

**MOLECULAR CHARACTERIZATION OF BACTERIAL DIARRHOEAGENIC
AGENTS IN CHILDREN AGED FIVE YEARS AND BELOW FROM
KENYATTA NATIONAL HOSPITAL, NAIROBI, KENYA.**

BY

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**A dissertation submitted in partial fulfillment for the requirements of
the Master of Science in Medical Microbiology**

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DECLARATION

This dissertation is my original work and, to the best of my knowledge, has not been presented for a degree in any other University.

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DEDICATION

To Al-Rahman, Al-Rahim, Al-Kabir...*without whom this may never have happened.

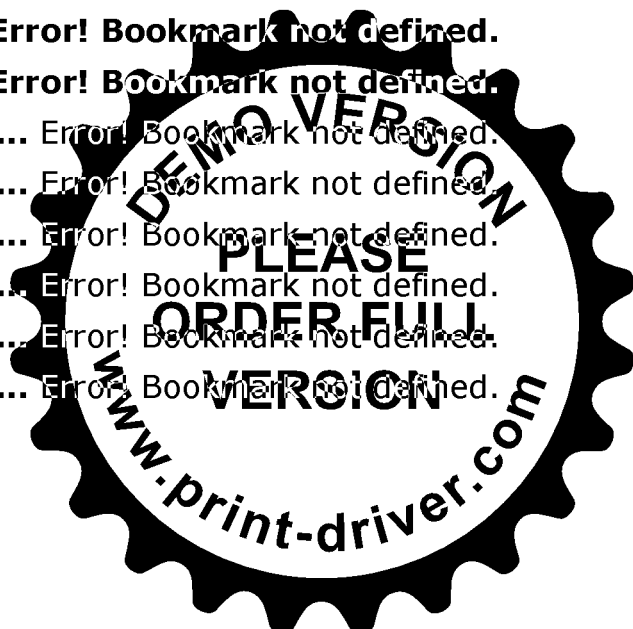
To my mother and my wife Nasra for all the sacrifices they made.

*The ninety-nine names of Allah.

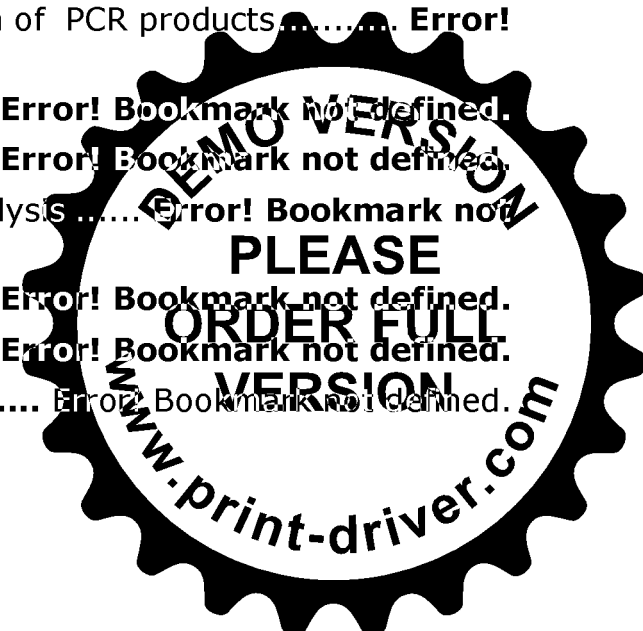


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LIST OF ABBREVIATIONS

AC	Amoxicillin
AIDS	Acquired Immunodeficiency Syndrome
AM	Ampicillin
<i>as</i>	Aspartokinase gene for <i>Campylobacter coli</i>



AST	Antimicrobial susceptibility test
AZM	Azithromycin
<i>bfpA</i>	Bundle-forming pilus of EPEC
<i>cadF</i>	Genus-specific virulence gene
CFU	Colony-forming units
CI	Ciprofloxacin
CL	Chloramphenicol
CLSI	Clinical laboratory Standards Institute
CM	Chloramphenicol
DC	Doxycycline
DCA	Deoxycholate Citrate Agar
DEC	Diarrhoeagenic <i>Escherichia coli</i>
DNA	Deoxyribonucleic Acid.
EAF	EPEC adherence factor
EAEC	Enterοaggregative <i>E. coli</i>
EIEC	Enterοinvasive <i>E. coli</i>
EM	Erythromycin
EMB	Eosin-methylene blue agar
EMBL	European Molecular Biology Laboratory
EPEC	Enterοpathogenic <i>E. coli</i>
ETEC	Enterοtoxigenic <i>E. coli</i>
GM	Gentamicin
HIV	Human Immuno-deficiency Virus
KEMRI	Kenya Medical Research Institute



KM	Kanamycin
KNH	Kenyatta National Hospital
LT	Heat labile enterotoxin
MCK	MacConkey agar
MDREC	Multidrug resistant <i>Escherichia coli</i>
MH	Müeller-Hinton agar
MICs	Minimum Inhibition Concentrations
MR-VP	Methyl red-Voges Proskauer
NA	Nalidixic acid
NCCLS	National committee for Clinical Laboratory Standards
ND	Not done
NTS	Non-typhi <i>Salmonella</i>
<i>NUITM</i>	Nagasaki University Institute of Tropical Medicine
OF	Ofloxacin
PBS	Phosphate-buffered saline
PCR	Polymerase Chain Reaction
RDTs	Rapid Diagnostic Tests
SM	Streptomycin
spp	Species
ST	Heat stable enterotoxin
TC	Tetracycline
TCBS	Thiosulfate citrate bile salt sucrose agar
TS	Trimethoprim-Sulfamethoxazole
TSI	Triple Sugar Iron
TX	Ceftriazone
UON	University of Nairobi
WHO	World Health Organization
XLD	Xylose lysine deoxycholate





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DEFINITION OF TERMS

Ambulatory care: medical care delivered on an outpatient basis.

Antibiotics: chemical substances that can inhibit the growth of, and even destroy, harmful microorganisms. They are derived from special microorganisms or other living systems, and are produced on an industrial scale using a fermentation process.

Antimicrobial agents: chemical compounds biosynthetically or synthetically produced which either destroy or usefully suppress the growth or metabolism of a variety of microscopic or submicroscopic forms of life.

Colitis: inflammation of the colon observed in various disease states.

Commensal: an organism that derives food or other benefits from another organism without harming it.

Diarrhoea: according to WHO diarrhoea is a disorder manifested by an individual having loose or watery stools at least three times per 24 hours, or more frequently than normal.

Enteric pathogen: A pathogen whose primary target is the gastrointestinal tissue.

Etiology: cause of a specific disease.

Gene: this is a functional unit of heredity.

Gel electrophoresis: is a widely used technique for separating electrically charged molecules. This is a technique used in DNA fingerprinting and other processes in which large molecules are to be identified. Fragments of DNA are placed in a semi porous gel, and an electrical field is turned on. The fragments move in response to the field, with smaller fragments generally moving faster.



Hypersecretion: excessive production of a bodily secretion.

Immunoglobulin: Any of a group of large glycoproteins that are secreted by plasma cells and that function as antibodies in the immune response by binding with specific antigens. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM.

Immunocompromised: Incapable of developing a normal immune response, usually as a result of disease, malnutrition, or immunosuppressive therapy.

Multiplex polymerase chain reaction (Multiplex PCR): this is a modification of polymerase chain reaction in order to rapidly detect deletions or duplications in a large gene. Multiplex-PCR consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences

Thermal cycler (also known as a Thermocycler, PCR Machine or DNA Amplifier): a laboratory apparatus used to amplify segments of DNA via the polymerase chain reaction (PCR) process.

Lamina propria: the layer of mucosal tissue directly below the epithelial cell monolayer.

Nosocomial infection: a secondary disorder associated with being treated in a hospital but unrelated to the patient's primary condition.

Oral rehydration therapy: Solutions designed to replace fluids and electrolytes lost in cases of dehydration, especially caused by diarrhoea. Oral rehydration therapy solutions contain salts, such as sodium chloride, potassium chloride, sodium citrate, and sodium bicarbonate, together with glucose or other forms of carbohydrate, which enhance their absorption.

Osteomyelitis: usually bacterial infection of bone and bone marrow in which the resulting inflammation can lead to a reduction of blood supply to the bone.



Outpatient: a patient who is not hospitalized for more than 24 hours. The patient visits a hospital, clinic, or associated facility for diagnosis or treatment.

Polymerase chain reaction (PCR): a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Primer: is a strand of nucleic acid that serves as a starting point for DNA synthesis.

Virulence gene: a gene in any pathogen which codes for the virulence factor like protein or polysacchride is called virulence gene and is denoted by Vir genes.



ABSTRACT

Relatively few studies have been carried out in Kenya to detect and classify diarrhoeagenic bacterial agents. This was a prospective cross-sectional study whose objective was to characterize bacterial etiological agents of diarrhoea using multiplex polymerase chain reaction (PCR) in children aged 5 years and below. In addition the study aimed at producing data on bacterial diarrhoea prevalence and antimicrobial susceptibility patterns in low income urban settings. Rectal swabs were collected from three hundred children and transported in Amie's transport media to University of Nairobi and NUITM-Kenya Medical Research Institute laboratories for processing and expertise management. The rectal swabs were inoculated on selective agars for direct bacterial isolation, identification and antimicrobial susceptibility testing. Standard methods of identification of bacterial agents were used for initial diagnosis. Multiplex PCR with several loci was then applied for detection of the bacterial virulence genes: *ipaH* (for identification of *Shigella*), *invA* (for identification of *Salmonella*); *cadF* (genus-specific virulence gene), *hipO* (hippuricase gene for *C. jejuni*) and *asp* (aspartokinase gene for *C. coli*); *eaeA* (for identification of EPEC), *aggR* (for identification of EAEC), *Est/elt* (for identification of ETEC), *vt/eae* (for identification of EHEC) and *invE/ipaH* (for identification of EIEC). The prevalence of bacterial diarrhoea was found to be 19.7% with 20.5% of isolates having the virulence gene. Diarrhoeagenic *E.coli* (DEC) was responsible for 82.3%, *Shigella* for 9.7% and *Salmonella* contributed to 8.1 % of all bacterial diarrhoea. In the 62 pathogenic isolates EAEC accounted for 37.1%, ETEC distribution was 24.2%, EPEC had 21%, *Shigella* had 9.7% and *Salmonella* contributed to 8.1%. *Campylobacter* spp., *Vibrio* spp., EHEC and EIEC strains were not isolated. . *aggR* and *aat* genes of EAEC had highest prevalence with other DEC, *Salmonella* spp. and *Shigella* spp. demonstrating multidrug (MDR) resistance patterns. DEC, *Shigella* and *Salmonella* were resistant to Amoxicillin (MIC: 0.016- 256ug/ml), Ampicillin (MIC: 0.016-256ug/ml), and



Trimethoprim-Sulfurmethoxazole (MIC: 0.002- 32ug/ml). In addition *Salmonella* was found to be resistant to Ceftriazone (MIC: 0.002- 32ug/ml) in which DEC were susceptible within 0.047-0.064ug/ml and *Shigella* within 0.023-0.032ug/ml (MIC). The odds ratio (OR) of having the detected gene in those who drank treated water was 20% less and 25% higher in those who did not treat drinking water. In conclusion, *E.coli*, *Salmonella* and *Shigella* are still a major cause of diarrhoea in children aged five years and below in Kenya. *aggR* and *aat* genes are the major cause of diarrhoea among the DEC group. EAEC strain of DEC was a major cause of diarrhoea at KNH. Use of molecular technique increased sensitivity for detection of DEC. Antimicrobial susceptibility tests results demonstrated MDR resistance pattern especially where multiple virulence genes caused diarrhoea in the patient attending KNH. The multidrug resistance and the relationship with the virulence genes need further investigations. Larger surveillance studies to monitor changes in diarrhoeal causative agents and trends in MICs over longer periods of time is required. In addition larger study to test for ESBL in resistant bacteria strains and species should be carried out.

