

AIN SHAMS UNIVERSITY  
FACULTY OF MEDICINE  
DEPARTMENT OF COMMUNITY, ENVIRONMENTAL & OCCUPATIONAL MEDICINE

THE ROLE OF HEAT-STABLE ENTEROTOXIN PRODUCED BY ESCHERICHIA COLI IN  
DIARRHEA OF INFANTS IN EGYPT

618.92 344  
M. M

A THESIS SUBMITTED AS A PARTIAL FULFILMENT FOR THE MASTER  
DEGREE IN PUBLIC HEALTH



BY

MAHI MAHMOUD FAHIM

DEMONSTRATOR IN THE DEPARTMENT OF COMMUNITY,  
ENVIRONMENTAL AND OCCUPATIONAL MEDICINE,  
AIN SHAMS UNIVERSITY, FACULTY OF MEDICINE

M. S

13911

SUPERVISORS

PROF. DR. ALY MASSOUD,  
CHAIRMAN OF THE DEPARTMENT OF  
COMMUNITY AND ENVIRONMENTAL AND  
OCCUPATIONAL MEDICINE,  
AIN SHAMS UNIVERSITY

DR. AZIZ EL KHOLY,  
DIRECTOR BIOMEDICAL RESEARCH  
CENTER FOR INFECTIOUS DISEASES,  
CAIRO

1981

### ACKNOWLEDGMENT

I wish to express my highest appreciation and deepest gratitude to Prof. Dr. Aly Massoud, Head of department of community, Environmental and occupational medicine, Ain Shams Univ., for his skilful guidance, supervision, expert assistance and fruitful criticism to proceed with this work.

I would also like to express my sincere gratitude and thanks to Dr. Aziz El Koly, Director of Biomedical Research Center For Infectious Diseases, Cairo, for his experienced advice he kindly offered me throughout the preparation of this thesis.

I am also much obliged and greatly indebted to Dr. Rifki Faris, Assistant Prof., Department of community, Environmental and occupational medicine, Ain Shams Univ., for his valuable assistance and useful advices throughout this work.

I also wish to express my deepest gratitude to Dr. Ahmed H. Abdel Karim, Assistant Prof. of industrial, Med. National Research Center, for his continuous encouragement and advice.

I would also like to thank Dr. Ahmed Sherif Hafez, Lecturer, Department of community, Environmental and occupational medicine, Ain Shams Univ., for his endless help and advice.



I wish also to thank with my gratitude Dr. Nabil Guirguis, Ph.D., Head of Microbiology Unit, The Biomedical Research Center for Infectious Diseases, Cairo, for his useful advice, generous help and revision of this work.

I wish also to thank all the staff of Biomedical Research Center for Infectious Diseases, for their kind co-operation.

Finally I extend my gratitude to every one who gave me help and advice.



## C\_O\_N\_T\_E\_N\_T\_S

	<u>Page</u>
<u>CHAPTER I</u>	
INTRODUCTION .....	1
REVIEW OF LITERATURE .....	4
✓ - Epidemiology of diarrheal disease .....	6
✓ - Etiology of diarrheal disease .....	8
- <u>Escherichia coli</u> .....	14
✓ - Antigenic structure of <u>E. coli</u> .....	16
- Enterotoxins produced by <u>E. coli</u> .....	19
✓ - Heat-labile enterotoxin (LT) .....	19
- Mechanism of action of LT .....	20
- Heat-stable toxin (ST) .....	21
- Mechanism of action of ST .....	23
- <u>E. coli</u> as pathogenic organism .....	25
✓ - Other factors that may be related to diarrhea caused by <u>E. coli</u> .....	30
- Vascular permeability factor .....	30
- Piliation of <u>E. coli</u> and both colonization and susceptibility to phagocytosis .....	31
- Objectives of the study .....	34

## CHAPTER II

MATERIALS AND METHODS .....	35
- Population .....	35
- <u>E. coli</u> strains .....	37
- Culture media .....	37
- Other requisities .....	41
- Experimental animals and tissue cultures.....	42
- Studies on fecal specimens .....	42
- Collection and transportation of specimens...	42
- Isolation of causative organisms .....	44
- Reading of Enterotube (Identification of Enterobacteriaceae) .....	47
- Heat-stable enterotoxin preparation .....	48
- Test for detection of heat-stable enterotoxin.	49
- Reproducibility of suckling mouse assay.....	53
- Heat labile enterotoxin preparation .....	53
- Test for detection of heat labile toxin .....	54

## CHAPTER III

RESULTS .....	58
---------------	----

Page

CHAPTER IV

DISCUSSION .....	83
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS .....	96
REFERENCES .....	103
ARABIC SUMMARY	

## LIST OF TABLES

<u>Table No.</u>		<u>Page</u>
I	Distribution of cases and controls according to age and sex .....	59
II	Distribution of cases and controls according to leucocytic count per high power field in fresh stool specimens .....	60
III	Distribution of isolated <u>E. coli</u> from stool specimens of cases and controls according to lactose fermentation .....	62
IV	Enterobacteriaceae other than <u>E. coli</u> isolated from stool of cases and controls	64
V	Number and percent of individuals with <u>E. coli</u> producing heat stable toxin in their stool specimens .....	66
VI	Number and percentage of individuals with <u>E. coli</u> producing heat labile toxin in their stool specimens .....	67
VII	Distribution of Enterotoxigenic <u>E. coli</u> isolated from stool specimens of cases and controls according to ST and LT production	68



<u>Table No.</u>		<u>Page</u>
VIII	Distribution of <u>E. coli</u> isolated from fecal specimens of cases and controls according to toxigenicity and lactose fermentation...	71
IX	Distribution of isolated <u>E. coli</u> according to different biochemical reactions (ID value) by Enterotube .....	73
X	Reproducibility of suckling mouse-assay....	74
XI	Distributions of signs and symptoms among diarrheal cases with ST or LT producing <u>E. coli</u> .....	77

## LIST OF FIGURES

<u>Fig.No.</u>		<u>Page</u>
1	Enterotube showing negative and positive reactions of the eight included media...	38
2	Represents the documentation of biochemical reactions, name of patient, date of test and organism identified .....	38
3	Disposable container for collection of fecal specimens .....	43
4	Rotating shaker .....	50
5	Suckling mouse assay .....	52
6	Chinese hamster ovary cells treated with LT -ve <u>E. coli</u> strain showing no elongation of the cells .....	57
7	Chinese hamster ovary cells treated with LT +ve <u>E. coli</u> strain showing elongated spindle-shaped cells .....	57
8	Distribution of cases and controls according to the presence or absence of toxigenic and non toxigenic <u>E. coli</u> .....	69

<u>Fig.No.</u>		<u>Page</u>
9	Distribution of controls group according to age and weight .....	80
10	Distribution of cases with nontoxigenic <u>E. coli</u> according to age and weight pre the onset of diarrhea .....	81
11	Distribution of cases with toxigenic <u>E. coli</u> according to age and weight pre the onset of diarrhea .....	82

## **CHAPTER I**

# **INTRODUCTION**

## INTRODUCTION

In developing countries, diarrheal diseases, together with acute respiratory infection are the leading cause of infantile and childhood mortality. In Cairo at 1961, 60% of infant deaths were due to gastroenteritis (Infant mortality rate was 151.2 per 1000 live births, while specific infant mortality rate from gastroenteritis was 92.6 per 1000 live births), (Shawki, 1965).

Every year, up to 1000 million diarrheal episodes cause four to five million deaths. In careful community study, children have been found to spend about 2 months a year (i.e. 16% of the time) with diarrhea (Barua, 1980).

Diarrhea and vomiting are common symptoms in infants and young children, but these symptoms may occur as a feature of many infectious disease.

With regard to the aetiological diagnosis, the position is not so clear. In many cases no bacterial or viral agent is isolated from the feces, even in acute outbreaks of the disease (Gordon et al 1964, Gurwith and William 1977 and Evans et al 1977-a). This probably means that laboratory techniques are not sensitive enough to detect the infective agents, but it may also means that no infective agent is

involved. It may be that some organisms not usually regarded as pathogenic, but not present at birth, may when it first colonizes the infant gut cause a temporary upset of the flora or lead to the production of toxic metabolites which irritate the bowel wall (Christie 1974).

Although Escherichia coli strains (E. coli) predominants among aerobic commensal organisms present in the baby gut, yet it stands now among the common agents producing diarrhea (Cook 1974, Gurwith and William 1977).

The pathogenicity of certain strains of E. coli attributed either to presence of certain surface antigen giving invasiveness properties and causing dysentery like syndrom (Ogawa et al 1968) or to production of enterotoxins that cause fluid accumulation giving salmonella like enteritis with no invasion or ulceration (Sakazaki et al 1967).

Enterotoxigenic strains of E. coli produce two known types of enterotoxin, one heat-labile toxin (LT), the other is heat-stable toxin (ST) (Smith and Halls 1967a-b, Gyles and Barnum 1969, Smith and Gyles 1970 a,b, and Gyles 1971). LT is partially identical to cholera toxin of Vibrio cholera and has the same mechanism of action. LT has given much attention and its role in diarrhea seems well established.

As regards the role of ST in diarrhea of infants the position was not so clear till recent studies showed that ST producing strains of E. coli were responsible for some cases and some outbreaks of diarrhea (Serafim et al 1977, Luke et al 1978 and Bl'aha et al 1978), and this was supported by the study done by Levine et al (1977) on volunteers which proved that strains of E. coli that produce ST are important in etiology of diarrheal disease.

The aim of this study is to determine the role of this heat stable enterotoxin produced by E. coli as a factor in infantile diarrhea in Egyptian rural area.