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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School of Medicine, College of Health Sciences

University of Nairobi
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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Signature ___________________ Date ___________________

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Signature ___________________ Date ___________________
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.
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AC</td>
<td>Amoxicillin</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>AM</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>as</td>
<td>Aspartokinase gene for Campylobacter coli</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>AST</td>
<td>Antimicrobial susceptibility test</td>
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<tr>
<td>AZM</td>
<td>Azithromycin</td>
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<tr>
<td>bfpA</td>
<td>Bundle-forming pilus of EPEC</td>
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<tr>
<td>cadF</td>
<td>Genus-specific virulence gene</td>
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<tr>
<td>CFU</td>
<td>Colony-forming units</td>
</tr>
<tr>
<td>CI</td>
<td>Ciprofloxacin</td>
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<tr>
<td>CL</td>
<td>Chloramphenicol</td>
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<tr>
<td>CLSI</td>
<td>Clinical laboratory Standards Institute</td>
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<tr>
<td>CM</td>
<td>Chloramphenicol</td>
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<tr>
<td>DC</td>
<td>Doxycycline</td>
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<tr>
<td>DCA</td>
<td>Deoxycholate Citrate Agar</td>
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<tr>
<td>DEC</td>
<td>Diarrhoeagenic <em>Escherichia coli</em></td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid.</td>
</tr>
<tr>
<td>EAF</td>
<td>EPEC adherence factor</td>
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<tr>
<td>EAEC</td>
<td>Enteroaggregative <em>E. coli</em></td>
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<tr>
<td>EIEC</td>
<td>Enteroinvasive <em>E. coli</em></td>
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<tr>
<td>EM</td>
<td>Erythromycin</td>
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<tr>
<td>EMB</td>
<td>Eosin-methylene blue agar</td>
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<tr>
<td>EMBL</td>
<td>European Molecular Biology Laboratory</td>
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<tr>
<td>EPEC</td>
<td>Enteropathogenic <em>E. coli</em></td>
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<td>ETEC</td>
<td>Enterotoxigenic <em>E. coli</em></td>
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<tr>
<td>GM</td>
<td>Gentamicin</td>
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<tr>
<td>HIV</td>
<td>Human Immuno-deficiency Virus</td>
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<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>KM</td>
<td>Kanamycin</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
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<tr>
<td>LT</td>
<td>Heat labile enterotoxin</td>
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<td>MCK</td>
<td>MacConkey agar</td>
</tr>
<tr>
<td>MDREC</td>
<td>Multidrug resistant <em>Escherichia coli</em></td>
</tr>
<tr>
<td>MH</td>
<td>Mueller-Hinton agar</td>
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<tr>
<td>MICs</td>
<td>Minimum Inhibition Concentrations</td>
</tr>
<tr>
<td>MR-VP</td>
<td>Methyl red-Voges Proskauer</td>
</tr>
<tr>
<td>NA</td>
<td>Nalidixic acid</td>
</tr>
<tr>
<td>NCCLS</td>
<td>National committee for Clinical Laboratory Standards</td>
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<tr>
<td>ND</td>
<td>Not done</td>
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<tr>
<td>NTS</td>
<td>Non-typhi <em>Salmonella</em></td>
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<tr>
<td>NUIITM</td>
<td>Nagasaki University Institute of Tropical Medicine</td>
</tr>
<tr>
<td>OF</td>
<td>Ofloxacin</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>RDTs</td>
<td>Rapid Diagnostic Tests</td>
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<tr>
<td>SM</td>
<td>Streptomycin</td>
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<tr>
<td>spp</td>
<td>Species</td>
</tr>
<tr>
<td>ST</td>
<td>Heat stable enterotoxin</td>
</tr>
<tr>
<td>TC</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>TCBS</td>
<td>Thiosulfate citrate bile salt sucrose agar</td>
</tr>
<tr>
<td>TS</td>
<td>Trimethoprim-Sulfmethoxazole</td>
</tr>
<tr>
<td>TSI</td>
<td>Triple Sugar Iron</td>
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<tr>
<td>TX</td>
<td>Ceftriazone</td>
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<tr>
<td>UON</td>
<td>University of Nairobi</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>XLD</td>
<td>Xylose lysine deoxycholate</td>
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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), aspU/aggR (for identification of EAE) 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.