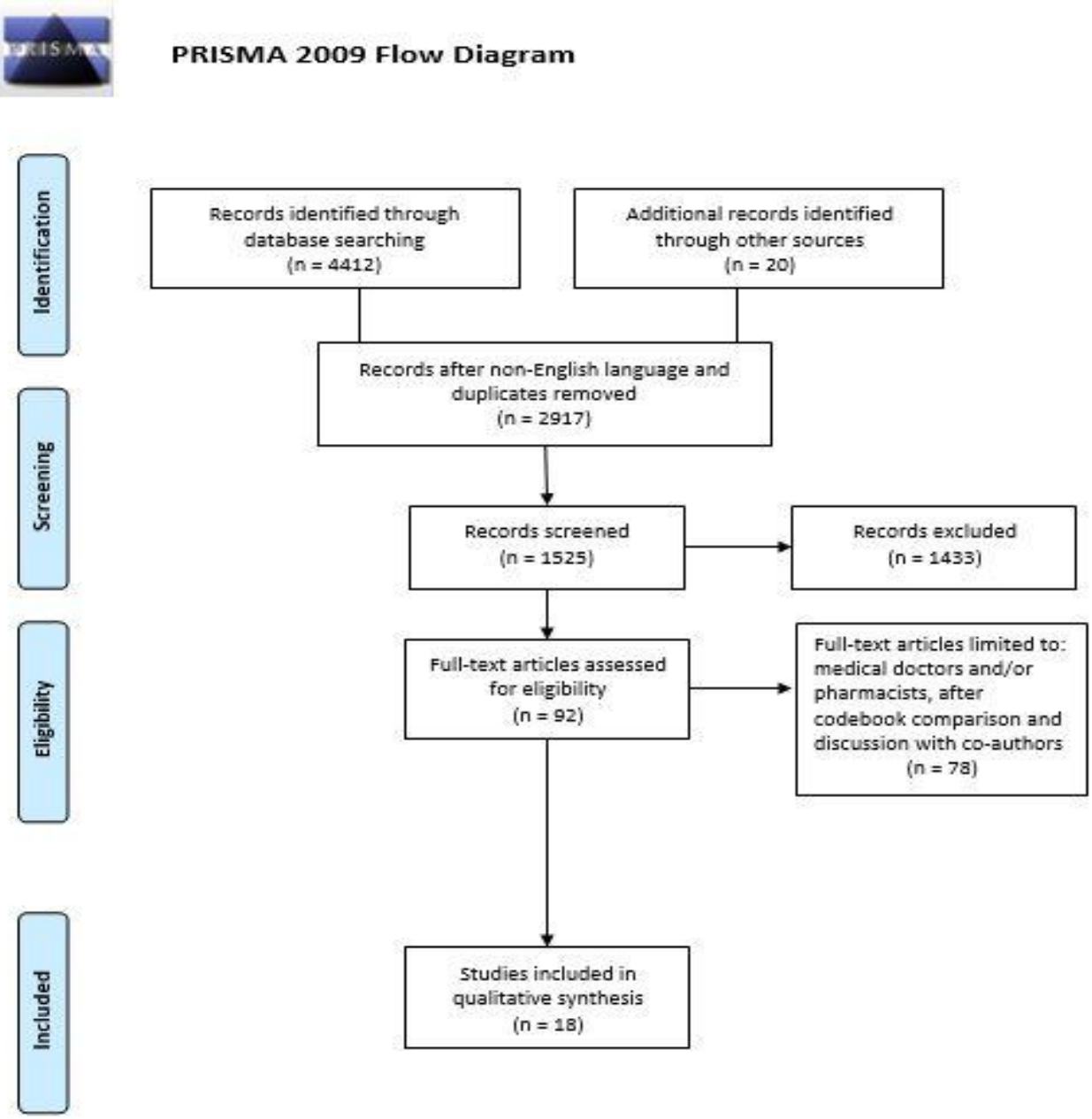

Table 1. Characteristics of Included Studies

Authors/ Year/ Country	Study design, study objectives	Study population/ Number of participants	Intervention	Use of WHO guidelines	Proportion of ORT prescriptions	Proportion of prescription of therapies other than ORT
Gani et al., 1991. Indonesia	Design not specified To investigate both reported and observed prescribing practices for treatment of acute diarrhea in children under 5 years of age	122 physicians participated in survey 73 physicians participated in observational study	Survey interview, observations of physicians' clinical practices and an in- depth interview.	None	ORT prescriptions by physicians :70% reported 67% observed physicians	Antibiotics prescriptions by physicians: 61% of physicians 94% of observed physicians
Kasi et al., 1995. Pakistan	Design not specified To screen the practices of a small sample of doctors confronted with children with diarrhea	30 doctors	Each survey team member introduced herself as the mother of the infant, and stated only that the child had diarrhoea.	None	Prescription of ORT by doctors: 60%	Mixture of kaolin and pectin: 46% Other drugs: 80%
Raghu et al., 1995. India	Design not specified To evaluate the awareness and practice of oral rehydration therapy amongst mothers, medical practitioners and pharmacists.	48 medical practitioners, 56 pharmacists and 55 mothers of children with diarrhoea	Interviews using a prepared questionnaire	WHO	ORT prescriptions: by pharmacists: WHO formulation (n = 1; 1.7%); non-WHO formulation (n = 27; % = 48%). By doctors: WHO formulation (n = 8; %16.5); Non Who formulation (n = 29; % = 60.5)	
Nizami et al., 1996. Pakistan	Design and objective not clearly stated	996 children with diarrhea	Observation of encounters between children with diarrhea and general practitioners (n = 62) and pediatricians (n = 28)	None	ORT prescription per qualification: general practitioners: 53.2%; pediatricians: 60.7%	Antibacterials: 50% of pediatricians Antidiarrhoeals: 60% general practitioners and 28% pediatricians Antiamoebic: 39% of general practitioners,32% of pediatricians
Buch & Bashir, 1997. India	Design not specified To focus on the inadequacies in the current management practices of acute diarrhea	1030 infants with acute diarrhea	Information regarding nature of diarrhea and its management were collected from the details mentioned in the prescriptions of the infants	None	ORT/SSS prescriptions: 4.1% of infants treated at private clinics and health units	Antidiarrheal + antispasmodic drugs: 46.8% of infants treated at private clinic or health units Parenteral antibiotics: 11.3% of infants

Choudry et al., 1997. Pakistan	Survey To study the current practices and factors affecting the management of acute watery diarrhea in children below 5 years of age	262 general physicians	Physicians were interviewed at their place of work by a team of three physicians using a pretested, semi-structured questionnaire	Yes	ORT alone by physicians: 19%. ORT + drugs: 61%	Prescriptions of drugs alone: 15%
Howteerakul et al., 2003. Thailand	Design not specified To explore Thai physicians' rationales about their prescribing practices for treating childhood diarrhoea	424 cases of child diarrhea	A clinical audit and observations of 424 cases treated by 38 physicians used to estimate the prevalence of sub-optimal prescribing practices.	Yes	ORT prescriptions: 91.3% of child cases	Antimicrobial: 75.5% of child cases Antiemectics + Antispasmodic: 32.4% of child cases ORT+ other drugs/IV:94.6% f child cases
Ahmed et al., 2009. India	Cross sectional survey To study the treatment practices of diarrhea and symptoms at health facilities (medical practitioners)	1052 children under 5	Household survey visits were conducted at the end of each season and details regarding any current episode of diarrhea and any past episodes of diarrhea were recorded from the mother	None	ORT only: 8.7% of children treated at the health facilities	Antibiotics: 77.9% of children treated at health facilities
Saengcharoena et al., 2010. Thailand	Simulated client survey To compare practice behavior and attitudes of pharmacy personnel in the management of childhood diarrhea	63 Pharmacists	simulated client survey and questionnaire survey	None	ORT prescriptions: 4.2% of pharmacists	Antibiotics: 25.2% of pharmacists
Chakraborti et al., 2011. India	Design not specified To determine the prescribing practices of doctors in management of acute diarrhea in children.	388 children suffering from acute diarrhea	Not specified	IAP guideline	ORT prescriptions by doctors: 90.2%	Antibiotics: 82.5% Pro/prebiotics: 56.4% Antiemectics: 43%
Pathak et al., 2011. India	Prescriptions chart review To determine the levels of adherence to treatment guidelines for acute diarrhea in children up to 12 years seen in drug prescriptions	843 prescriptions	Pharmacy assistants and medical officers assisted in the collection of information from outpatient prescriptions	None	ORT alone: 58% of reviewed prescriptions	Antibiotics: 71% of reviewed prescriptions

Aung et al., 2013. Myannmar	Survey study To determine the norms for both home-based care and health-seeking behavior	253 children under 5	A structured questionnaire was administered by trained healthcare workers.	Yes	Any ORT: Government health facilities: 16.6% of infants Private providers: 13.4% Pharmacy: 0.8%	
Pham et al., 2013. Vietnam	Pre intervention survey. To report knowledge and practice of staff in pharmacy settings regarding management of childhood diarrhea	220 pharmacies 281 pharmacy staff	survey was used to record knowledge and reported practice and simulated client surveys	None	Any ORT prescriptions: 41.9% of pharmacists and pharmacy staff	Antibiotics: 14.8% of pharmacists and pharmacy staff
Kanungo et al., 2014. India	Cross-sectional study using interviews To asses diarrhea-related knowledge, practice, their correlates and interrelationship among allopathic practitioners treating diarrhea patients in the slums of Kolkata	264 practitioners	Interviews collected on practitioners' knowledge attachment, year of practice, average age	None	Prescription of ORT: Odds Ratio = 1.24 of qualified and government physicians	Antibiotics: 49.24% of physicians
Naeem et al., 2014. Pakistan	Cross-sectional survey To appraise general practitioners i n the management of acute watery diarrhea for children under 5 years of age	380 general practitioners	Semi structured questionnaires assessing the knowledge and practices regarding the management of acute watery diarrhea	Yes	Prescription ORT based on general practitioners' qualification: MBBS only 48.1%. MBBS and Minor diploma pediatrics: 100%	Antidiarrheal drugs: MBBS 74.4% MBBS + minor diploma in pediatrics: 100%
Diwan et al., 2015. India	Cross-sectional study using simulated client survey To assess the treatment of childhood diarrhea in private pharmacies	164 private pharmacies	Four simulated clients visited the pharmacies and filled out a questionnaire within 15 minutes after leaving the pharmacy.	Yes	ORT prescribed: 2.44% pharmacies	Antibiotics: 40.24% by pharmacies Anti-motility drug: 31.1% by pharmacies
De et al., 2016. India	Prospective observation: Audit of prescriptions To study the prescription practices, regarding adherence to WHO protocol and deviations in the management of acute diarrhea in children	402 children 6 months- 5 years with acute diarrhea episode	Cases were evaluated in detail for clinical history, physical examination, prescription given by treating doctor at the hospital and associated co-morbidity. Each prescription was scrutinized to	Yes	ORT: 100% of cases	Probiotics: 78.1% of the cases Antibiotics: 12.2% of the cases

			determine whether WHO protocol was followed or not			
Fisher Walker et a., 2016. India	Survey using: Interview and direct observation To characterize the childhood diarrhea treatment knowledge and practice of both formal and informal private sector providers	232 private providers	Interviews and direct observation conducted on diarrhea treatment knowledge and practice and access to ORS and zinc supplies	Yes	Interviews: ORT prescription: 68.1% Direct observations:ORT prescriptions: 56.7%	Antibiotics: 65.9% Zinc: 35.8%.

CHAPTER THREE

Impact of breastfeeding duration before 6 months on the reported incidence of diarrhea in infants between the ages of 7 to 12 months: A United States Birth Cohort Study

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Abbreviations:

What is known:

Even in the developed country like the United States, diarrhea associated infections remain a health issue in infancy and early childhood. Breastfeeding during the first 6 months after birth has been proven to provide optimal immunity to prevent diarrheal infections.

What this study adds:

Breastfeeding cessation prior to six months of age significantly increases the risk of having a diarrheal episode prior to one year of age by 31-48% according to a US database.

This publication is intended to be submitted to Pediatrics for publication..

AMA style

Contributors' Statement Page

Ana F Diallo conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted.

Dr Walsh assisted Mrs Diallo in carrying out the data analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Drs Henderson, Lucas, McGrath, and Cong critically reviewed the manuscript, and approved the final manuscript as submitted.

Abstract

Background: Diarrhea diseases remain a major cause of childhood morbidity in the United States. Optimal breastfeeding has been identified as one of the most effective measure to prevent diarrhea diseases in childhood.

Objective: To evaluate the impact of breastfeeding duration during the first 6 months of age on the incidence of diarrhea in infants between 7 and 12 months in the United States.

Methods: A secondary data analysis was performed using mothers and infants participating in the Infant Feeding Practices Study II (2005-2007).

Results: Compared to those who breastfed for 6 months or more, infants who discontinued breastfeeding between 0 and 3 months were at 48.4% greater risk to have diarrhea at 7 and 12 months and those who discontinued breastfeeding between 3 and 6 months were at 31% greater risk of having diarrhea between 7 and 12 months.

Conclusion: Breastfeeding cessation before the first 6 months is a significant predictor of diarrhea between 7 and 12 months.

Word count: 2989

3.1 Introduction

The major burden of diarrheal related morbidity and mortality is reported to be in the developing regions of the world. However, children living in richer countries are also affected by diarrheal diseases.[4] While accounting for fewer deaths in developed countries, diarrheal infections are associated with high rates of doctors and emergency hospital visits, hospital admissions and medical costs. In the US, diarrheal related infections account for approximately 300 deaths each year in children under the age of 5.[4]

Causing approximately 200, 000 hospitalizations, it is estimated that diarrhea cost about $250 million in direct medical expenses each year.[4-5] Low birth weight, being male and black are all factors that have been found to significantly increase the risks of dying from diarrheal diseases in the US.[6] Preventive measures to control and reduce the impact of diarrhea in the US includes the promotion of rotavirus vaccines and optimal breastfeeding for the first 6 months of life are among the key strategies to prevent diarrhea.[5]

Before the introduction of rotavirus vaccines, the pathogen was reported to cause the most frequent episodes of diarrhea in US children with 55,000 to 70,000 hospital admissions each year and approximately $1 billion to the US government in medical cost.[7] Following its introduction between 2007 and 2009, the rotavirus vaccine was estimated to reduce gastroenteritis hospitalizations by at least 53% in US infants living in the Western part of the country.[7] The vaccine reduced by more than 70% diarrheal related infections among children between 1 to 4 years old in the Southern, Northeastern and Midwest regions of the US.[7] Besides the rotavirus vaccine, the promotion of breastfeeding for the first 6 months of life has been another cost-effective preventive measure against diarrheal diseases in the US.

Since 1984, breastfeeding was identified as one of the most important and cost-effective intervention, significantly reducing the incidence of infectious diseases among children worldwide, especially diarrhea and pneumonia.[4-6] Human breastmilk contains bioactive molecules mostly secretory antibodies, lactoferrin and glycans offering protection and maturation of the gastrointestinal track and immune system during infancy and early childhood.[7-8] However, the 2014 CDC report card on breastfeeding indicates that only 18.8% of US infants are exclusively breastfed at 6 months while 49.4% receive some breastmilk through 6 months.[9] These numbers depict the perpetuation of suboptimal breastfeeding in the US since they remain far from the Healthy People 2020 objectives of 25.5% exclusive breastfeeding at 6 months and 60.6% infants receiving some breastmilk at 6 months.[9-11]

In the most recent meta-analysis on breastfeeding practices published in the Lancet, it was reported that compared to middle-income and low-income countries, the prevalence of any breastfeeding at 12 months is lower in high-income countries.[17] The weighted prevalence of breastfeeding at 6 months in high income countries was approximately 45% (compared to more than 95% in low income countries) and was 20% at 12 months (compared to more than 90% in low income countries).[17] Breastfeeding practices among US mothers are not an exception. The prevalence of any breastfeeding at 12 months in the US is around 27%.[17]

While the protective effect of breast milk is undeniable, significant gaps still exist in the promotion and practice of early breastfeeding initiation and continuation for the first 6 months of life, even in countries like the US. Non-exclusive or early interruption of breastfeeding, also known as suboptimal breastfeeding, was ranked in 2010 by the Global Burden of Disease Study as the second leading risk factor of disease burden in children under the age of 5 worldwide regardless of whether the child was in a developed or under-developed country.[18]

The prevention of persistent diarrhea through the promotion of optimal breastfeeding is imperative in reducing the burden of the symptom in early infancy and childhood. Thus providing more evidence on the outcomes related to the immunologic properties of breastfeeding using a national survey database, will strengthen the empirical evidence and encourage the promotion of optimal breastfeeding practices of US mothers. The Infant Feeding Practice Survey II (IFPS II) dataset was utilized as it is the most recent survey that compiled comprehensive information on US mothers and infant feeding practices and their associated health status.[12] By our best knowledge, this is the first study to examine the IFPS II data to assess the longitudinal effect of breastfeeding duration on the occurrence of diarrhea.[13]

The goal of this study was to evaluate the impact of breastfeeding duration during the first 6 months after birth, on the maternal reports of diarrhea episodes in infants during their first year of life. You could add research questions/hypotheses here. A secondary data analysis was performed to answer the following research question: Is the incidence of diarrhea episodes in infants between the ages of 7 to 12 months influenced by mothers' breastfeeding duration between 0 to 6 months? The research question was based on the hypothesis that compared to infants who discontinued breastfeeding between 0 and 6 months, the infants who were breastfed up to 6 months or longer would have a lower count of diarrheal episodes, reported by mothers within 2 weeks prior to completion of questionnaires at 7, 9, 10, and 12 months.

3.2 Patients and Methods

A secondary data analysis method was used to in evaluate the effect of breastfeeding duration during the first 6 months of life on the reported incidence of diarrhea in infants between the ages of 7 and 12 months. The study population included mothers and their infants enrolled in the IFPS II. Longitudinal data was collected between May 2005 and June 2007 on a nationwide

consumer opinion panel of 4902 women who were at least 18 years old, in their third trimester of pregnancy and expecting a singleton birth.[12]

Mothers were excluded from the study if: they refused to participate; did not return the questionnaire; the infant was born before 36 weeks gestational age; the infant was born with a long-term illness. Mothers were also excluded from the original study if they resided in areas where the United States Postal Service could not deliver mail after the 2005 Gulf Coast hurricanes.[12] Based on these exclusion criteria, a total of 3033 mothers completed all the survey questionnaires.[12]

3.2.1 Participants

The sample size was limited to mothers who completed the questionnaires with reports on breastfeeding cessation, last time a questionnaire was retuned and diarrhea episodes between 7 and 12 months after delivery. We excluded data missing study variables (no survey responses reported) on the study's variables. A total sample of 2344 dyads (mother/infant) were included in the final analysis (Figure 1).

3.2.2 Study variables

Breastfeeding duration. Breastfeeding duration was the primary exposure variable and corresponds to the infant's age when the mother reported to completely discontinue breastfeeding and provision of expressed breastmilk. The variable indicates the infant's age when he/she stopped receiving breastmilk either directly from the breast or expressed (pumped). The variable was categorized into four subset variables: 1) infants reported to have never breastfed (bfduratn= 0); 2) infants who stopped receiving breast milk between 0 and 3 months

(0.1 to 12.0 weeks); 3) infants who stopped receiving breast milk between 3 and 6 months (12.1 to 23 weeks); and 4) infants who receiving breast milk at 6 month or beyond (≥ 24 weeks?).

A variable, last questionnaire received, was created to identify the number of mothers who dropped out before the 6 month study period. The last questionnaire received was coded the last time (in month), mothers were reported to return an IFPS II questionnaire. The code allowed estimation of the number of mothers who discontinued breastfeeding between 0 and 6 months after delivery. This variable was also used to estimate the sample size of each breastfeeding duration groups.

Reported episodes of diarrhea. The main outcome variable was reported diarrheal episodes within the 2 weeks prior to receiving the questionnaire at 7, 9, 10 and 12 month of infant age. Data on infants' diarrheal episodes were obtained on the questionnaire by asking the following question: "Which of the following problems did your baby have during the past 2 weeks?" Diarrhea was listed as one of the multiple choice options including fever, vomiting and ear infection. The occurrence of a diarrheal episode was defined as a mother reporting at least one episode of diarrhea within the 2 weeks prior to receiving questionnaires in months 7, 9, 10 and 12. For the purpose of the analysis, the dependent variable (reported episodes of diarrhea) was recoded into a binary variable: 0 = no episodes of diarrhea; 1 = 1 or more episodes of diarrhea reported at months 7, 9, 10 and 12.

A total of 59 mothers did not respond to the question on diarrhea at least once between months 7 and 12 month questionnaires. A sensitivity analysis was completed to determine whether treating the lack of response as occurrence (condition 1) or non-occurrence (condition 2) of diarrhea would modify the study finding. No statistically significant difference were found. The statistical analysis was completed using the more conservative condition, condition 1.

Offset variable. The number of times a mother reported an episode of diarrhea between 7 and 12 months depended on the number of questionnaires she returned during that given period. Therefore, the number of questionnaires returned by each mother could affect the total count of reported diarrhea episodes. For this reason a variable "number of questionnaire returned" was created to account for the total number of time mothers returned the survey questionnaire between 7 and 12 months. A value from 0 to 4 was given based on the number of times a mother returned a postnatal questionnaire at months 7, 9, 10 and 12. This variable was considered an offset variable and was used as a control variable during statistical analysis using Poisson regression.

Other variables. Following consultation with a certified lactation specialist and an extensive literature review, a series of mother and infant characteristics were identified as potential confounders were therefore controlled for in the Poisson regression. The variables included sociodemographic characteristics of the mothers such as age, race, level of education and employment status. Infant characteristics included gender, weight and occurrence/non-occurrence of infant breastfeeding during the neonatal period.

3.2.3 Statistical Analysis

Frequency and descriptive statistics were completed for the demographic characteristics of the sample using SPSS version 22.[14] To assess the association between breastfeeding cessation and the report of diarrhea episodes in a longitudinal manner, generalized linear modeling using Poisson regression was used. Recoding of the variables of interest was completed using SAS version 9.4.[13]

Diarrhea episodes were self-reported by the participating mothers and returns of the questionnaires occurred at different times for each participants; so these variables could not be controlled. For these reasons, rate ratios rather than relative risk were conducted to estimate the possible relationship between the independent and dependent variables. Poisson regression was applied to estimate the rate ratio (RR) and a *p value* was obtained based on the Wald test and 95% confidence interval of the reported occurrence of diarrhea between months 7 and 12 as it related to breastfeeding cessation between 0 and 6 months. All analyses were completed using SPSS version 22.[15]

3.3 Results

3.3.1 Participants' demographics

Demographic statistics for maternal and infant characteristics are presented in Table 1. In the infant cohort, the male to female ratio was almost equal with 49.7% male and 50.2% female. A majority of the infants were delivered vaginally (71.3%) with an average birth weight of 7.64 pounds. Descriptive analysis of the maternal participants indicated that mothers' average age was 29 years old with the majority being White (85.5%) and married (77.4%). Most mothers were highly educated (65% had some college education) and 45% lived in a household with an income level higher than $50,000. Maternal and infant characteristics in this cohort mirror the larger IFPS II sample of 3033 mother- infant dyads. A majority of the mothers in the initial IFPS II study were White (84.4%), between 25 to 34 years old (61.4%) with a college education (40.2%).[12]

3.3.2 Breastfeeding duration and reported episodes of diarrhea

Of the 2344 mothers included in this study, 84.3% (n= 2000) either breastfed or tried breastfeeding their infant during the neonatal period. More than a quarter of the infants (25.8%) stopped receiving any breastmilk before 3 months. In this study, 14.2% of the infants were never breastfed either directly from the breast or expressed breastmilk from the bottle; while 38.5% stopped being breastfed between 0 and 6 months. A total of 1109 (47.3%) infants were reportedly breastfed for the first 6 months or more. Approximately 28% of the mothers reported one or more episodes of diarrhea within the last 2 weeks after receiving questionnaires in months 7, 9, 10 and 12. The reported episodes of diarrhea increased over time with 6.4% mothers reporting at least one episode at 7 months compared to 12% reported at least one episode at 12 months.

The incidence of diarrhea for infants who breastfed for 6 months or more was approximately 93 per 1000 infants (p = 0.000). The results from the Poisson regression model that assessed the relationship between the four-level breastfeeding cessation variable and reported episodes of diarrhea are described in Table 2. Repetition of the analysis using either condition 1 (considering “no answer on diarrhea occurrence question” as no occurrence of diarrhea) or condition 2 (considering “no answer on diarrhea occurrence question” as occurrence of diarrhea) did not change the rate ratio for breastfeeding cessation. Further analyses were completed using the more conservative approach, condition 1.

Breastfeeding cessation during the first 6 months of life is a significant factor predicting the number of reported episodes of diarrhea between 7 and 12 months. Compared to the infants who breastfed for 6 months or more, infants who discontinued breastfeeding between 0 and 3 months were at 48.4% greater percentage to have a reported episode of diarrhea at least once

between 7 and 12 months (p = 0.000 CI [1.18- 1.71]). Infants who stopped receiving any breastmilk between 3 and 6 months were at 31.1% greater risk of having a diarrhea episode reported by their mother between 7 and 12 months (p = 0.016, CI [1.17- 1.90]) compared to those who breastfed for 6 months or more.

When adjusting for the demographic variables by entering them as covariates in the Poisson regression modeling, the results indicate three maternal characteristics significantly affected reported episodes of diarrhea. These variables were household income, maternal age, and maternal education level. Among the mothers who breastfed their infants, every $1,000 increase in their household income was linked to a 2% decrease in reported episodes of diarrhea at months 7, 9, 10 and 12 (p = 0.006). The results also showed that reported episodes of diarrhea decreased with older (RR = .98; p = 0.000) and more educated (RR= .92; p = 0.02) mothers (Table 2).

In addition, none of the reported infant demographic characteristics were found to significantly influence the reported episodes of diarrhea between 7 and 12 months. When infant birth weight was added as a covariate in the regression model, the estimate rate ratio was .970 (p = .367). Gender or delivery method also did not show a statistical significant impact on the relationship between breastfeeding duration and report of diarrhea episodes (p = .143 and p= .684 respectively). In other words, inclusion or exclusion of the infants' demographic variables in the regression model did not change the rate ratio estimates of reported episodes of diarrhea between 7 and 12 months based on breastfeeding duration before the age of 6 months.

3.4 Discussion

Breastfeeding is considered the most cost effective intervention to assist in preventing diarrhea in early infancy and childhood. In a 2011 meta-analysis of studies performed in developing countries between 1980 and 2009, Lamberti et al., reported that no breastfeeding increased by 26% the risk of incidence of diarrhea in infants between the ages 0 to 5 months.[16] While exclusive breastfeeding is the recommended practice for infants up to 6 months of age, the authors reported that any breastfeeding for more than 6 months had a protective effect on outcomes such as diarrhea. Increasing evidence indicates that antibodies and glycobiome found in breastmilk provide an important source of protection against infectious diseases.[15] These breastmilk components are reported to provide protection against diarrhea by decreasing the incidence and duration of diarrheal episodes.[16]

Consistent with the previous research, the present study demonstrates that breastfeeding for 6 months or more could significantly decrease the incidence of diarrhea for infants younger than 12 months. The results of the secondary data analysis indicated that approximately 93 in 1000 infants who breastfed for 6 months or more had at least one episode of diarrhea reported by their mothers between 7 and 12 months. The number of infants experiencing an episode of diarrhea increased by 48.4% if they stopped receiving breastmilk before 3 months and by 31.1% if they stopped receiving any breastmilk between the first 3 to 6 months of life. The findings showed that infants who breastfed for less than 3 months had the highest risk of diarrhea prior to 12 months of age.

This could be due to the decrease in breastfeeding intensity and introduction of solid foods. In a previous secondary analysis of the IFPSII, Grummer-Strawnn et al. (2008) reported that by 3 months, majority of the infants (80%) were either fed breastmilk or formula milk. By 4

and 5 months, 61% of the infants were fed with formula.[36] Supplementation and early introduction of food other than breastmilk have been linked with suboptimal breastfeed and increase likelihood of infections like diarrhea- related diseases.[14]

When adjusting for mothers' age, education and household income, breastfeeding duration was significantly associated with reported episodes of diarrhea. Adding each of these variables individually in the regression model was associated with variation in the event of having a reported episode of diarrhea varied statistically significantly. Higher income households decreased the likelihood of reporting an episode of diarrhea by 2%. Infants whose mothers had a higher level of education decreased the risk of reporting an episode of diarrhea by 8%. These results are consistent with previous research and publications reporting that higher income and more educated mothers tend to breastfeed longer and thus, have healthier infants with less risks of diarrheal symptoms.[37-40]

Jones et al, assessed the factors associated with breastfeeding practices in US infants using the 2007 National Survey of Children's Health. The authors reported that children living in families with a household poverty status between 200% and 399% of the federal poverty level were 85% less likely to ever breastfeed compared to their counterparts living in household with a poverty level higher than 400% the federal poverty level.[37] In addition, the authors also found that 20 years old or younger mothers are .88 times less likely to ever breastfeed compare to 30 years old or older mothers.[37] Results from Jones' study and the present study reflect the 2011 "Surgeon General's Call to Action to Support Breastfeeding", the US Department of Health and Human Services noted the significant disparities in breastfeeding based on race and ethnicity, socioeconomic characteristics and geographical location.[41]

This study is the first to explore a longitudinal relationship between breastfeeding duration and diarrhea episodes using the IFPSII dataset. The use of a more recent database to explore the protective effect of breastfeeding strengthens the literature and provides more evidence toward the promotion of breastfeeding in early infancy at the clinical practice and community level.

3.4.1 Limitations

While consistent with the current literature, the results are subject to certain limitations. Because the IFPS-II dataset is derived from a self-selected costumer opinion panel rather than random sampling, it is not a necessarily representative sample of the US population. Participating mothers were predominantly white, medium to high income with higher level of education. Therefore, the study findings cannot be generalized to the entire US population.

Second, breastfeeding cessation was a composite variable that did not distinguish between infants who exclusively breastfed and those who partially breastfed. Had these items been available in more precise variables it may would have allowed the formulation of a stronger relationship between exclusive breastfeeding duration and the occurrence of diarrhea. More specific information on the breastfeeding type may have also allowed for exploration in the differences between the effects of exclusive versus any breastfeeding duration on reported onset of diarrhea episodes.

Thirdly, the code 77, which represented both mothers who dropped out during the study and those who continued breastfeeding beyond the study period, did not allow a clear distinction between the mothers who dropped out early during the study and when did they drop out versus the mothers who continued breastfeeding beyond the 12 months study period. Finally, the lack of

data collected between the neonatal period and month 3 was a missed opportunity to depict a clearer picture of early breastfeeding cessation and potential influencing factors. Additionally, the reported episodes of diarrhea were self-reported by the mothers, so the definition of diarrhea might have varied based on mothers' knowledge and perception.

3.4.2 Implication for practice and research

The study's findings support US and WHO clinical practice guidelines regarding breastfeeding during infancy. The results also highlight the issue of suboptimal breastfeeding during the first 6 months of life and its impact on the increased incidence of diarrhea later during the first year of life. By providing statistically significant evidence in the protective effect of breastfeeding duration for 6 months or more, the study adds to the existing research and evidence-based policies for the promotion of optimal breastfeeding at the clinical and community level.

While already established and practiced, these strategies need to be re-evaluated to identify and address the factors influencing limited adherence to optimal breastfeeding during this critical period in infancy. The study sample did not include additional minority groups. Therefore, further studies using a prospective longitudinal design must be done to better capture breastfeeding cessation and diarrhea incidence across a nationally representative sample thereby reflecting the racial/ethnic and economic diversity of the US population.

3.5 Conclusion

Diarrhea related infections remain an issue for children under the age of 5 years worldwide. Optimal breastfeeding for at least the first 6 months of life has been considered one of the most effective interventions for protecting infants against diarrhea. The findings from the

present secondary data analysis using a nationwide dataset provides more evidence for the protective effect of breastfeeding against diarrhea. While this has been well-known for the last three decades, adherence to optimal breastfeeding for the first 6 months of life remains limited, even in more developed countries like the US and within a subgroup of the population with higher income, education level and better access to healthcare services and support. Further research leading to evidence based health promotion programs are warranted to identify and address factors predicting early breastfeeding cessation. Additionally, these programs need to offer healthcare providers the means to detect early in the post-partum period (during hospitalization) and follow-up periods, mothers at-risk for suboptimal breastfeeding practices to offer the best protection against diarrhea illnesses.

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Table 1 Maternal and Infants Demographics

	Total N= 2344 (%)
Mother's Characteristics	
Race	
White	2003 (85.5)
Black	89 (3.8)
Asian/Pacific Islander	64 (2.7)
Other/Missing	188 (8.0)
Age (averaging years)	
18 - 28	1092 (46.5)
29 - 39	1158 (49.4)
40 or older	94 (4.1)
Marital status	
Married	1811 (77.3)
Unmarried	402 (17.2)
Missing	131 (5.6)
Household Income (US dollars)	
less than 5000	47 (2.0)
5000-24999	428 (18.3)
25000- 49999	819 (34.9)
50000-74999	591 (25.2)
75000-99999	271 (11.6)
100000 or over	188 (8.1)
Education Level	
High-school or less	435 (18.6)
Some college	827 (35.3)
College graduate or higher	940 (40.1)
Missing	142 (6.1)
Employment status	
Full time	864 (37)
Part time	271 (11.6)
Unemployed/missing	1209 (52)
Infant's characteristics	
Sex	
Male	1165 (49.7)
Female	1177 (50.2)
Weight (average)	7.64 lbs
Delivery method	
Vaginal	1671 (71.3)
Cesarean section	673 (28.7)
Ever breastfed or tried breastfeeding	
Yes	2000 (85.3)
No	344 (14.7)

Table 2 Effect of breastfeeding duration before 6 months and other variables on reported episodes of diarrhea in months 7, 9, 10 and 12.

Parameter	**Rate Ratio (RR) estimate for reported episode of diarrhea**	
	RR (*p value*)	95% CI
Intercept	0.093 (.000)	[0.083-0.103]
Breastfeeding cessation Never breastfed Breastfed between 0-3 months Breastfed between 3-6 months	 1.24 (.043) 1.48 (.000) 1.31 (.016)	 [1.007 - 1.52] [1.26 - 1.74] [1.05 - 1.64]
Household income	.98 (.003)	[.97 - .99]
Mother's age	.98 (0.000)	[.96 - .99]
Education	.92 (.02)	[.86 – 0.99]

Figure 1 Participants' selection process

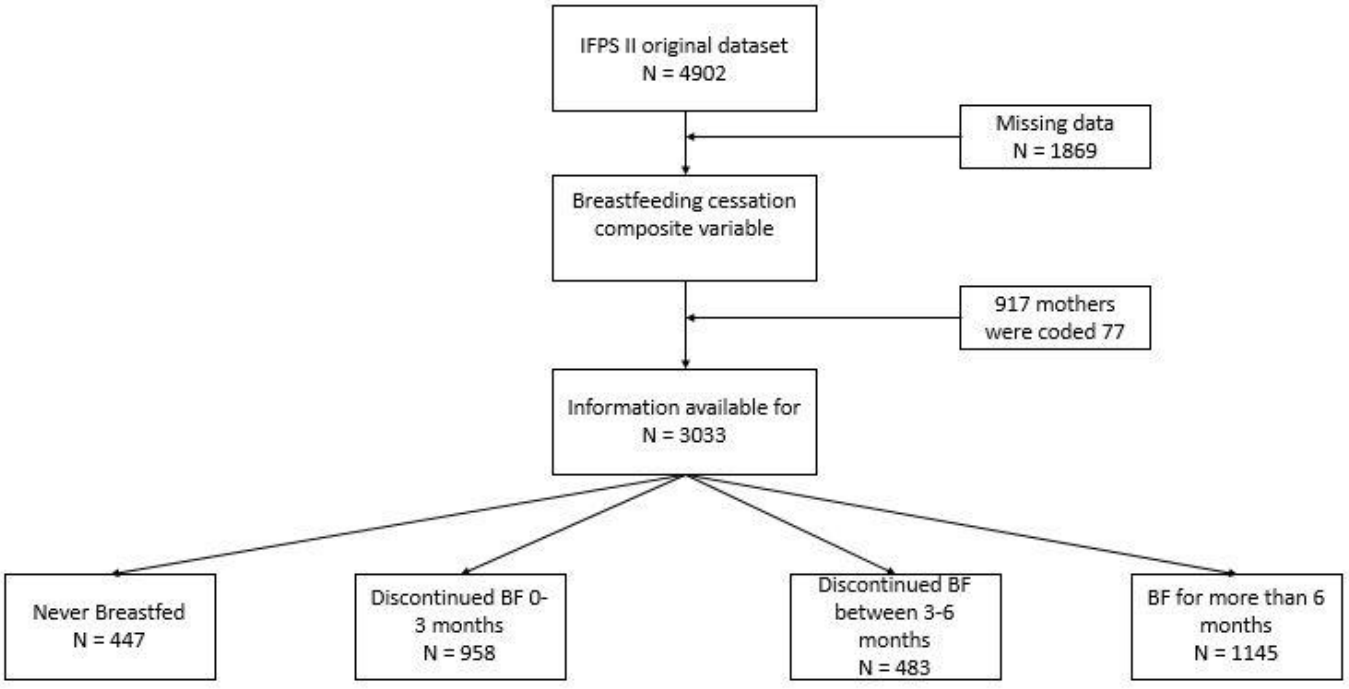
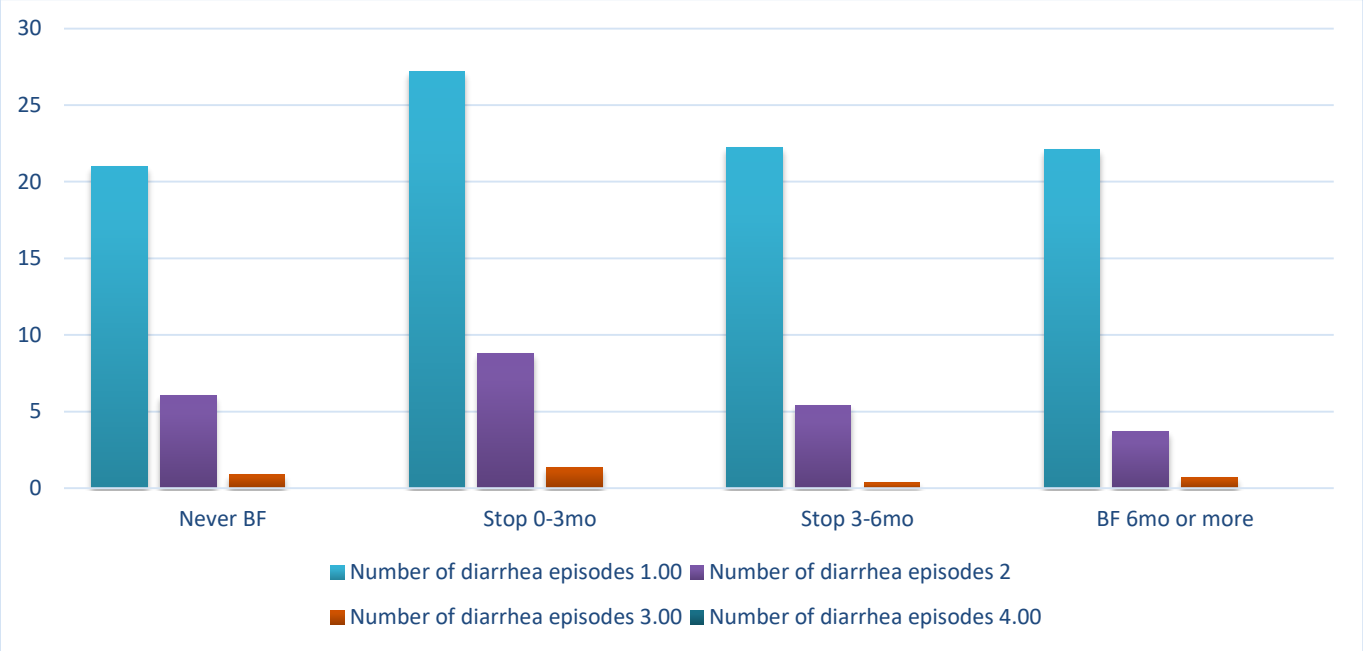


Figure 2 Percentage of reported number of diarrhea episodes at 7,9,10 and 12 months based on breastfeeding duration.



CHAPTER FOUR

Testing the sensitivity of a pathogen identification tool for diarrhea in two laboratory simulated temperatures: a feasibility study

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1- Journal of infection in developing countries

2- BMC infectious diseases

3- Clinical infectious diseases

Abstract

Background: In limited resources countries, the diagnosis and management of diarrheal diseases are mainly symptom based. A pathogen identification tool suited to resources limited settings will enable accurate identification of diarrheagenic pathogens, thereby improving management of diarrhea.

Method: This feasibility study aims to test the sensitivity of a point-of-care lateral flow assay strip under two different environmental conditions to mimic temperate compared to a hot climate to Specifically, the goal is to correctly detect potential diarrheagenic pathogens present in stool, such as *Escherichia coli*, under two different temperatures (69°F and 91°F). Time before the appearance of positive signals and color intensity of the signals at the test and control zones were used for sensitivity and performance analysis.

Results: In the presence of target DNA bound with gold nanoparticle and detection probe complex, interaction with the capture probe at the test zone produces a red signal observable by the naked eyes. At 69°F, optimum color intensity of the red signal was detected at 10 minutes with 10 and 25nM target DNA concentrations. At the 91°F, interaction with the capture probe at the test zone produced light pink signal observable by the naked eyes after the first 5 minutes. The signal was only observable at 10 and 25nM target DNA concentrations. Generalized linear model regression suggests that for every 1nM increase in target DNA concentration, color intensity of the signal decreased by 3.8% in pixel value.

Conclusion: The tool was able to positively detect the presence of *Escherichia coli* at different concentrations in a 69°F environment. Further optimization may improve the tool's sensitivity at 91°F. This is a step forward in the utilization of a Point-of-Care pathogen identification tool to be reliable in resource-limited settings.

4.1 Introduction

The diagnosis and management of diarrheal episodes are mainly based on physical symptoms. Laboratory diagnostic tests are expensive, time consuming, require significant training and are often not available in a timely way (Kotloff et al., 2013; Kaushik., 2016). The diagnosis and treatment of diarrheal diseases, based solely on patient or caregiver reported clinical symptoms, is often inaccurate, causes preventable medical complications and unnecessary use of other therapies such as broad spectrum antibiotics. In addition, inpatient hospitalization for unresolved diarrhea episode create a significant economic burden to the household and healthcare system. The average medical cost of hospitalization for a household is approximately $44 in Rwanda, $3 in India and $6 in Pakistan (Ngabo et al., 2016; Rheingans et al., 2012).

Molecular diagnostic methods, mostly with polymerase chain reaction (PCR), are the current gold standard tests for diagnosis of infectious diseases (Platts-Mills et al., 2012). However, PCR equipment and reagents are expensive, require skilled personnel and are subjected to amplification bias (Platts-Mills et al., 2012; Sjöling et al., 2015). Reliable and valid diagnostics are fundamental to identify the causes of a disease, formulate appropriate treatment, limit unnecessary use of antibiotics and reduce the disease burden. There is currently, a growing interest to develop lateral flow assays (LFA) for Point-of-Care (POC) diagnostic tool relevant to the realities of limited resources settings (Reid, Fidler, & Cooke, 2013; Wang et al., 2016).

These novel diagnostic platforms need to be developed to address the WHO seven characteristics of POC test for low resources settings. Also known under the acronyms ASSURED the POC tests must be **a**ffordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end users (Mabey, Peeling, Ustianowski, & Perkins, 2004;

Dittrich et al., 2016).

Given the recommendations of the ASSURED criteria, the present study is a feasibility study that aims to assess the sensitivity of a rapid, simple to use, diarrhea pathogen identification tool. Climate variation has been associated with an increase relative risk of diarrhea diseases by 22-29%; mainly by adversely impacting the quality of water supply and increasing temperature and rainfall worldwide (Kolstad & Johansson., 2011; Mellor et al., 2016). It is estimated that for every 1 degree Celsius increase in temperature there is approximately a 7% increase in diarrhea (Carlton, Woster, DeWitt, Goldstein, & Levy, 2016).

The tool was developed as a collaboration with the National Institutes of Health, National Institute of Nursing Research and GoDx Inc. It consists of a paper-based, colorimetric LFA strip, using nucleic acid detection. The aim of this feasibility study was to evaluate the ability of this tool to positively detect potential diarrheagenic pathogen under simulated environment such as hot temperatures. Since LFA are intended to be used in the field, in resources limited settings, the strips may be affected by the surrounding environmental conditions. In addition, increasing evidence suggests that environmental factor such as temperature, significantly affect the sensitivity of LFA (Choi, 2016). The successful completion of this study is expected to result in further optimization of a tool; allowing healthcare providers, to formulate better management of diarrhea by accurate identification of diarrheagenic pathogens regardless of the surrounding environmental condition.

4.2 Methods

4.2.1 Pathogen Identification Tool

The tool was developed as a collaboration with the National Institutes of Health, National Institute of Nursing Research and GoDx Inc. It consists of a paper-based, colorimetric LFA strip,

using nucleic acid detection. Nucleic acid detection provides critical genetic information in detecting the presence of infectious pathogens, the diagnosis and treatment of genetic diseases and cancers (Sun, Xianyu, & Jiang, 2014). Paper has many natural properties making it adequate to use in resource-limited settings. The most important features include its ability to passively transport fluids through capillary flow; it is made of cellulose making it compatible with various biological samples; its white color allows contrast background for visible and colorimetric readouts; it is cheap, thin and light, hence easy to transport and store (Zhao, Brook, & Li, 2008).

The major principle in LFA involves an interaction or hybridization of a single-stranded target DNA region of commercial *Escherichia coli (E. coli)* with complementary capture probe forming a double-stranded nucleic acid. The hybridization will produce a colorimetric signal and allowing the detection of the bacteria target DNA. Gold nanoparticles (AuNP) are used as colorimetric probes in the tool. The red predictable color change of AuNP aggregation provides a platform for colorimetric detection using AuNP as signal label to trace hybridization of the target DNA (Zhao, Ali, Aguirre, Brook, & Li, 2008).

The LFA strip is about 6.0 cm long and is made of: a glass fiber sample pad, a conjugate pad loaded with gold nanoparticles and detection probe complex, a nitrocellulose membrane containing a test zone and a control zone and a cellulose absorbent pad facilitating fluid flow. Colorimetric responses at the test and control zones of the LFA strip indicates the presence of the target DNA diarrhea pathogens. More details on the tool are mentioned in another publication.

4.2.2 Study procedure

Commercial *E. coli* (2011C- 3911) DNA target sequence was purchased and cultured in 100µl of broth to a final concentration of 100µM. Five different dilutions of the target DNA solution were made in 4X SSC buffer. They included: 1nM, 10nM, 25nM, 100nM and 200nM.

Six strips were used, each deposited with 85ul of the target DNA solution at each of the five aforementioned concentrations. A strip deposited with a solution containing 0nM target DNA was considered as the control.

Next, the sample solution containing the target DNA was deposited onto the sample pad of the LFA strip. Target DNA first hybridized with AuNP and detection probes (AuNP-DP) to form AuNP-DP-target DNA complex at the conjugate pad. Then the solution containing AuNP-DP-target DNA complex migrated by capillary action, passing the glass fiber part of the strip into the test zone. At this point, a second hybridization took place between the AuNP-DP-target DNA complex and the capture probes, producing a red signal at the test zone, observable by the naked eyes (Figure 1.1). In the absence of target DNA in the sample solution, no colored signal is observed at the test zone. The excess complex then reached the control zone where hybridization with the control probe occurred, producing a second red signal observable at the control zone. The red signal at the control zone indicates that the tool is working (Figure 1.2).

The tool was then placed in an incubator set at 69◦F (experiment 1) and 91◦F (experiment 2). These temperatures correspond to the average range of temperature variation reported in regions of Sub-Saharan Africa and South Asia (National Centers for Environmental Information, 2016). The presence or absence of a colored signal at the test and control zones was observed and noted at 5, 10, 15 and 30 minutes.

4.2.3 Image analysis

The LFA strips were imaged using an iPhone 6S Plus camera (Apple) on HDR (high dynamic imaging) mode. To obtain the same lighting, the pictures were taken at the same time and location on the laboratory bench. The strips were displayed on a blank sheet of white paper. The images were processed without modification. Image analysis followed the steps described in

Lathwal & Sikes (2008) supplemental publication. The color intensity of the signal at test and control zones were analyzed in RGB (red, green, and blue) color space using ImageJ software (National Institutes of Health). RGB is the most common color space used in image processing devices and corresponds to the red, green, and blue elemental components defining a color (Capitan-Vallvey et al., 2015). The darker an image is, the lower the color intensity; inversely, the lighter the image, the higher the color intensity.

Each image was separated into a red, green, and blue channel. The minimum, maximum, and average intensity of the signal at the control and test zones were measured on each channel and the results were exported on MS Excel. Color intensity values of the signal was defined as the mean intensity of all three channels at the test and control zones. Statistical analysis was performed using Generalized Linear Model to evaluate the main effect of temperature, time and target DNA concentration on the color intensity. Color intensity was expressed as the log10 of the mean pixel value obtained from each channel.

4.3 Results

Colorimetric assessment of the signal was quantified as the mean color intensity of each RGB channel at the test and control zones. Comparison of the colorimetric responses, under each temperature, was done by tracking the changes in color intensity based on dilution series and time interval (5 and 10 minutes). While the tool was observed for a total of 30 minutes, we are only reporting on color change occurring within the first 10 minutes because no change was noted after that time point.

4.3.1 Effect of time on color intensity

69^{o}F processing environment. A red signal was progressively observed at the test zone (Figure 2). Color intensity values of the signal observed at the test and control zones were similar in each

RGB channels. So analysis of the colored intensity of the tool focused on the values obtained from the Red channel. Change in color intensity of the signal occurred progressively over the first 10 minutes, then remained constant. Except for the strip loaded with 1nM target DNA solution, red signal at the test zone was observed on all strips at 5 minutes after application of the sample solution. After 10 minutes, a light pink signal was also observable on the strip loaded with 1nM target DNA solution. These findings are suggestive that the optimal maximal time for colorimetric readout is 10 minutes when the tool is tested at 69◦F.

91ºF processing environment. In a 91◦F environment, a light pink signal was observed after five minutes at the test zone of the strips loaded with 10 and 25nM target DNA solution (Figure 1). On the remaining strips (loaded with 1nM, 100nM and 200nM target DNA solutions), no colored signal appeared at the test zone. At the control zone, reddish- pink signal was observable on each strip. Lowest colored intensity, i.e., darkest colored signal, was obtained on the strip loaded with 10nM DNA concentration. After the first 10 minutes, a pink signal at the control zone of each strip was observed. No colored signal was observed at the test zone expect for two strips (loaded with 10 and 25nM target DNA solution).

Contrary to the results obtained under the 69◦F experiment, under 91◦F temperature, the signal reduced, i.e., lighter colored signal is observable at the test zone. The findings suggest less DNA hybridization at 91◦F. It seems that as temperature increases, the tool's sensitivity decreases.

4.3.2 Effect of concentration on color intensity

Concentration series of the target DNA was also used to assess the effectiveness of the tool to detect the genetic material of targeted diarrheagenic pathogen in the two simulated environments. Concentration gradient ranged from a minimum of 1nM to maximum of 200nM,

and included dilution of the solution at 10nm, 25nm and 100nM. Observable red signal and their change in color intensity were recorded at both 5 and 10 minutes. Color intensity of the test zone decreased significantly by 3.8% in pixel value with every 1nM increase in target DNA concentration ($p = 0.035$) (Figure 3). The findings indicate that target DNA concentration at 10 and 25nM concentration produced greatest color intensity; suggesting an increase hybridization rate between the target DNA and capture probe at the test zone with these concentrations. In addition, the results also suggest that the detection limit was achieved as low as 10nM and optimum colorimetric response was obtained at 25nM target DNA concentration.

4.4 Discussion

The present study is a feasibility study testing the sensitivity of a diarrhea pathogen identification tool to positively detect target sequence DNA of commercially available *E. coli* in two different temperature environments. The tool is a LFA strip using gold nanoparticle as a colorimetric probe to detect the presence of target DNA in the sample solution at different concentrations. Red signal was observed at the test and control zones when target sequence DNA solution is applied on the sample pad.

Previous studies have been done testing LFA strips using AuNP based on time, temperature and target DNA concentration (Mao et al., 2009; Choi et al., 2016). In Mao and colleagues' study (2009), the authors tested the sensitivity of a low-cost nucleic acid biosensor to detect commercial DNA based on time and target DNA oligos at various dilution series. Optimal time for detection was reported to be 15 minutes. Their study's findings suggest a linear relationship between color intensity and concentration ranging from 1 to 100nM target DNA. When looking at temperature variation and its impact of the LFA ability to detect target nucleic acid, Choi et al. (2016) reported that optimal temperature for detection ranged between 131 and

140^{o}F.

In an environment of 69^{o}F in our study, the tool showed the most visible signal at the control and test zones. Increase in temperature from 69^{o}F to 91^{o}F seemed to be linked with a decrease in color intensity of the red signal at the test zone. This may be due to various factors including, AuNP precipitation, evaporation of the sample solution or melting point of the primers or target DNA was reached or higher temperature might cause the double-stand DNA to dissociate, hence reducing the hybridization with the capture probe (Choi et al., 2016).

We found the color intensity of the signal was negatively influenced by the target DNA concentration gradient. In 69^{o}F environment, the lowest color intensity values corresponding to the darkest red signal was observed on the strips loaded with 10 and 25nM. The findings suggest that 10 minutes is the optimal colorimetric readout time and the detection limit is achieved at 10nM while optimum red signal was achieved with 25nM target DNA concentration.

4.4.1 Limitations

This study assessed the tool sensitivity using only the target DNA of one bacterial species at two different temperature conditions. The experiments were not repeated to assess replicability and further validation of the tool. Additional optimization is needed at each step of development of the LFA strips to address these limitations. Sensitivity and specificity of the tool will be tested using various target DNA species at different concentration, as well as various concentration of reagents, capture probes and SSC buffer which can promote DNA hybridization. Colorimetric assessment will be further explored as method of analysis. Future optimization of the tool will involve endogenous stool sample and testing the sensitivity at wider temperature variation and under other environmental condition such as humidity and stool acidity.

4.4.2 Implication for research and practice

Future research will involve reproducing the experiment at ambient conditions and comparing it to various temperatures, target DNA concentrations and environmental conditions such as relative humidity and stool acidity. In addition, tool sensitivity will be tested using other commercial bacterial DNA and endogenous diarrhea and healthy stool.

Diagnostics for poverty-related conditions such as diarrheal diseases are limited because they are mostly expansive and not adapted to the realities of many countries of the Developing World. Currently, management of diarrheal diseases are symptom based. However, this approach falls short to accurately identify the etiology and the severity of the disease as well as distinguishing asymptomatic diseases. Developing low cost, precise and user-friendly POC diagnostics is crucial in improving the management of diarrheal diseases at the bedside and the community level. Development of such tool has the potential to continue reducing the impact of diarrhea worldwide.

4.5 Conclusion

Diarrheal diseases remain the cause of millions of unnecessary deaths. Well-established interventions have proven to significantly reduce the mortality rates, but their impact have been limited in the last decade and even reversed in some areas. Identification of the etiologic agent causing a diarrheal episode will result in reduction of diarrhea related morbidity and mortality and prevent the unnecessary use of antibiotics. Existing standard diagnostics typically require expensive material, sophisticated infrastructure, and highly trained personnel. POC testing has been at the center of investigation to address global health issues such as diarrhea. The properties of paper-based LFA offer the ability to develop low cost, rapid and accurate testing.

This study is significant because it evaluates the effectiveness of a diarrheal pathogen

identification tool to positively detect the presence of pathogens causing diarrhea under laboratory simulated climatic conditions. Testing the ability of the research tool under such conditions, in a controlled laboratory environment, allows identification of the tool's limitation under high temperature. In addition, it also offers guidance for further optimization of the tool to make it more sensitive and specific. The utilization of a simple, robust and accurate tool for the identification of diarrheal pathogens is important in addressing the burden of diarrhea worldwide.

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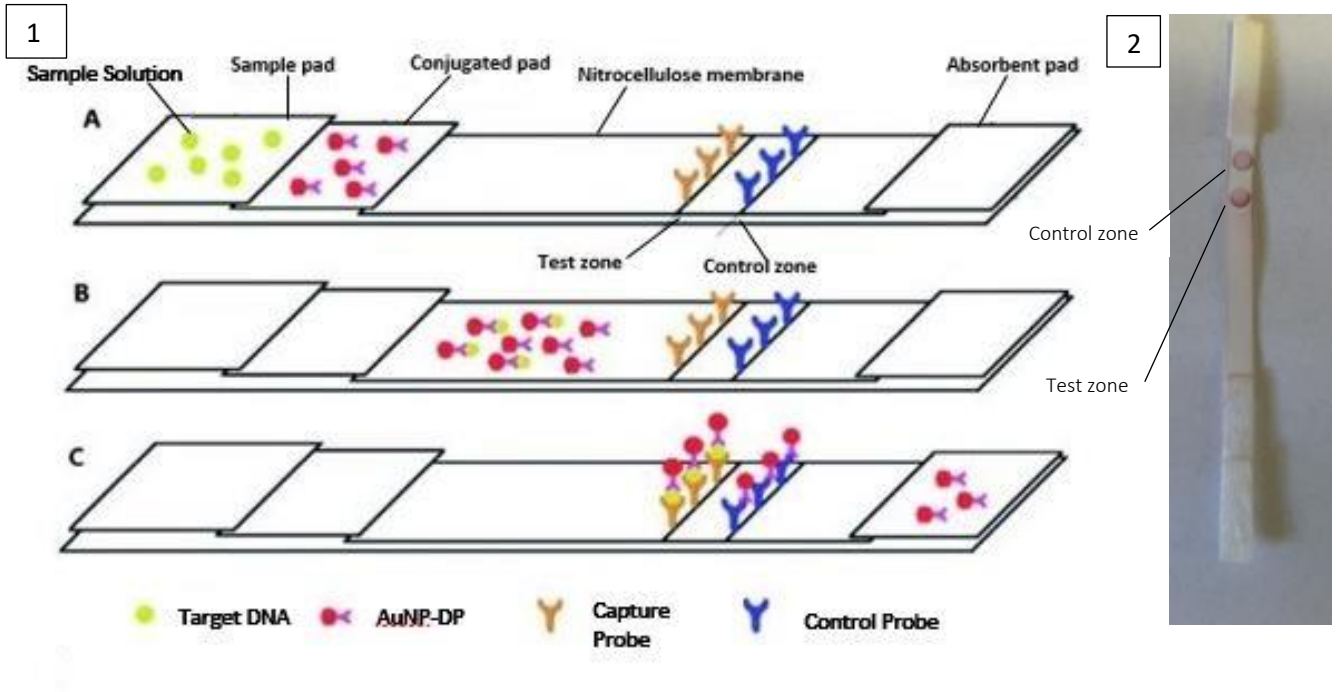
Figure 1. Schematic illustration of the pathogen identification tool



1) A. Sample solution containing target DNA is applied on the sample pad. Hybridization of target DNA with AuNP-DP to form AuNP-DP-target DNA complex. B. Migration of AuNP-DN-target DNA complex through the nitrocellulose membrane. C. Hybridization of AuNP-DP-target DNA with capture probe at the test zone. Hybridization of excess AuNP-DP-target DNA complex with control probe at control zone. 2) Red signal observable at the test and control zones.

Figure 2. Effect of temperature, target DNA concentration and time on the pathogen identification tool

69◦F Environment

5 minutes

200nM 100nM 25nM 10nM 1nM Control

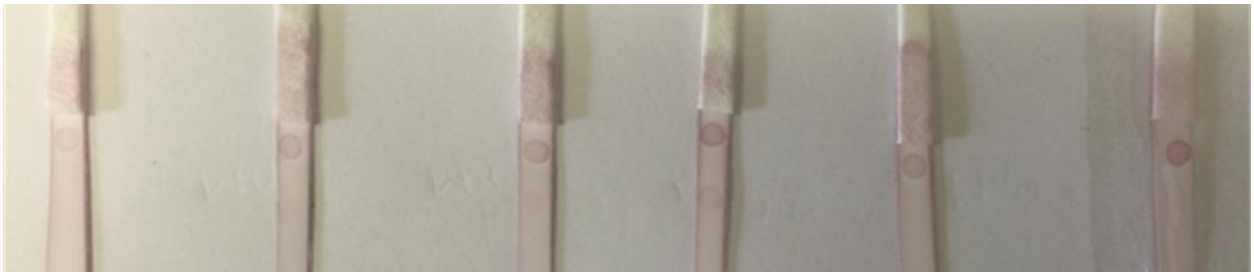
10 minutes

200nM 100nM 25nM 10nM 1nM Control

91◦F Environment

5 minutes

200nM 100nM 25nM 10nM 1nM Control



10 minutes

200nM 100nM 25nM 10nM 1nM Control

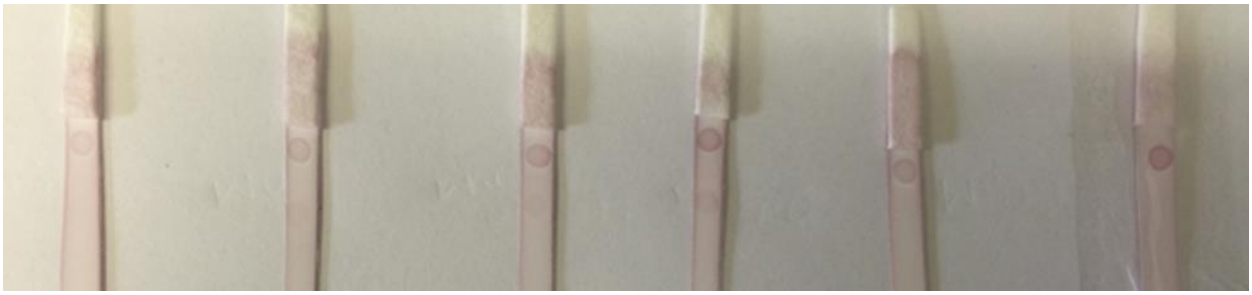
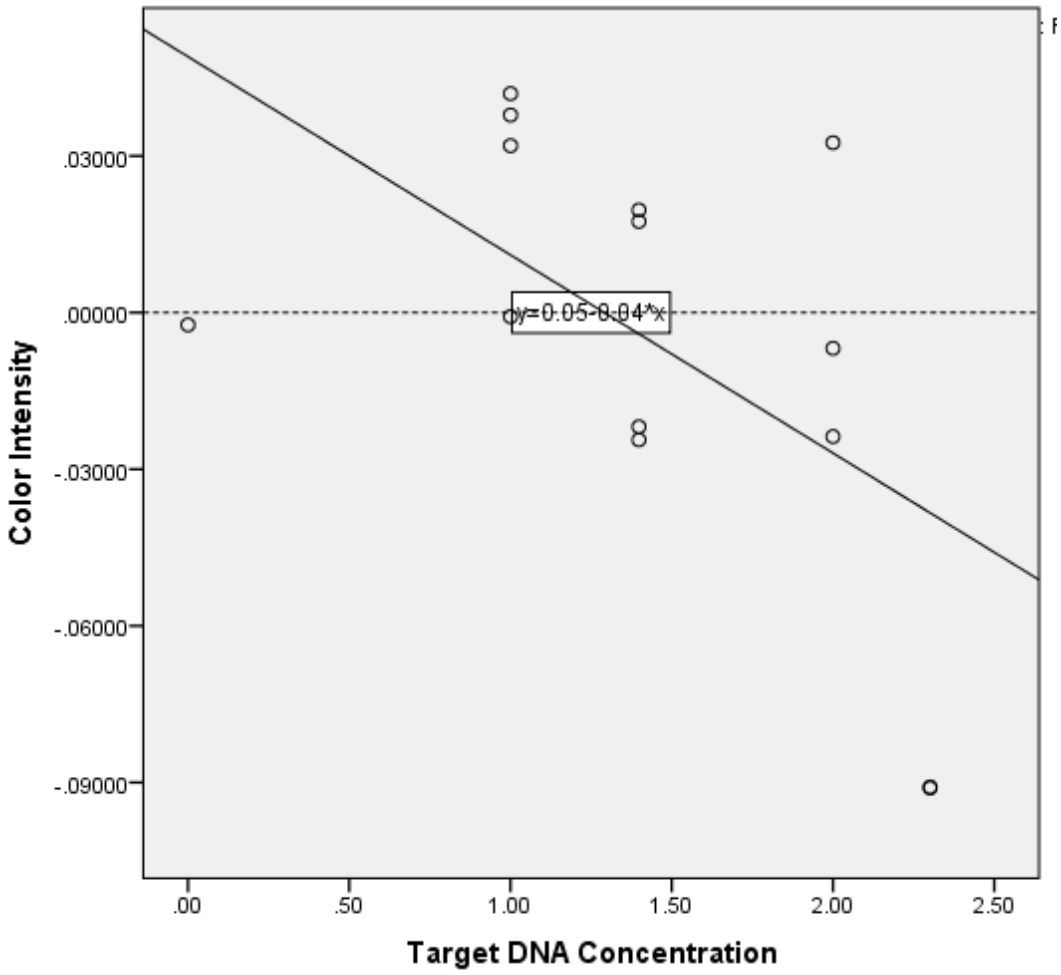


Figure 3. Main effect of Target DNA concentration on intensity of color signal



CHAPTER FIVE

5.1 Introduction

The ultimate goal of this dissertation was to contribute to our understanding of how the management of childhood diarrhea has evolved over the last four decades, to identify the major gaps in the current management and introduce a novel approach to address these gaps. The research in Chapters 2 and 3 examined the limited use of ORT and breastfeeding, interventions known to effectively prevent and treat diarrhea since the 1970's. The study in Chapter 4 took a closer look at the properties of a pathogen identification tool for diarrhea in two laboratory simulated environments.

5.2 Major findings from Chapter 2

Observational studies reporting on physicians and pharmacists use of ORT in the management of childhood diarrhea in South Asia were systematically reviewed. The main objectives of the study was to assess what the literature reported on the use of ORT in treating cases of childhood diarrhea and comparing the prescription behaviors before and after the publication of the 2004 WHO guideline on the management of childhood diarrhea.

A systematic review of the available evidence suggested significant variability in physicians and pharmacists reported ORT prescription rates in studies published before and after the 2004 WHO guideline. Physicians appeared to prescribe more frequently ORT compared to pharmacists. Reported knowledge and referencing of the WHO guideline for the management of childhood diarrhea and its recommended ORT formulation were limited. These findings reiterates the numerous reports on the limited use of ORT in the management of childhood diarrhea.

Most importantly, this study highlighted the methodological limitations of the published research and their reports. Inconsistencies in the quality of the data collection and reporting calls for the urgent need to develop standard methods to measure the outcomes, perform data collection and report the results. Studies with stronger methodology would have the potential to provide evidence that could identify the currents gaps and be the basis for the formulation of more effective interventions addressing global health issues such as diarrhea.

5.3 Major findings from Chapter 3

A secondary data analysis of the IFPS2 survey on breastfeeding cessation during the first 6 months on the onset of diarrhea shows that: 1) breastfeeding for 6 months or more significantly decreases the incidence of diarrhea before the first year of life; 2) infants who breastfed for less than 3 months had the highest percentage of reported episodes of diarrhea at seven to 12 months; 3) increase in maternal age, higher income households and higher maternal education were associated with a decrease in reported episodes of diarrhea episodes.

Since it is free, breastfeeding is considered the most cost-effective intervention to prevent and treat diarrhea in infancy and early childhood. The results of the study reported in this chapter provides evidence supporting the practice of exclusive breastfeeding for the first 6 months of life. Infants who were breastfed for less than three months were the most affected by diarrhea which might be associated with early introduction of food other than breastmilk. Most importantly, the findings depicts the impact of social determinants such as education and household income on early breastfeeding cessation and subsequent onset of diarrheal episodes.

5.4 Major findings from Chapter 4

Findings from Chapter 4 present the properties of a diarrheagenic pathogen identification tool to detect the presence of E coli at different dilution points. The tool was tested in laboratory environments simulating temperatures characteristic of region of the world that are the most affected by diarrhea. This feasibility study offers insight on the tool sensitivity based on temperature, time and concentration variations, allowing further optimization.

The tool is a paper based lateral flow assay strip using gold nanoparticles as an indicator of the presence of a target pathogen. Positive detection of *E. coli* was translated by the presence of a hue of red colored signal at the test region on the strip. Ability to detect a pathogen was assessed by the presence or absence of a colored signal at the test and control zones, the time before observation of colored signal by the naked eyes and the color intensity of the signal.

At 69^{o}F, 10 minutes after the sample solution was applied, the tool was able to detect the presence of *E. coli* at a concentration varying from 1nM to 200nM. The colored signal were the darkest on the strips containing 10 and 25nM solutions. In a 91^{o}F environment, the strips were only able to positively detect *E. coli* at 10 and 25nM after 5 minutes. Very light pink signal was visible on the test and control zones at these concentrations. Generalized linear regressions assessing the main effect of temperature, time and concentration on the color intensity suggested that concentration gradient had a negative relation on the color intensity signal visible on the strip.

These findings suggest that optimal detection of bacterial DNA occurs at concentration between 10 and 25nM, 10 minutes after the initial application of the sample solution. Temperature and increase in DNA concentration appear to limit the detection properties of the

tool. The results will guide further optimization of the tool to ultimately become reliable and relevant to resource-limited settings.

5.5 Limitations

The studies in this dissertation have several limitations. First the study in Chapter 2 is based on the existing peer- reviewed literature limited to English language. Quality of the articles included in the review was not assessed, which increase the risk of bias from inclusion of studies with misrepresentative findings. Across studies, measurement of ORT prescriptions was inconsistent. The studies differed greatly in their design and methodology, population and settings. This variation limited the availability of quality data; hence unabling combination of the studies' findings into a meta-analysis and identification of defined ORT prescription patterns across countries, settings and time periods.

Second, due to the nature of the data, i.e secondary data analysis, the study in Chapter 3 has some limitations. The major challenge was the fact that the study's population was not representative of the US population, so the findings could not be generalized at the national level. In addition, the lack of data collected between the neonatal period and month 3 was a missed opportunity to depict a clearer picture of early breastfeeding cessation and potential influencing factors. Nationally representative studies with quality data collected monthly from birth would produce stronger evidence on the preventive properties of breastfeeding on childhood diarrhea.

The major limitation of the study in Chapter 4 is the sample size. Only one sample solution was used to test the tools' properties. Therefore, evaluation of the potential effect of temperature, concentration and time on the color intensity is limited. Further test will be

performed using commercial DNA of other bacteria such as *Clostridium difficile* and endogenous stool sample under diverse environmental conditions such as wider temperature range, relative humid and stool acidity.

5.6 Implication for policy and practice

The findings highlight the magnitude of inconsistencies in the current management of childhood diarrhea and the need to develop alternative approach to contain the effect of the health condition on the most vulnerable. The research furthers previous studies and international institutions' efforts to address the limited use of ORT and breastfeeding in the management of diarrhea for the profit of unnecessary use of antibiotics and others therapies. These findings support the on-going development of new technologies, economic and requiring minimal instrumentation, to address global health issues.

In resource limited settings, where the medical system lacks modern laboratory infrastructures and trained human resource, user-friendly technologies are urgently needed to improve therapeutic management. The development of low cost lateral- flow immunoassays used to identify etiologic agents causing diarrhea diseases, offers considerable potential to reduce the burden of the disease and change the current practice guideline which is symptom based (Yetisen, Akram, & Lowe, 2013). In addition, distinguishing bacterial from viral diarrheal episodes would better inform healthcare providers to make the right prescriptions, hence reducing the risk to unnecessarily prescribe antibiotics.

Regardless of the advances in health and technology, the porosity of geographical boundaries and population migration due to globalization, foodborne illnesses, zoonotic and waterborne diseases remain health issues that continue to fuel diarrheal diseases. This is mainly

due to limited access to clean water and adequate sanitation, close human-animal contact and ingestion of contaminated food. Programs testing the food and water supplies designated for human consumption are limited in developing countries. Development of such tests has the potential to play an essential role in medical diagnosis, food safety and control as well as environmental monitoring for prevention and control of epidemics (Yetisen et al., 2013).

5.7 Implication for research

Given this dissertation's findings, two areas for future research were mainly defined. First and most importantly, there is an urgent need to design and perform observational studies with strong methodology, data collection and reporting. Standard guideline for global health research based on conceptual framework could be defined by international institution such as the WHO to insure actions based on quality evidence and report of the findings. Research settings might differ greatly based on the culture, language and demographics, but regardless of these distinguishing features, common factors are associated with diarrhea. Therefore, overarching guideline could be develop to collect and measure outcomes related to diarrheal diseases.

Multicountry surveys such as the Demographic Health Survey and Multiple Indicator Cluster Survey (MICS) can also contribute to our understanding of global health issues as they occur in the natural settings. Managed by the United States Agency for International Development, DHS consists of numerous surveys lead to monitor and evaluate different indicators of countries' needs (Rutstein & Rojas, 2006). The analysis of such large dataset can be another alternative to picture the prevalence of health behaviors such as diarrhea management.

Second area of research is the development of alternative platform technologies that could address the identified gaps in the management of childhood diarrhea. The WHO has set

seven criteria for the development of point of care diagnostic tests for resources-limited settings (Kettler et al., 2004). These characteristics should guide research and prioritize the development of low cost, rapid, sensitive and specific diagnostic assays. Besides the development of new diagnostics, more scientific efforts should be invested in ensuring that these technologies are adapted to the climate and environment specific to resource-limited settings.

5.8 Conclusion

A shift in priority away from diarrhea management has contributed to the slowing progress in the full coverage of ORT and breastfeeding as preventive and treatment measures. The development of point-of-care diagnostics at the service of global health issues is critical to address the gap in using adequate management measures, hence improving child survival and reducing morbidity and mortality rates caused by diarrheal diseases. Understanding the fundamentals of capillary flow of the paper-based assays, detection of bacterial genetic material and other characteristics of the technology will allow development of assays with better control over the impact of temperature, moisture and other environmental factors.

The characteristics of paper and potential simplicity of the tool offer the potential for this paper-based lateral flow assay to be utilized in settings with very limited resources while offering accurate and highly sensitive information. However, having laboratory controlled data does not always yield to accurate performance of the tool once deployed in the real world. Testing the specificity and sensitivity of the tool in different clinical and community settings is required in order to produce strong evidence to adopt the test within the standard management of diarrhea. Such research will allow transformation of the tool from a prototype into a reproducible diagnostic platform.

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