FREQUENCY AND DISTRIBUTION OF DIFFERENT PATHOGENS IN ACUTE DIARRHEAL EPISODES AMONG EGYPTIAN CHILDREN USING A COMBINATION OF PHENOTYPIC AND MOLECULAR TESTS

Thesis submitted for partial fulfillment of M.D degree in pediatrics

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2011

Acknowledgement

My deepest gratitude and thanks to **Allah**, whose help is the first factor in everything we do in our lives.

My sincere thanks to **Prof. Dr. Mohamed Emad El-din Salem**, professor of pediatrics, faculty of medicine, Cairo university for his kind help and supervision.

My deep gratitude to **Prof. Dr. Mortada El-Shabrawy**, professor of pediatrics, faculty of medicine, Cairo university for his kind help, indispensable advice and valuable criticism.

My sincere thanks to **Dr. Suzan El-Naghy**, consultant of pediatrics and head of pediatrics department, National Hepatology and Tropical Medicine Research Institute (NHTMRI), for her supervision, encouragement and unlimited support.

My deep thanks to **Dr. Hanan El-Mohamady**, consultant of immunology, NAMRU-3, Cairo, for her kind supervision and understanding.

I am deeply grateful to **Dr. Maha Abo Zekri**, consultant of pediatrics, faculty of medicine, Cairo university for her continuous supervision, valuable suggestions and guidance.

My sincere thanks to **Dr. Adel Mansour**, NAMRU-3, Cairo, for his continuous advice, outstanding support and active contact with statistician, **Manal Mostafa,** for data management and analysis.

Ayman Mohamed

To my father's soul,

kind mother,

supporting wife,

and lovely kids.

With love and gratitude.

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LIST OF ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
CMV	Cytomegalovirus
CSPM	Center for Social and Preventive Medicine
EAEC	Enteroaggregative Escherichia coli
EHEC	Enteropathogenic Escherichia coli
EIE	Enteroinvasive Escherichia coli
ELISA	Enzyme-Linked Immunosorbent Assay
EMR	Eastern Mediterranean Region
EPEC	Enteropathogenic Escherichia coli
ETEC	Enterotoxigenic Escherichia coli
GE	Gastroenteritis
GIT	Gastrointestinal Tract
HIV	Human Immunodeficiency Virus
HUS	Hemolytic Uremic Syndrome
LT	Heat-Labile Toxin
NAMRU-3	Naval Medical Research Unit No.3
NHTMRI	National Hepatology and Tropical Medicine Research Institute
ORS	Oral Rehydration Solution
PCR	Polymerase Chain Reaction
RAPPD	Random Amplified Polymorphic DNA
RS	Reiter Syndrome
RT-PCR	Reverse-Transcreptase Polymerase Chain Reaction
ST	Heat-Stable Toxin
URI	Upper Respiratory Infection
VPEC	Verotoxin-Producing Escherichia coli
WHO	World Health Organization

Abstract:

Key words: Diarrhea – Enteropathogens.

To identify frequency and distribution of enteropathogens in acute diarrhea, this case-control study was conducted in Center for Social and Preventive Medicine, Cairo university. Stool samples were obtained from 356 cases and comparable controls below 5 years and processed at NAMRU-3, Cairo, for microbiology. Results pointed to 13% bacterial against 11% being due to rotavirus and 4% due to *Cryptosporidium*. Conclusion: Rotavirus is a principal pathogen in acute diarrhea followed by ETEC, *Campylobacter* and *Cryptosporidium.*

Introduction:

Diarrhea is a leading cause of childhood morbidity and mortality in developing countries, and an important cause of malnutrition. Worldwide, children younger than 5 years experience an estimated 1.4 billion episodes of diarrhea each year, leading to 123 million clinic visits, 9 million hospitalizations, and 1.87 million deaths, with more than 98% of these deaths occurring in the developing world ***(Boschi et al., 2008)***.

Diarrheal disease attack rate remains largely unchanged because of the continuing high prevalence of bacterial, viral and parasitic enteropathogenics. Most deaths are caused by failure to treat acute dehydration and to correct electrolyte imbalance and to provide adequate nutrition ***(Vernacchio, 2006)***.

Disease specific rates for enterotoxigenic *Escherichia coli* (ETEC), *Campylobacter*, rotavirus, and *Shigella* were 1.5, 0.6, 0.24, and 0.2 episodes per child year ***(Rao et al., 2001 and Wierzba et al., 2006)***.

While *Campylobacter*, *Salmonella*, and *Shigella* remain major contributors to acute enteric infections, few studies on these pathogens have been conducted in Egypt ***(Momtaz et al., 2000)***.

Cryptosporidium parvum is common in Egyptian children and may be associated with severe diarrhea ***(Ibrahim Adib et al., 2005)***.

A clinic-based study for the surveillance of severe diarrhea conducted in Northern Egypt identified bacterial or viral enteropathogens in almost 45% of the acute diarrhea cases studied. However, additional available data suggests that another 18% of the diarrhea cases might caused by diarrheagenic *E. coli* pathovars, in particular, enteropathogenic *E. coli* (EPEC) [~ 6%] and enteroaggregative *E. coli* (EAEC) [~ 12%]. It is possible that by including these additional pathogens, the majority of diarrheal pathogens of Egyptian children will be accounted for ***(Wierzba, et al., 2006)***.

Objective of the study:

The aim of this study is to determine the frequency and distribution of different pathogens of diarrheagenic *E. coli*, *Campylobacter*, *Shigella*, *Salmonella*, rotavirus, *Cryptosporidium* and *Entamoeba* in the feces of healthy and diarrheagenic children using a combination of phenotypic and molecular tests.

CHAPTER I

PATHOPHYSIOLOGY OF ACUTE DIARRHEAL DISEASE

The pathophysiologic mechanisms of diarrhea include osmotic diarrhea, secretory diarrhea, mutations in apical membrane transport proteins, a reduction in anatomic surface area, alteration in intestinal motility, and inhibition of transport of electrolytes by inflammatory mediators ***(Walker et al., 2000)***.

Osmotic diarrhea:

In osmotic diarrhea, osmotically active solutes pull water from the body into the gut lumen ***(Stefano, 2000*)**. The commonest cause of osmotic diarrhea and acute diarrhea disease worldwide is viral enteritis due to the rotavirus **(*Parashar et al., 2006*)**.

This virus stimulates shedding of mature absorbing epithelial cells from the intestinal villi and replace it with immature underdeveloped transport processes. When unbalanced sugar-electrolyte solutions, such as fruit juices, soda pop, or both are provided, the intestine`s transport capacity is overwhelmed, and the osmotic forces created by non-absorbed nutrients that remain in the lumen stimulate watery diarrheal fluid losses ***(Walker-Smith et al., 1996*)**. Diarrheal stools promptly regress with discontinuation of the

offending nutrient and the stool ion gap is high, exceeding 100 mOsm/kg ***(Stefano, 2000*)**.

Interestingly, since the implementation of treatment protocols that stress prompt oral rehydration and early feeding, the incidence of this complication has decreased in children with mild to moderate degrees of dehydration ***(Walker et al., 2000*)**.

Malabsorptive osmotic diarrhea also occurs with generalized small intestinal injury due to celiac disease, autoimmune enteropathy, or graft versus-host disease, as well as infections due to *Giardia Lamblia*, *Cryptosporidium, Salmonella*, or EAEC **(*Wilson, 2005*)**.

Medications, including laxative, that contain unabsorbable sugars such as sorbitol, lactulose, or manitol, or poorly absorbable ions such as magnesium, sulfate, phosphate, or citrate, may provoke osmotic diarrhea **(*Stefano, 2000*)**.

Neonates with congenital chloridorrhea, sodium secertory diarrhea, and congenital glucose- galactose deficiency develop severe watery diarrhea in the first week of life due to defects in intestinal chloride bicarbonate exchangers, sodium hydrogen exchangers, or sodium-dependent glucose transporters, respectively ***(Wilson, 2005*)**.

A more common genetic cause of osmotic diarrhea is ``late`` or `adult-onset`` lactase deficiency. Intestinal lactase activity declines to 5-10% of

levels present at birth. Clinical lactose intolerance with resultant osmotic diarrhea may develop at 5 to 7 years of age but can be delayed into adolescence in some ethnic groups ***(Stefano, 2000*)**.

Secretory diarrhea:

The mechanisms of secretory diarrhea include activation of intracellular mediators such as c AMP, c GMP, and intracellular calcium, which stimulate active chloride secretion from the crypt cells and inhibit the neutral coupled sodium chloride absorption. These mediators alter the paracellular ion influx because of toxin-mediated injury to the tight junctions **(*Field, 2003*)**.

Known luminal secretagogues include (1) bacterial enterotoxins (*cholera*, heat-labile and heat-stable *E. coli*, *staphylococcal* enterotoxins, *Clostridium perfringens*, *Bacillus cerus*); (2) hydroxy fatty acids produced by the bacterial digestion of malabsorbed dietary lipids; and (3) Nonabsorbed bile acids **(*Stefano, 2000*)**.

Endogenous secretagogues include (1) hormones often secreted by tumors (vasoactive intestinal peptide, substance p, serotonin, gastrin, and calcitonin) and (2) inflammatory mediators released in response to food allergy, inflammatory bowel disease, or systemic infections **(*Wilson, 2005*)**. Direct stimulation of the enteric nervous system also provokes secretory diarrhea **(*Gastro-Rodrigues et al., 1997*)**.