CHAPTER ONE

INTRODUCTION

Adenoviruses (family Adenoviridae) are non-enveloped viruses with linear double-stranded DNA enclosed by a protein capsid that can cause diseases in humans and other animals (Echavarria, 2009). Adenovirus is a contagious disease that causes respiratory tract infection, gastrointestinal tract infection (GTI) conjunctivitis etc. It is a nonenveloped DNA virus and it belongs to the family Adenoviridae under the genus Mastadenovirus (Kapikan *et al.,* 2001). Human Adenovirus (HAdV) was first discovered by Wallace Rowe and his colleagues in 1953 from an adenoid tissue hence the family name Adenoviridae was created (Hilleman and Werner, 1954; Rowe *et al*., 1953). An essential characteristic of adenoviruses is their ability to establish latency using the lymphoid tissue of the gut as a reservoir for many species. (Kosulin *et al*., 2019)

There are 52 human adenovirus serotypes (A to G), divided into seven species based on their ability to fuse various types of red blood cells (Jones *et al.,* 2007). Human adenovirus is usually associated with respiratory tract infection, some serotypes, which are called denominated enteric adenoviruses (EAds) are associated with diarrheal disease (Aminu *et al.,* 2007*)*. The human adenoviruses that are mostly associated with diarrheal diseases or inflammation of the intestine in young children are as a repercussion of serotypes A, F and G (Aminu *et al.,*2007). Enteric Adenoviruses are not quite known to also partake in the cause of gastroenteritis in Africa but South Africa is an exception since etiological importance in pediatric gastroenteritis has been established (Moore *et al.,* 1998).

Enteric Adenovirus (EAds) rank as the second most serious viral agent causing gastroenteritis in infants. They are more common in young children because they lack humoral immunity i.e., they lack adaptive immunity manifested by the ability of B lymphocytes to produce antibodies. This virus rapidly disseminates in close and clustered environments such as military recruits, hospitals, job training centers, children's homes, public swimming pools etc. and through that, it could affect healthy children and even adults (Lynch *et al.,* 2011). Adenovirus usually spreads in bodies of water without adequate chlorine (Sinclair *et al.,* 2009).

Adenovirus infection occurs throughout the year but is more active during winter or early spring majorly because they cause respiratory infection and respiratory infection occurs in cold weather which leads to individuals constantly clearing their noses due to mucus thereby paving a way for the virus to invade the body (Opere,2019). Asymptomatic carriers of adenovirus may last for weeks or even months at most without developing any symptoms (Dennehy,2005).

Electron microscopy for direct examination of the stool cultures which were associated with a hospital outbreak of infectious diarrhoea in children failed to grow in tissue cultures and it later became clear that the pathogen causing the diarrhoea was a result of the adenovirus. They were therefore referred to as uncultivatable (Brandt *et al.,* 1979), fastidious (Kidd and Marley, 1981) or enteric adenoviruses (EAds) (Jacobsson *et al.,* 1979; Retter *et al.,* 1979)

1.1 Statement of Problem

Diarrhea is the second largest cause of mortality in children under the age of 5 and it is responsible for about 1.5 million deaths every year. Diarrhoea is caused by non-infectious etiologies including food, medication, inflammatory conditions, and hormonal imbalances but the most common cause of diarrhoea is gastroenteritis, which is an infection or inflammation of the intestines caused by a virus, bacteria or parasite. This study focuses on detecting Adenovirus in children from age 0-5 presenting with diarrhea.

1.2 Justification of study

This study creates awareness of the virus causing diarrhea in children and the implications of the infection in children.

The result of this study guilds parents on the measures to take when it comes to the health management of their child.

1.3 Aim and objectives of this study

This study aims to investigate the molecular epidemiology of Adenovirus in children under the age of 5 presenting with diarrhea in Ogun state, Nigeria

CHAPTER TWO

LITERATURE REVIEW

Human Adenovirus is the leading common cause of severe gastroenteritis or diarrhea in children under the age of five. This human adenovirus was first identified by Rowe and colleagues in the year 1953 (Rowe *et al.,* 1953). It was initially recognized when it was isolated from the adenoidal tissue of children thereby the name "Adenovirus" was coined. It was discovered that adenovirus was the cause of several outbreaks in hospitals, nurseries, and military camps, the virus compromised environmental sources including food and water (Rowe *et al.,* 1953).

According to The United Nations Children's Fund (UNICEF) "Diarrhea is the second largest cause of mortality in children under the age of 5 and it is responsible for the 1.5 million deaths worldwide every year" (UNICEF 2009). Diarrhoea causative agents can be bacteria, parasites, viruses and rarely fungi. Viral Diarrhea is caused by Rotavirus (being the commonest virus), Calicivirus, Astrovirus and Enteric Adenovirus (Shetty *et al.,* 2014).

Diarrhoea (diarrhoea) is defined as experiencing at least three loose, liquid, or watery bowel function per day. It is also a change in the normal bowel movement accompanied by an increase in the moisture content, volume or frequency of stools (Guerrant *et al.,* 2001). It typically lasts a few days and can lead to dehydration as a result of fluid loss and diarrhoea is more fatal than HIV/AIDS and malaria combined. Diarrhoea is caused by non-infectious etiologies including food, medication, inflammatory conditions, and hormonal imbalances but the most common cause of diarrhoea is gastroenteritis, which is an infection or inflammation of the intestines caused by a virus, bacteria or parasite (Mead *et al.,* 1999*)*. These illnesses are frequently acquired through feces-contaminated food or water, or direct contact with an infected person (Saeed, 2014). Clinically diarrhoea and gastroenteritis are used interchangeably (Guerrant *et al.,* 2001).

Signs and symptoms of diarrhoea in children include vomiting, acute weight loss, abdominal pain, nausea, swelling (bloating), fever, the passage of blood (dysentery), anorexia (eating disorder), and dehydration occurs if diarrhoea is severe or prolonged.

2.1 Classification and Structure of Adenovirus

Adenovirus is from the family Adenoviridae and it has 6 genera which are Mastadenoviruses i.e., humans and mammals are their natural host and Aviadenoviruses i.e., birds are their natural host, Atadenovirus i.e., vertebrates serve as a natural host, Ichtadenovirus i.e., fish is their natural host, Siadenovirus i.e., Vertebrates serve as their natural host, Testadenovirus (Echavarria, 2009) (Li *et al.,* 2005). Human adenovirus is divided into 7 sub-genera (A-G) with different tropisms from 52 serotypes. In recent years, subgenus F adenovirus types 40 (Ad40) and 41 (Ad41) are the causative agents of intestinal infections, the second leading cause of neonatal stomach inflammation after rotavirus (Boloursaz *et al.,* 2013). It is called an intestinal adenovirus based on the associated disease pattern (Li *et al.,*2005).

Adenovirus genome is linear, non-segmented double-stranded (ds) DNA that ranges between 26 and 48 Kbp. This enables the virus to carry 22 to 40 genes. It is an icosahedral capsid ranging from 70 to 100nm and 252 capsomeres (subunits) make up its structure, with 240 hexons constituting its face and 12 pentons at its vertices (Doerfler, 1996). (Harper., 2011). The double-stranded linear DNA in each penton contains a thin fiber, two key core proteins are linked to it, and a 55-kDa protein is covalently bonded to its 5' end (Doerfler, 1996). (Harper., 2011). The fiber is present on each of the 12 pentons on the vertices and it has a protein which is made up of three similar units of polypeptides that is a homotrimer. The homotrimer consists of a tail, a shaft and a knob domain (Weigele *et al.,*2003). The tail is parallel to the N-terminal of the fiber and it forms a non-covalent interlinkage with the penton base. The shaft contains recurrent patterns of 15 amino acids (Green *et al.,*1983). The length of the shaft is dependent on the quantity of repetitions, allowing it to vary from one serotype to another (Vann *et al.,* 1999). The Knob corresponds to the spherical C-terminal of the fiber and interacts with the Heparan Sulfate cellular receptor accompanied by the secondary binding of the penton base to integrins which allows the penetration of the virus into the cells (Rajan *et al.,* 2018). The non-covalent complex formed by the penton base and the fiber is called the penton (Besson *et al.,* 2020).

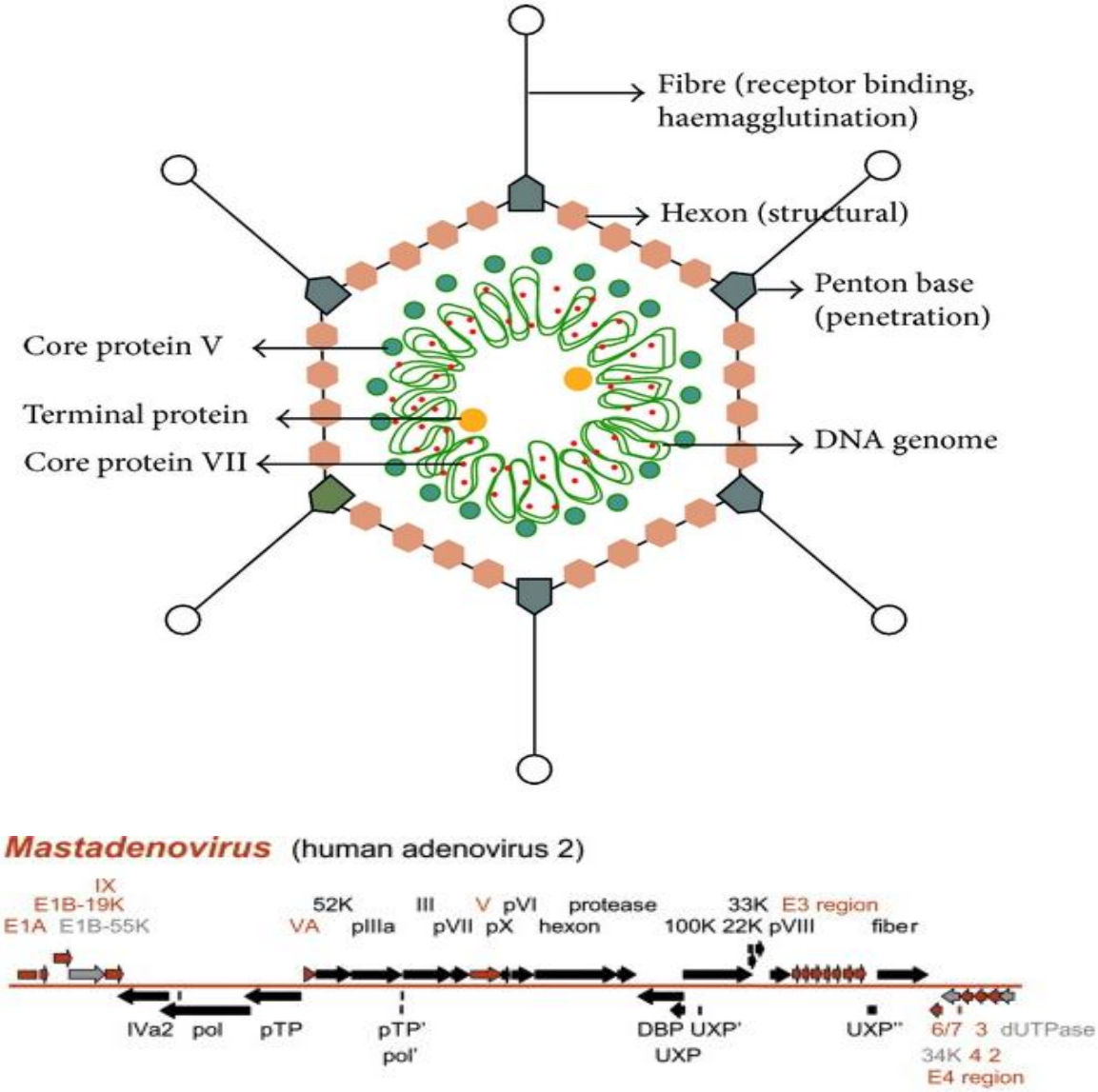

Figure 2.1. Schematic view of adenovirus and its genome. The icosahedral capsid is formed by the hexon. The penton base is located at the 12 vertices and forms a non-covalent complex with the trimeric fiber. The fiber's knob domain is responsible for the interaction with the receptors (Hierholzer *et al.,* 1992) (Singh *et al.,* 2019).

2.2 Epidemiology

Adenovirus is distributed all over the world and is ubiquitous in human feces and sewage-contaminated environments, infections can occur all year round (Okoh *et al.,* 2010). Outbreaks were common in late winter, spring and early summer (Pscheidt *et al.,* 2021). Most adenovirus infections are mild, but deaths and a series of sequelae are associated with immunocompromised patients (Hierholzer, 1992). Infection with adenovirus is quite widespread and is estimated to account for 2-5% of all respiratory infections. All age groups are susceptible to adenovirus infections, which usually occur in childhood. Acute lower respiratory tract infections in children are a major health problem worldwide, and the virus is second only to rotavirus, which is an important cause of pediatric gastroenteritis (Boloursaz *et al.,* 2013). During the winter, adenovirus caused many cases of illness among military recruits, with about 25% requiring hospitalization for fever and lower respiratory tract disease. In some cases, it can lead to death (Lewis *et al*., 2009*)*. Sero-epidemiological studies show that intestinal adenovirus infections occur worldwide. Enteric adenoviruses have been discovered in the feces of young children with viral gastroenteritis in Asia, Latin, Europe and North America (Uhnoo *et al.,* 1990). During a serological survey, antibodies that disrupt Ad40 and Ad41 were observed in more than three-quarters of the sera from children resident in New Zealand, Hong Kong, the United Kingdom and Gambia (Allard *et al.,* 1992). Another research discovered that the occurrence of enteric adenovirus antibodies cautiously rose through childhood. Serum antibody was found less in 20% of children who were less than 6 months, in children and young adults about 50% was found, people who were over 70 years old about 10% of the serum antibody was found. This discovery indicates that the enteric adenovirus is primarily a childhood pathogen and corresponds well with clinical pathogens that the intestinal adenovirus gastroenteritis affects infants in most cases (Shinozaki *et al.,* 1987). There are quite many occurrences of adenovirus diarrhoea in developing countries such as Brazil in which 2% of the infection was discovered in infants and Johannesburg, South Africa where 6,5% of the infection was discovered (Leite *et al.,* 1985) (Kidd *et al.,* 1985). In follow-up study carried out in Africa Ad40 and Ad41 were discovered in 13.2% out of 310 children with diarrhoea (Tiemessen *et al.,* 1989). There is no characteristic seasonal difference that has been observed with Enteric adenovirus-related gastroenteritis. virtually all research done have indicated Ad40 and Ad41 as the serotypes which occurred

throughout the year (Brandt *et al.,*1985) while in Sweden and Africa a disproportionate number of enteric adenoviruses are detected during the summer months (Kidd *et al.,* 1985).

2.3 Pathogenesis

Adenoviruses attach themselves to the epidermal tissues of the enteric organs and the respiratory organs of a host. When an adenovirus invades its host, it replicates in the epidermal cells that line up the respiratory organ or the enteric organ. Human adenoviruses initiate infection when the host cell receptors and the knob domain of the fiber capsid protein interact. Adenoviruses make up the majority of the capsid, with hexons present in 240 copies, penton bases present in 5 copies on each of the 12 vertices, and fiber 3, a homotrimer protein that protrudes from each apex. To date, more than 50 human serotypes of adenovirus have been identified, the species of adenovirus best studied are species C including adenovirus serotype 2 (AdV2) and adenovirus serotype 5 (AdV5) (Fauquet *et al.,* 2005)**.** The major receptor for species C is assumed to be Coxsackie-Adenovirus (CAR) receptor (Zhang *et al.,*2005). Coxsackie-Adenovirus (CAR) receptor is highly recognized in most human adenovirus classes. It is a protein that is inscribed by the CXADR gene in humans (Bergelson *et al.,* 1997). The protein encoded by this gene is the type I membrane receptor for Coxsackievirus group B and Adenovirus in groups A, B, C, D, E, and F. CAR protein manifests in various tissues, comprising the heart, lung, liver, pancreas, central and peripheral nervous system and the intestine (Wobus *et al.,* 2019). The CAR receptor binds to the globular knob of the domain fiber, this interlinkage causes the virus to attach to the cell, the fiber then binds to the CAR and the penton base attracts Alpha v Beta 3 (αvβ3) and Alpha v Beta 5 (αvβ5) integrins to initiate endocytosis and viral entry (Wickham *et al.,* 1993). Adenoviruses can cohere to the integrins through an RGD motif present in the penton base but this binding between the penton base integrin interaction is aimed at virus internalization and not virus binding (Wickham *et al.,* 1993). There have been various reports on the alternate mechanisms for adenovirus entry. According to Huang *et al.,* adenovirus binds to hematopoietic cells through a penton base interaction with integrin αMβ2 which is not expressed in the epithelial cells but relies on integrin αv for virus internalization (Huang *et al.,* 1996)

Heparan sulphate glycosaminoglycans have also been identified as a receptor for serotype Ad5 (Dechecchi *et al.,* 2000) and lactoferrin has been identified as the bridge between the viral particles and cell surface (Adams *et al.,*2009).

Adenovirus binds to the coxsackie and adenovirus receptor (CAR) receptors on host cells with the help of its filamentous structure. The penton base links with the cellular integrins after the fiber attaches to its receptor on the host cell, enhancing receptor-mediated internalization (Flatt *et al.,*2019). The viruses are then engulfed by clathrin-coated endosomes, and the high pH of endosomes aids viral shedding and transport of viral DNA to the cell nucleus, viral nucleocapsids are transported from the cytosol to the nucleus by microtubules (Dom *et al.,* 2016). An early transcription phase occurs in which the viral DNA is transcribed to mRNA, it induces the host cell to enter into the S-phase of the cell cycle and create a condition most favorable for viral replication (Rampersad *et al.,* 2018). The DNA replication occurs in the nucleus, A virus-encoded protein at the 5' end of the viral DNA strand acts as a primer to initiate viral DNA synthesis. Late events begin simultaneously with the initiation of viral DNA synthesis (Pombo *et al.,* 1994). A single large primary transcript is synthesized from viral DNA and spliced into 18 fragments, each of which serves as mRNA and is transported to the cytoplasm. Translation takes place and viral structural proteins are created in the cytoplasm (Nevins *et al*., 1978). In the nucleus, adenoviral morphogenesis takes place. Viral DNA is packaged into preformed capsids that form mature viral particles (Condezo *et al.,* 2017). Mature virus particles are stable, infectious, and resistant to host cell nuclease enzymes. Adenoviral infection does not lyse host cells. Mature viruses are released from the host cell by budding (Ryu., 2017).

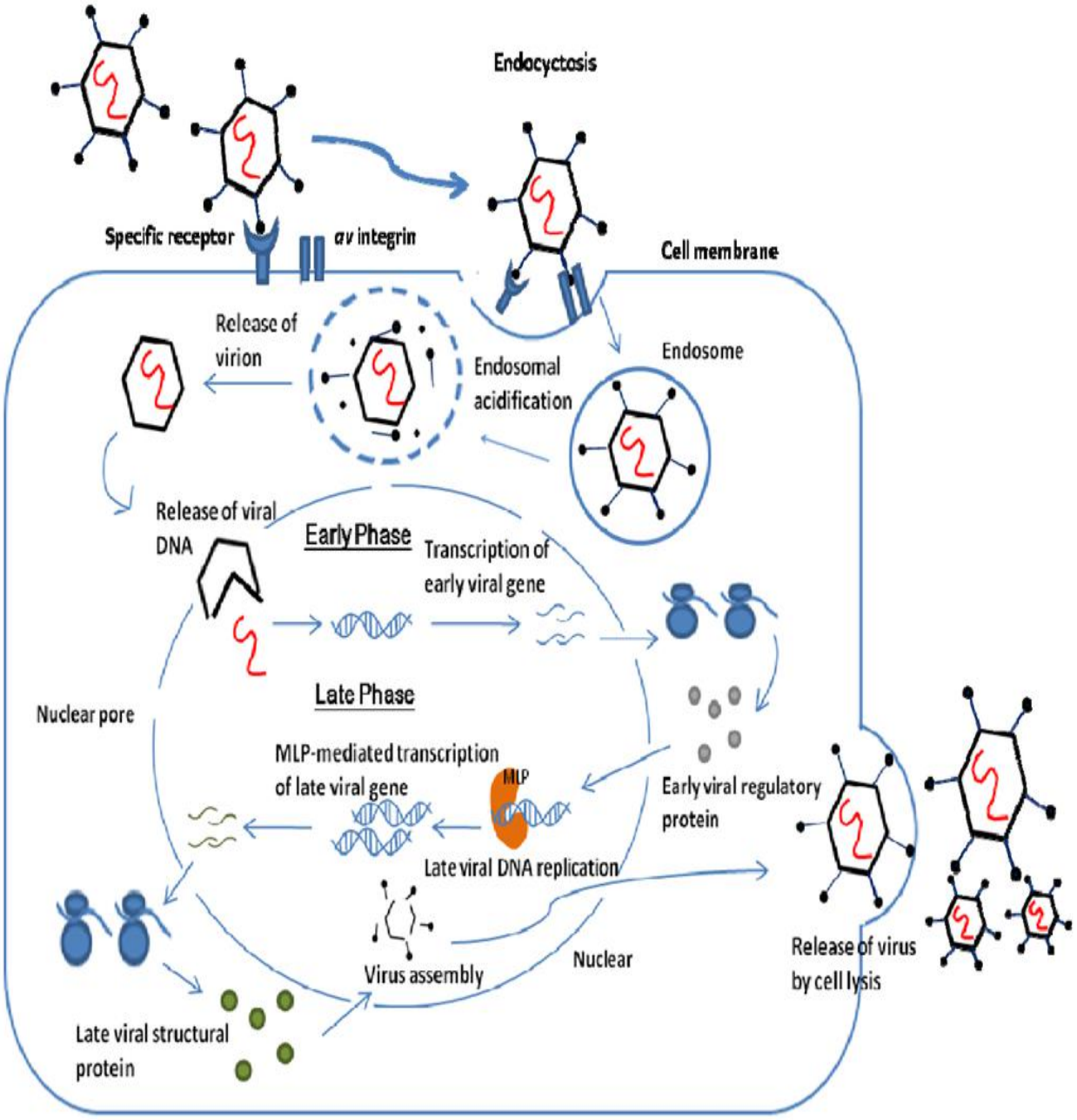

Figure 2.2: Replication Cycle of Adenovirus Infection (Dom *et al.,* 2016).

2.3.1 Mode of Transmission

Adenovirus can be spread from person to person in different ways such as: direct contact with an infected person's droplet when he/she coughs or sneezes, the virus can transmit through contact with surfaces that are contaminated with the virus, through contact with a person's hand who has touched an infected eye also adenovirus can be transmitted fecal orally. The virus can survive for about 30 days on environmental surfaces (Abad *et al.,* 1994).

2.3.2 Clinical Manifestation

According to Khanal *et al.,* 2018 the signs and symptoms that occur when an individual is infected with adenovirus include common cold or flu-like symptoms, fever, sore throat, acute bronchitis (swelling of the lung airways), pneumonia (infection of the lungs), pink eye(conjunctivitis) etc. (Khanal *et al.,* 2018). The incubation period of adenovirus ranges from 2 days to 2 weeks. some people tend to show symptoms 5-6 days after exposure to the virus.

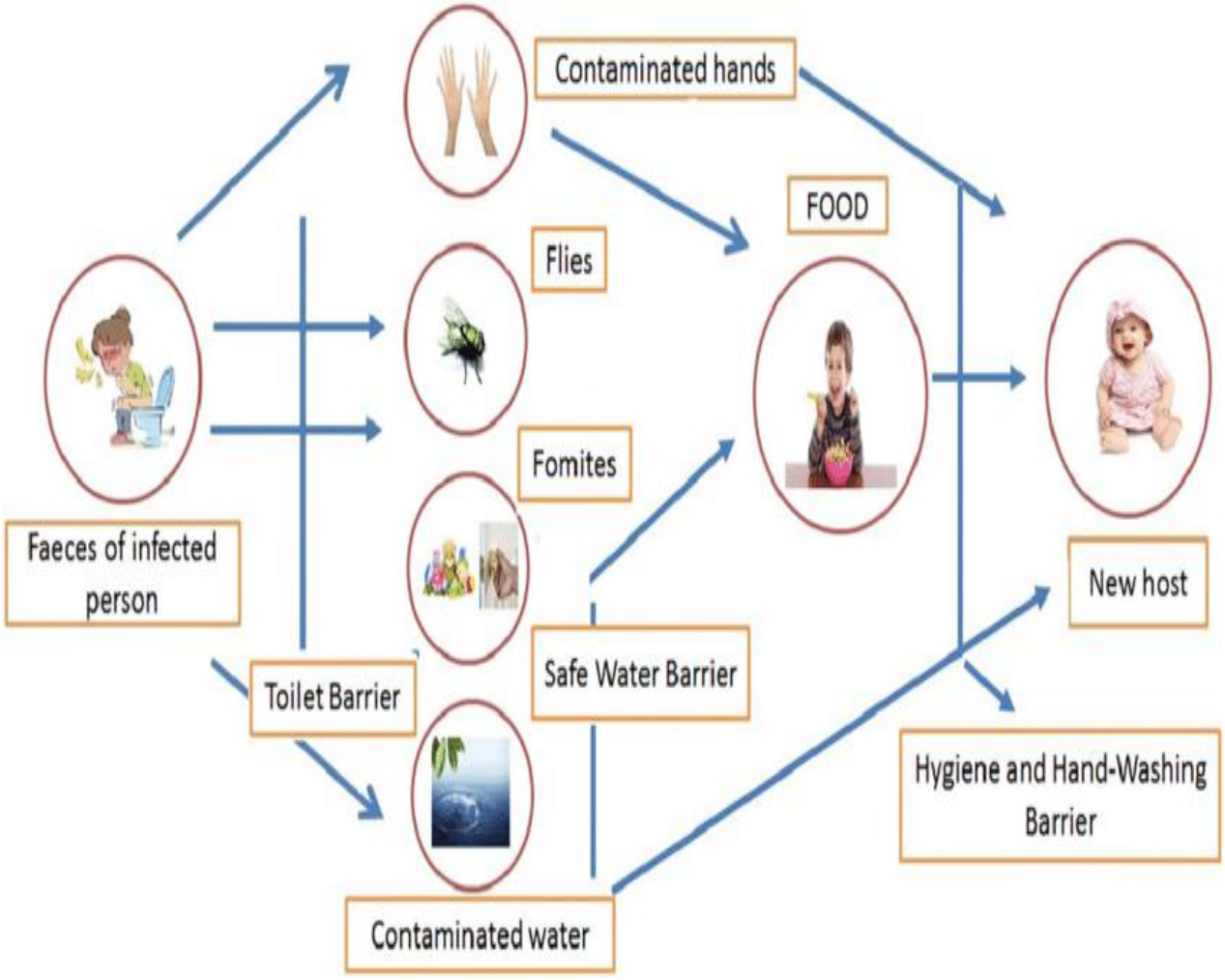

Figure 2.3: Transmission cycle of Adenovirus (Lynch *et al.,*2011)

2.4 Laboratory Diagnosis

Adenoviral infection can be diagnosed in various ways using different specimens such as throat swabs, stool, urine, blood, bodily fluids, nasal swabs etc. (Dilnessa *et al.,* 2017). Antigen detection, polymerase chain reaction (PCR), virus isolation, and serology can all be used to detect adenoviral infections, but the mostly used diagnosis of enteric adenovirus include ELISA, Electron microscopy and PCR technique because enteric adenovirus serotypes 40 to 42 do not grow readily in available cell cultures (Herrman et al., 1987). Adenovirus typing is usually done using molecular techniques. Adenovirus infection does not always result in the exact sickness that a person experiences (Allard *et al.,*1992).

2.4.1 Electron Microscopy

An electron microscope source of illumination is an accelerated electron beam due to the 100,000 times longer wavelengths of electrons which are smaller than photons of visible light, electron microscopy has a higher resolution than optical microscopy, enabling the visualization of the structure of small objects (William *et al.,*1996). It was invented in the year 1931 by physicist Ernst Ruska and electrical engineer Max Knoll. Electron microscopy makes use of electron beams and their wave-like properties to magnify images of objects, unlike optical microscopes, which use visible light to magnify images (Croft.,2006). The most common samples obtained for viral testing in diagnostic EM laboratories are bodily fluids, particularly stool and urine samples, but any fluid sample (e.g., cerebrospinal fluid [CSF], bronchoalveolar lavage fluid, tear fluid), bladder fluid, or aspirate) can be used. processed with negative staining and displayed in minutes (Goldsmith *et al.,*2009) (Hazelton *et al.,* 2003).

2.4.2 Enzyme-Linked Immuno-Sorbent Assay

It is a widely used analytical biochemical assay that was first introduced in 1971 by Peter Perlman and Eva Engvall (Ahsan., 2021). Using an antibody against the target protein, this assay uses a solid-phase enzyme immunoassay (EIA) to identify the presence of a ligand (often a protein) in a liquid sample (Butler., 2000). ELISA is used as a diagnostic tool in the fields of medicine, plant pathology, biotechnology, and quality control in various industries (Butler., 2000). The antigen of the sample to be tested is bound to the surface. Appropriate antibodies are

then applied to the surface so that they can bind to the antigen (Barnstable *et al.,* 1978). This antibody binds to the enzyme and removes any unbound antibody. In the last step, a substance containing the substrate for the enzyme is added. Once binding occurs, subsequent reactions produce a detectable signal Barnstable *et al.,* 1978). The most common is a color change. It is a helpful tool for figuring out serum antibody levels (like in an HIV or West Nile virus test) (Pierson *et al.,* 2006). Additionally, it has uses in the food industry for the detection of potential food allergens such as milk, peanuts, walnuts, almonds and eggs and as a serological blood test for celiac disease (Pierson *et al.,* 2006). Toxicology can also make use of ELISA as a rapid putative screen for specific classes of drugs (Collins *et al.,* 2008).

2.4.3 Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a popular technique for rapidly producing large number of duplicates (full or partial) of a specific DNA sample, allowing scientists to collect only a small portion of the DNA sample and expand it or acquire sufficient amounts to examine in detail (Kadri., 2019). American biochemist Kary Mullis created PCR at Cetus Corporation in 1983. Nucleic acid denaturation, the first step of PCR, is the process by which the two strands of the DNA double helix physically separate at high temperatures. The temperature is decreased in the second stage, and the primers bind to DNA sequences that are complementary to one another. The double strand of DNA acts as a template for DNA polymerase, which enzymatically assembles new DNA strands from free nucleotides, the building blocks of DNA (Kadri., 2019). As PCR proceeds, the generated DNA itself is used as a template for replication, initiating a chain reaction in which the original DNA template is exponentially amplified (Fakruddin *et al.,* 2013). The most common used samples for viral testing include plasma, serum, stool, nasal swabs etc. the types of PCR include: Real-time PCR, Quantitative real-time PCR (Q-RT PCR), Reverse Transcriptase PCR (RT-PCR), Multiplex PCR, Nested PCR, Long-range PCR, Single-cell PCR, Fast-cycling PCR, Methylation-specific PCR (MSP), Hot start PCR, High-fidelity PCR, In situ PCR, Variable Number of Tandem Repeats (VNTR) PCR, Asymmetric PCR, Repetitive sequence-based PCR, Overlap extension PCR, Assemble PCR, Intersequence-specific PCR(ISSR), Ligation-mediated PCR, Methylation-specific PCR, Miniprimer PCR, Solid phase PCR, Touch down PCR, etc. (Fakruddin *et al.,* 2013).

2.4.3.1 Nested Polymerase Chain Reaction (PCR)

Nested PCR usually involves two sequential amplification reactions, each using a different pair of primers. The first amplification reactions result serves as a template for the second PCR, which is primed by oligonucleotides inserted inside the first primer pair (Green *et al.,*2019). The second set of primers is located internally to the first set of primers, while the first set of primers is designed to anneal to a sequence upstream of the second set of primers. A larger portion of the gene is amplified by the primary set of primers, also known as "outer primers," which is then used as a template in the second round of PCR, which targets a smaller portion of the amplicon using the second set of primers, also known as "nested primers" or "inner primers" (Green *et al.*,2019). Amplicons from this PCR assays are visualized by electrophoresing the reaction mixture in 2% ethidium bromide-stained agarose gel along with a molecular weight marker (Xu *et al.,* 2000).

2.4.3.2 Gel Electrophoresis

A method called gel electrophoresis is used to sort DNA fragments according to size and charge, as well as other macromolecules like RNA and proteins. In electrophoresis, a gel containing the target molecules is subjected to an electric current. The molecules can be divided from one another in the gel based on how quickly or in what directions they migrate there, which depends on their size and charge. A polymer called agarose is frequently used to make DNA separation gels, which are available as dry, powdered flakes (Lee *et al*.,2012). Agarose forms a solid, rather viscous gel when heated in a buffer (salted water) and chilled. The gel is made up of a matrix of agarose molecules that are joined by hydrogen bonds and have formed small pores at the molecular level. To solidify, the prepared gel must be put in a gel box (Maizel, 1971). (Mesapogou *et al.,*2013). The box's ends are joined at one end by a positive electrode and the other by a negative electrode. The inner part of the gel tank contains a salt buffer solution that can conduct current. The well-equipped end of the gel is facing the negative electrode, and the other end is facing the positive electrode (Mesapogou *et al.,*2013). Each PCR reaction is transferred into each of the wells, one well is set aside for a DNA ladder (a standard reference consisting DNA fragments of known lengths). When the gel box's electricity is turned on, current starts to flow through the gel. Due to the phosphate groups in their sugar-phosphate backbone

giving the DNA molecules a negative charge, they begin to move through the gel's matrix in the direction of the positive pole. The gel is said to be running when current is flowing through it. (Lee *et al.,*2012).

2.5 Prevention and Treatment

Adenovirus infection can be prevented through the following ways: Practice proper hand hygiene: wash your hands thoroughly with soap and water especially after using the toilet and also before and after eating (Baker *et al.,* 2001). Management at a hospital as management of patients and patients' rooms, patients are separated from healthy persons. Equipment for hand washing should be kept inside of the room (Atiken *et al.,* 2001). Patients wash their hands with disinfectant frequently when they go outside (Baker *et al.,* 2001). Tables, telephones, handrails, door knobs, and other places where the patients touch or vomit are washed with sodium hypochlorite or hypochlorous acid water (Akhter *et al.,* 1995). Patients should use disposable dishes. Health care providers and caregivers use disposable masks, globes and clothes. The toilet should be separated from healthy persons.

There is no specific treatment for adenovirus infection, and antibiotics do not work against adenovirus (Kaufman., 2011). There is insufficient evidence that antiviral drugs are effective against adenoviral infections in children with a healthy immune system (Lenaerts *et al.,* 2008)**.**

Oral rehydration using ORS which comprises of sodium, glucose, potassium and bicarbonate or citrate is important for infected people; they must drink plenty of liquids to replace fluid lost through vomiting and diarrhoea. In some cases, fluids may need to be given intravenously (Nedeljko *et al.,* 2015)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site and Target population

Stool samples were collected from children under the ages of five (5) visiting hospitals and clinics located in Abeokuta and Obafemi- Owode local government area in Ogun State, Nigeria (6.9980°N 3.4737°E) (Figure 3.2).

3.2 Sample Collection

Samples were collected from children under the ages of five years presenting with diarrhoea. Prior to sample collection, informed consent was obtained from the child's caregiver with the help of nursing staff. With the help of sterile plastic loop, stool was obtained from the diarrheic children and stored in a sterile universal bottle prefilled with Cary Blair transport medium (Cary and Blair, 1964). Samples were labelled, packed, sealed and sent to the laboratory within 24 hours for analysis.

3.3 Socio-Demographic

A systematic (closed-ended questions) questionnaire was used in a face-to-face interview with the patient's parents and guardians to obtain baseline demographic data on the patient, including gender, age, medical history, occupation, housing, and sanitary conditions. The information gathered was utilized to identify the risk variables that increase a child's likelihood of developing diarrhea. Data obtained from the questionnaires were statistically analyzed using SPSS version 20.

Figure 3.3 Map of the research region with red dots designating the sampling sites

3.4 Stool Sample Processing Using Normal Saline

Stool samples were homogenized with normal saline (Oxoid Limited, England) solution (1:1) by vertexing for 20 seconds, centrifuged (at 7 000 rpm for 5 minutes) and supernatant placed in another sterile 2ml snap vial for viral DNA/RNA extraction.

3.4.1 DNA Extraction

The genomic DNA of Adenovirus were extracted using the QIAamp Viral RNA extraction kit. The reagents where prepared according to the manufacturer instruction in QIAamp Viral RNA Mini Kits (QIAGEN, Germany) (Appendix 2)**.**

The procedure for extraction is as described:

- About 1000 µl of a viral lysis buffer AVL (Qiagen, Germany) was pipetted into a falcon tube after which 560 µl of the carrier RNA (cRNA) was dispensed into the AVL buffer and was gently swirled 10 times.
- An aliquot of 560 µl of prepared AVL buffer solution was dispensed in each Eppendorf tube and 140 µl of the stool sample was dispensed into the Eppendorf tube, incubated at room temperature for 10 mins and pulse vortexed (Scientific Industries, United State of America) and pulse centrifuged.
- About 560 µl of absolute ethanol (96-100%) was added to the sample-AVL solution and pulsed vortexed and centrifuged for 15 secs. 630 µl of the already prepared solution was dispensed into QIAamp Mini columns (Qiagen, Germany) (in a 2ml collection tube) carefully without wetting the rims and it was centrifuged at 6000 x g (8000rpm) for 1 min.
- Each QIAamp mini-column was placed into a clean 2ml collection tube and the tube containing the filtrate was discarded. 500 µl of wash buffer AW1 (Qiagen, Germany) was added to each QIAamp mini-column and centrifuged at 6000 x g (8000rpm) for 1 min. Each QIAamp mini-column was placed into a clean 2ml collection tube and the tube containing the filtrate was discarded.
- An aliquot of 500 µl of wash buffer AW2 (Qiagen, Germany) was added to each QIAamp Mini column and it was centrifuged at full speed (20,000 x g; 14,000 rpm) for 3mins. Each QIAamp mini-column was placed into a clean 2ml collection tube and the tube containing the

filtrate was discarded and it was centrifuged at full speed for 1 min. each of the QIAamp Mini columns was placed in a clean 1.5 ml microcentrifuge tube and the old collection tubes were discarded.

- About 60 µl of Buffer AVE (Qiagen, Germany) was added to each QIAamp Mini column and it was incubated at room temperature for 1 min. After the incubation, it was centrifuged at 6000 x g (8000 rpm) for 1 min and it was stored at -20 degrees Celsius.

3.5 Molecular Detection of Adenovirus

The Adenovirus genes were genetically typed using nested polymerase chain reaction (PCR) as described by oh *et al*. (2003). The primers used in this analysis are depicted in Table 3.0 and their working solution were prepared according to manufacturer instruction, Ligo (MACROGEN, South Korea) (Appendix 3).

Table 3.0 Primer sequences used in the Nested PCR assay and their required base pairs products (Oh *et al*., 2003)

Primer name	Sequence (5'–3')	Base pair products
AdV1	CAAGATGGCCACCCCCTCG	17,639–17,657
AdV2	CGATCCAGCACGCCGCGGATGTC	17,968–17,946
AdV3	AATGGTCTTACATGCACAT	17,668–17,686
AdV4	ACCCGGTTGTCGCCCACGGCCAG	17,920–17,898

The master-mix cocktail was primarily prepared for the 1st round as shown in (Table 3.4.1).

The first round PCR amplification were performed in a mixture (25 µl) consisting of 5 µl of Red Load Taq Master (Jena Bioscience, Germany), 5 µl of the DNA template, 13 µl of PCR grade water (Qiagen, Germany) and 1 µl of primer mix (0.8 µM of AdV1 and AdV2). The PCR reactions were carried out using a MiniAmp thermal cycler (Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) set at 94 ºc for 2 mins followed by 40 cycles of amplification; denaturation at 94 ºc for 30 secs, annealing at 72 ºc for 5mins and ending with a final extension at 10 ºc for ∞.

Following PCR, agarose gel electrophoresis was carried out using 1.8% agarose gel stained with ethidium bromide and viewed using the UV-transilluminator.

Amplicons with the required band size of 307bp were selected for the nested PCR.

The master-mix cocktail for the 2nd round is as shown in Table 3.4.2.

The nested-PCR amplifications were performed in a mixture (25 µl) consisting of 5 µl of Red Load Taq Master (Jena Bioscience, Germany), 2 µl of the DNA template, 16 µl of PCR grade water (Qiagen, Germany) and 1 µl of primer mix (0.8 µM of AdV3 and AdV4). The PCR reactions were performed using a MiniAmp thermal cycler (Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) set at 94 ºc for 2 mins followed by 40 cycles of amplification; denaturation at 94 ºc for 30 secs, annealing at 72 ºc for 5mins and ending with a final extension at 10 ºc for ∞.

Following PCR, agarose gel electrophoresis was carried out using 1.8% agarose gel stained with ethidium bromide and viewed using the UV-transilluminator. The amplicons with the required band size of 230bp were confirmed as positive for Adenovirus.

Table 3.1: Adenovirus 1st Round PCR Reaction Table for samples 1v to 100v. The primers used for 1st round polymerase chain reaction is**:** AdV1 and AdV2 (Oh *et al.,*2003

Reagents	**Initial Concentration**	**Final Concentration**	**Volume/Reaction (V/R)**	**Number of samples (n=102)**
Master Mix	5x	1x	5	510 µl
AdV1	20 µm	0.8 µm	1	102 µl
AdV2	20 µm	0.8 µm	1	102 µl
PCR Grade Water (dH20)			13	1326 µl
DNA Template			5	
Total			25 µl	

The calculation carried out for the first-round reaction table can be identified in Appendix 4

Table 3.2: 2nd Round Reaction Table for AdV3 and AdV4 Primers for 29 positive samples (NESTED PCR)

The primers used for 2nd round polymerase chain reaction are AdV3 and AdV4 (Oh *et al.,* 2003)

Reagents	Initial Concentration	Final Concentration	Volume/Reaction (V/R)	Number of samples (n=30)
Master Mix	5x	1x	5	150 µl
AdV1	20 µm	0.8 µm	1	30 µl
AdV2	20 µm	0.8 µm	1	30 µl
PCR Grade Water (dH20)			16	480 µl
DNA Template			2	
Total			25 µl	

The calculation carried out for the first-round reaction table can also be identified in Appendix 4

CHAPTER FOUR

RESULTS

A total of 100 stool samples were obtained during the period of this study. About 59% of the stool samples were females while 41% were males.

Amplification Adenovirus PCR

Fifteen (15%) females and Fourteen (14%) males tested positive for the Adenovirus infection making a total of twenty-nine (29%) samples positive for Adenovirus infection

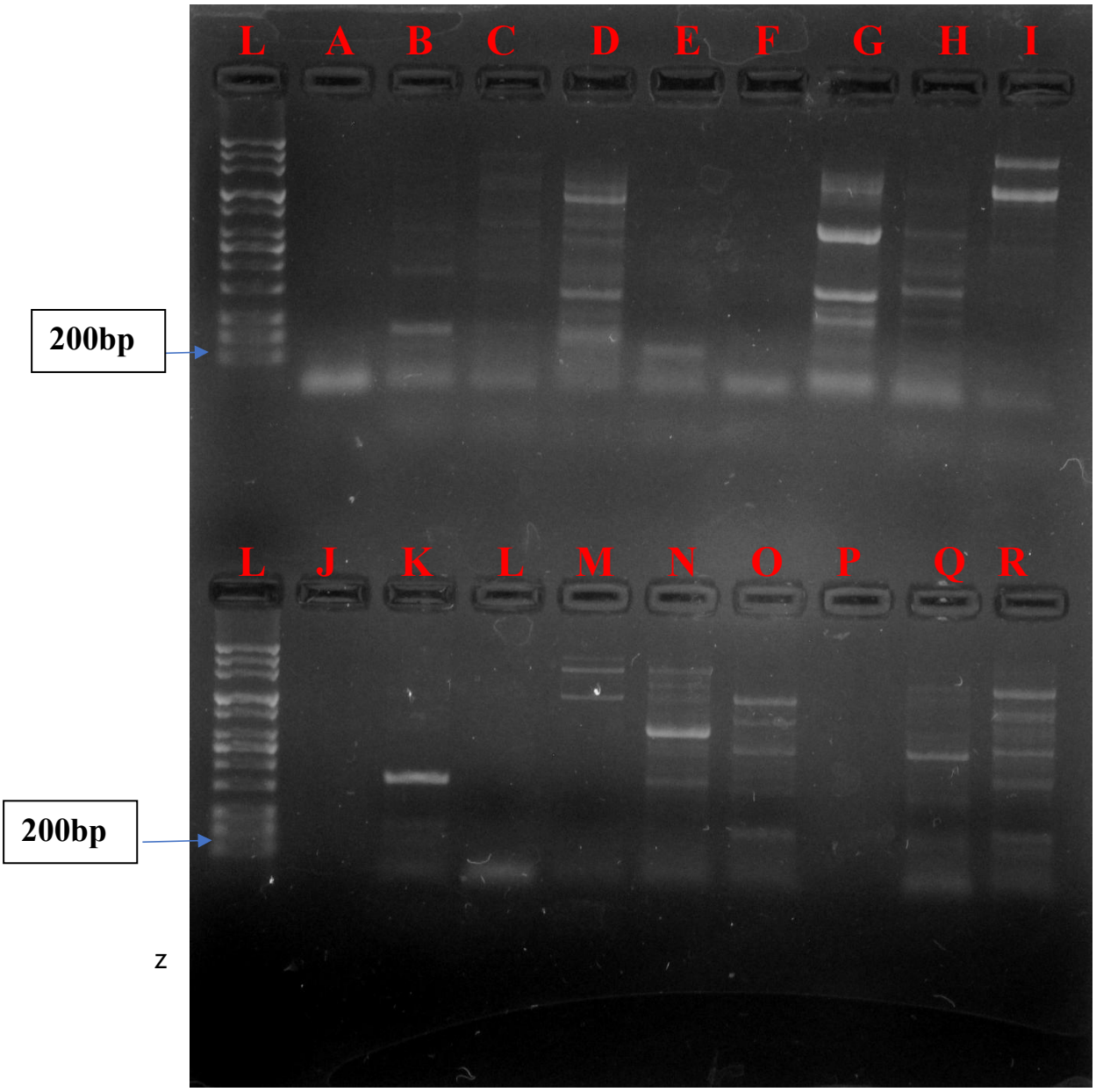

Plate 4.0- A representative gel image showing the 1st round of the polymerase chain reaction where the first two wells represent the DNA ladder (L) and letter A- R represents the samples.

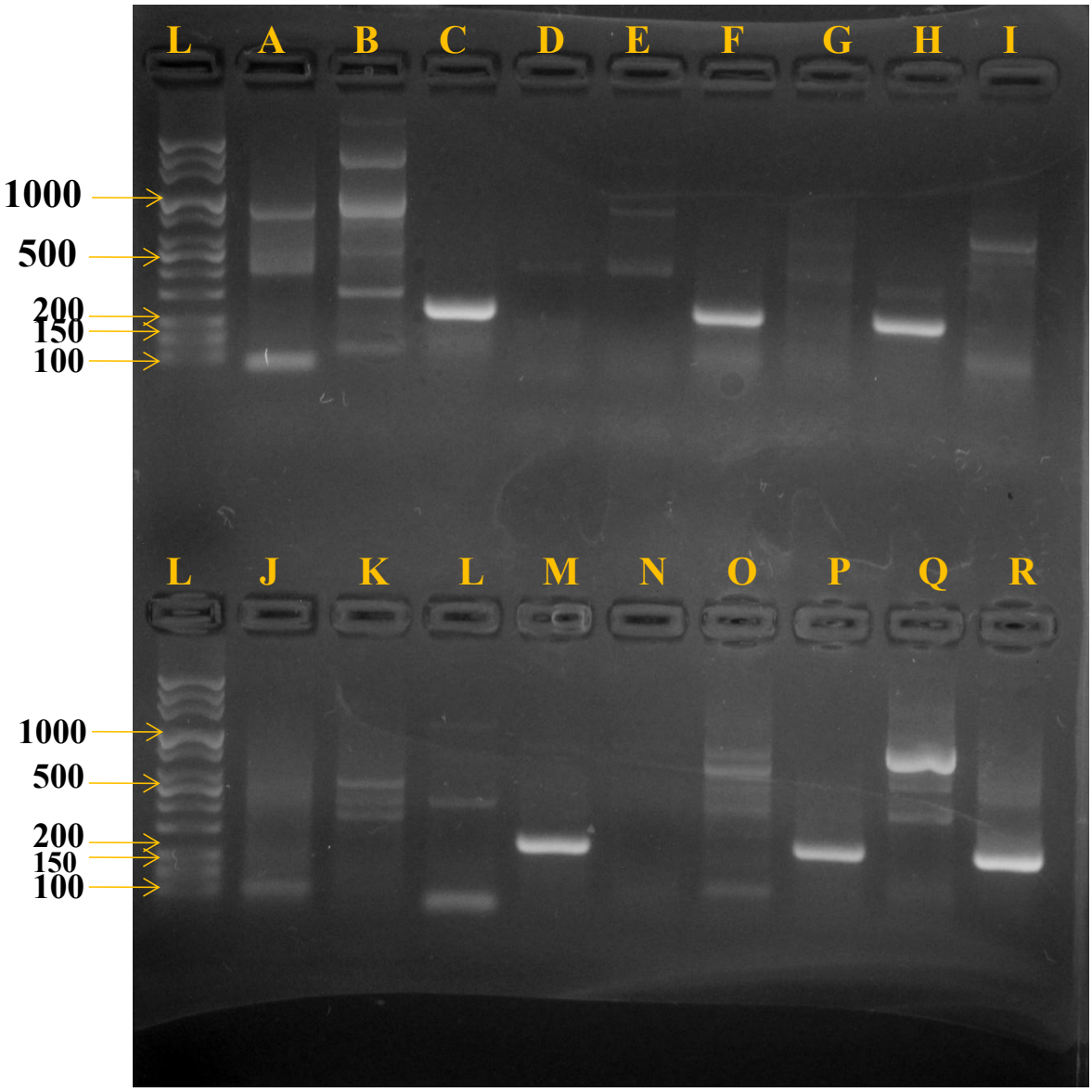

Plate 4.1- A representative gel image showing the positive 2nd round of the polymerase chain reaction where the first two wells represent the DNA ladder (L) and letter A- R represents the samples. **Positive samples (253bp)**.

Seasonal Distribution of Adenovirus Infection

Analysis of the seasonal distribution of adenovirus indicated that the virus peaked during the winter season as shown in **Figure 4.0**

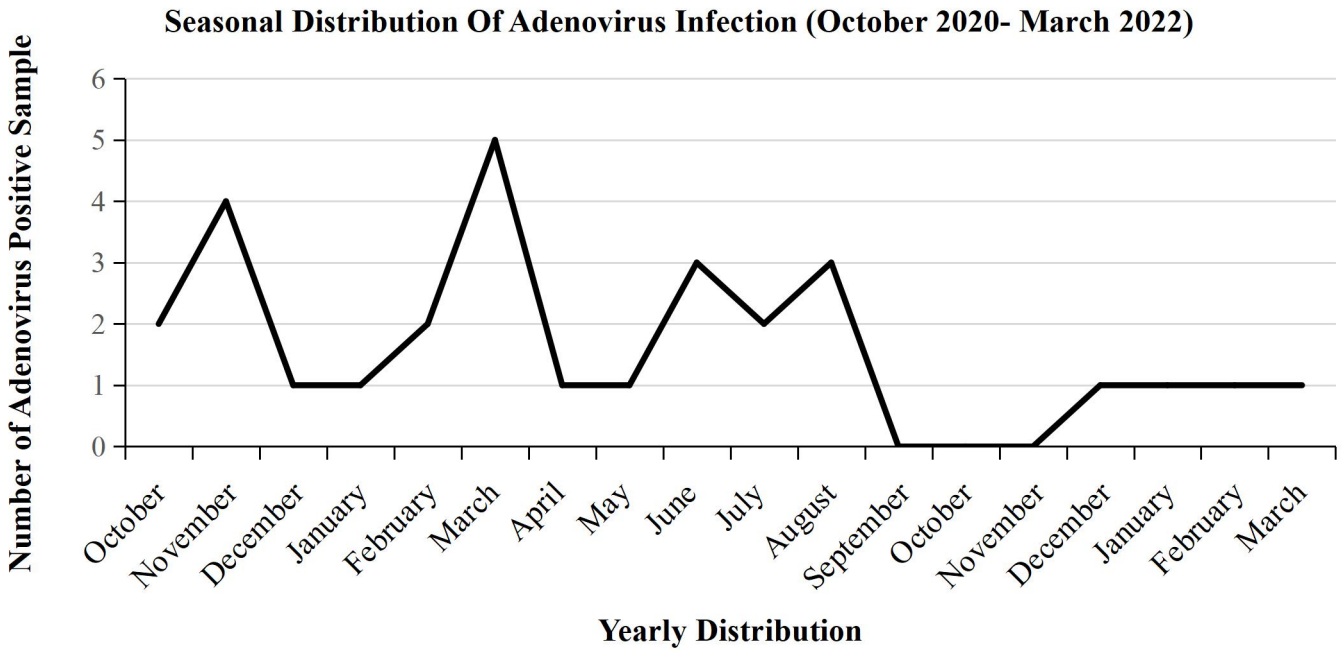

Figure 4.0 Seasonal Distribution of Adenovirus Infection from October 2020- March 2022

Statistical Analysis of Associated Risk Factors

A questionnaire was handed out to the caretakers of children who presented symptoms of diarrhea, and a total of one hundred (100) stool samples were obtained. Using SPSS version 20, these data were statistically analyzed. **Table** 4.0 shows the data analysis of the variable distribution of Adenovirus infection in children under the age of 5 and their respective gender. **Table** 4.1 shows the data analysis of the variable distribution of patients who presented diarrhea as well as the symptoms experienced by these patients and **Table** 4.2 shows the data analysis of the variable distribution of their drinking water source and also the occupation of the caregivers of the patients.

Table 4.0: Variable distribution of Adenovirus infection in children under the age of 5 and their respective gender (P value of < 0.05 was statistically insignificant).

Variable	Total no.	No. with Adenovirus	OR (95% CI)	P value
Gender				
Female	59	15	1	
Male	41	14	1.5 (0.6-3.60)	0.50
Age (year)				
< 2	67	21	1	
≥2	33	8	0.7 (0.3-1.7)	0.52

This table indicates that the females are more susceptible to Adenovirus infection and children less than the age of 2years are more susceptible to Adenovirus infection than children greater than or equals to 2years.

Table 4.1: Variable distribution of patients who presented diarrhea as well as the symptoms experienced by these patients (P value of < 0.05 was statistically insignificant).

Variable	Total no.	No. with Adenovirus	OR (95% CI)	P value
Fever				
No	33	10	1	
Yes	67	20	1.0 (0.4-2.4)	1.00
Vomiting				
No	62	18	1	
Yes	38	12	1.1 (0.5-2.7)	1.00
Appetite				
Yes	50	14	1	
No	50	16	1.2 (0.5-2.9)	0.83
Nausea				
No	82	27	1	
Yes	13	3	0.4 (0.1-1.5)	0.30
Abdominal pain				
Absent	78	26	1	
Present	22	4	0.4 (0.1-1.4)	0.3
ORS before Hospital presentation				
No	47	10	1	
Yes	53	20	2.2 (1.0-5.5)	0.11

The table identifies the symptoms and treatment associated with diarrhea and their prevalence in children experiencing them.

Table 4.2 Variable distribution of their site of enrollment, drinking water source, the occupation of the caregivers of the patients (P value of < 0.05 was statistically insignificant).

Variable	Total no.	No. with Adenovirus	OR (95% CI)	P value
Site of enrollment				
School/daycare	77	23	1	
Others	28	7	1.03 (0.4-3.0)	1.00
Street food consumption				
Absent	83	24	1	
Present	17	6	1.3 (0.4-4.0)	0.82
Type of toilet used				
Water closet	84	20	1	
Pit Latrine	6	5	0.9 (0.1-1.2)	
Potty	10	5	0.2 (0.4-59.7)	0.00
Source of drinking water				
Bottled water	21	8	1	
Sachet water	33	9	0.6 (0.3-2.2)	
Wells/borehole	46	13	0.2 (0.7-6.2)	0.30
Occupation of caregiver				
Office worker	27	7	1	
Merchant/trader	47	14	0.8 (0.2-4.0)	
Artisan/craftsman	18	6	1.0 (0.2-5.4)	
Housewife	8	3	0.2 (0.0-1.7)	0.5

CHAPTER FIVE

DISCUSSION

Diarrhea is one of the major causes of gastroenteritis worldwide and it can be as a result of viral agents (Norovirus, Rotavirus, Astrovirus, Adenovirus etc.) (Banyai *et al.,* 2018). In this case Adenovirus was the case study. Adenovirus affects all age groups but causes diarrhea majorly in infants. The samples were obtained from different primary health care center in Abeokuta Local Government Area and Obafemi Owode Local Government Area of Ogun State.

in Ogun state. This research was carried out using a molecular detection method polymerase chain reaction and about 100 patients were diagnosed using this method. The result of this study identified 29 (29%) patients comprising of 15 (15%) females and 14 (14%) males were positive for Enteric Adenovirus (EAds) which made up a prevalence rate of 29% in Ogun state which is a western state in Nigeria. The study indicated that children less than two years were more infected with Adenovirus than children greater than or equals to two years, additionally, this analysis revealed that the majority of the children were screened for diarrhea.

Adenovirus has no seasonal variation; Adenovirus infection occurs throughout the year but is more active during winter or early spring. This study indicates that adenovirus infection is active during the wet season.

Several research have shown different occurrence rates of adenovirus in different states in Nigeria and also in different countries across the globe. According to Audu *et al. (*2002), 16.7% of stool samples of young children were detected with Adenovirus infection in Lagos, Nigeria and a study carried out by Aminu *et al.*(2007) on four Northern states in Nigeria showed a prevalence rate of 22.3% of Adenovirus infection in Kaduna, Kebbi, Sokoto and Zamfara; In Liu *et al.* (2014), 10% of adenovirus infection in children were detected in stool samples in Beijing, China and in Lijuan *et al*. (2017) a lower prevalence rate of 4.7% of Adenovirus infection were detected in the stool samples of hospitalized children in Shanghai, China. Uhnoo *et* al (1984) identified a prevalence rate of 7.9% in Sweden and Harsi *et a*l (1995) reported a prevalence rate of 4.5% in Brazil. Adenovirus is primarily linked to respiratory tract infections, hence studies on adenovirus infections linked to gastrointestinal tract infections have not been conducted in great detail. However, the illness is seen to be more common in Nigeria than in other nations (Aminu *et al.,* 2007).

This study has a prevalence rate of 29% which suggests that Nigeria has a much higher prevalence than other countries with females more prone than males. Adenovirus prevalence in Nigeria is getting to its peak and it causes a wide variety of symptoms including respiratory symptoms, conjunctivitis, pneumonia which are quite common in infants and it could also be the reason for the high prevalence rate (Audu *et al.,*2002; Aminu *et al.,* 2007).

The relatively large number of Enteric Adenovirus infections observed in infants or young children with diarrhea suggest that there is high risk of exposure to Enteric Adenovirus, it is especially likely for infants to become infected or ill when exposed to the virus. This infection could have been as a result of fecal-oral transfer, also through contaminated water. Children tend to play around in water and the water could be contaminated when sewage waste leaks into the water or when the swimming pool has been contaminated with feces of individuals that may carry the infection (Wigginton *et al.,* 2015), even wells/boreholes where individuals get most of their drinking water from could also be a source of infection in children. The reason for this crucial presentation of diarrhea in the infants is due to their frequent visit to the restroom because infants tend to eat a lot. During this period, these infants or young children tend to get in contact with their stool and may end up not washing their hands when they use the restroom and then they use that same hand to eat food and due to the weak immune system of children they end up getting infected with the virus (Guerrant *et al.,* 2001).

In addition to infants, food handlers and parents may also infect children because they are primarily in charge of what their wards consume as food. Most parents risk infecting their kids if they don't practice good personal hygiene; if they use the restroom and don't properly wash their hands before using them to prepare food for their kids, they end up introducing the infection into their kids' systems (Baker *et al.,* 2001). Symptoms of diarrhea experienced by infants or younger children include abdominal pain, nausea, vomiting, etc., once they begin to experience this symptoms parents usually do not visualize it as a big issue and end up not going to the hospital until the symptoms eventually worsen (Guerrant *et al.,* 2001).

5.1 Conclusion

In conclusion, Adenovirus was discovered in this report's study population. This confirms Adenovirus as a significant cause of diarrhea in Ogun state. Since the Adenovirus can be transmitted fecal-orally, through contaminated food and water and also through contaminated surfaces etc. and there seems to be no active treatment available for the infection, parents should pay rapt attention to their child's personal hygiene and surrounding and they should ensure that their meals are well prepared. Careful handling of patients is also needed to decrease outbreaks in hospitals and families.

5.2 Recommendation

This study has identified that this infection may be as a result of lack of personal hygiene and also contaminated water and surfaces, therefore the recommendation proposed are as follows:

- Parents should ensure that their children observe proper personal hygiene measures which includes; washing of their hands after using the toilet and also before and after eating.
- Hospitals should take precautionary measures to ensure that restrooms and door handles of hospital wards are cleaned with bleach so as to prevent the virus from spreading leading to an outbreak in the hospital.
- Amusement parks or swimming areas should also ensure that the water the children or other individuals use should be treated before use and after use.

REFERENCES

Abad, F. X., Pinto, R. M., & Bosch, A. (1994). Survival of enteric viruses on environmental fomites. *Applied and environmental microbiology*, *60* (10), 3704-3710.

Adams, W.C., Bond, E., Havenga, M.J., Holterman, L., Goudsmit, J., Karlsson, G.B., Koup, R.A., Lore, K. (2009). Adenovirus serotype 5 infects human dendritic cells via a coxsackievirus-adenovirus receptor-independent receptor pathway mediated by lactoferrin and DC-SIGN. *Journal of General Virology*, 90: 1600-1610.

Ahsan, H. (2021). Monoprix and multiplex immunoassays: Approval, advancements, and alternatives. *Comparative Clinical Pathology*, 1-13.

Akhter, J., Al-Hajjar, S., Myint, S., & Qadri, S. M. (1995). Viral contamination of environmental surfaces on a general paediatric ward and playroom in a major referral centre in Riyadh. *European journal of epidemiology*, *11*(5), 587-59

Allard, A. (1992). *Enteric adenovirus type 41: genome organization and specific detection procedures* (Doctoral dissertation, Umeå Universitet).

Allard, A., Albinsson, B., & Wadell, G. (1992). Detection of adenoviruses in stools from healthy persons and patients with diarrhoea by two-step polymerase chain reaction. *Journal of medical virology*, *37*(2), 149-157.

Aminu, M., Ahmad A.A., Umoh J. U., de Beer M. C., Esona M.D., Steele A. D. (2007). Adenovirus Infection in Children with Diarrhea Disease in Northwestern Nigeria. *Annals of African Medicine*. 6 (4), 168 – 1s73

Anup, K.S., Faizan M.K., Sibin J.M., Rekha B., Beena A. (2014). Detection of Rotavirus and Adenovirus diarrhoea in children below five years, in Dakshina Kannada District, a coastal region of Karnataka State, India. Muller Journal of Medical Science and Research | Vol 5 | Issue 2

Aitken, C., & Jeffries, D. J. (2001). Nosocomial spread of viral disease. *Clinical microbiology reviews*, *14*(3), 528-546.

Audu, R., Omilabu A.S., Peenze I., Steele A.D. (2002). Isolation and identification of adenovirus recovered from the stool of children with diarrhoea in Lagos, Nigeria. *African Journal of Health Sciences.* (9) 105-11

Barker, J., Stevens, D., & Bloomfield, S. F. (2001). Spread and prevention of some common viral infections in community facilities and domestic homes. *Journal of applied microbiology*, *91*(1), 7.

Barnstable, C. J., Bodmer, W., Brown, G., Galfre, G., Milstein, C., Williams, A. F., & Ziegler, A. (1978). Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens-new tools for genetic analysis. *Cell*, *14*(1), 9-20.

Besson, S, Vragniau C, Vassal-stermann E, Dagher MC (2020). The Adenovirus Dodecahedron: Beyond the Platonic Story, 12,718

Boloursaz, M. R., Lotfian, F., Aghahosseini, F., Cheraghvandi, A., Khalilzadeh, S., Farjah, A., & Boloursaz, M. (2013). Epidemiology of lower respiratory tract infections in children. *Journal of Comprehensive Pediatrics*, *4*(2), 93-8.

Brandt, C. D., Kim, H. W., Rodriguez, W. J., Arrobio, J. O., Jeffries, B. C., Stallings, E. P., ... & Parrott, R. H. (1985). Adenoviruses and pediatric gastroenteritis. *Journal of Infectious diseases*, *151*(3), 437-443.

Butler, J. E. (2000). Enzyme-linked immunosorbent assay. *Journal of immunoassay*, *21*(2-3), 165-209.

Cary, S.G., Blair E.B. (1964). New transport medium for shipment of a clinical specimen. *Journal of bacteriology, (*88) 96-8

Christiane E.W., Sophia A. (2019) Svoboda in Encyclopedia of Microbiology (Fourth Edition), pages 156-162

Collings, F. B., & Vaidya, V. S. (2008). Novel technologies for the discovery and quantitation of biomarkers of toxicity. *Toxicology*, *245*(3), 167-174.

Condezo, G. N., & San Martin, C. (2017). Localization of adenovirus morphogenesis players, together with visualization of assembly intermediates and failed products, favour a model

where assembly and packaging occur concurrently at the periphery of the replication centre. *PLoS pathogens*, *13*(4), e1006320.

Croft, W. J. (2006). *Under the microscope: a brief history of microscopy* (Vol. 5). World Scientific.

Dechecchi, M.C., Melotti, P., Bonizzato, A., Santacatterina, M., Chilosi, M., Cabrini, G. (2001) Heparan sulfate glycosaminoglycans are receptors sufficient to mediate the initial binding of adenovirus types 2 and 5. *Journal of Virology*,75: 8772-8780

Dennehy, P. H. (2005). Acute diarrheal disease in children: epidemiology, prevention, and treatment. *Infectious Disease Clinics*, *19*(3), 585-602.

Dilnessa, T., & Zeleke, H. (2017). Cell culture, cytopathic effect and immunofluorescence diagnosis of viral infection. *Journal of Microbiology. Mod. Tech*, *2*, 1-8.

Doerfler, W. (1996). Adenoviruses. *Medical Microbiolo\gy. 4th edition*.

Doms, R. W. (2016). Basic concepts: A step-by-step guide to viral infection. In *Viral pathogenesis* (pp. 29-40). Academic Press.

Echavarria, M. (2009) Adenoviruses. In: Zuckerman AJ, Banatvala JE, Schaub BD, Grifhs PD, Mortimer P, Wiley-Blackwell (editor) Principles and practice of clinical virology 6th edition. Wiley, Hoboken, p 463–488

Fakruddin, M., Mannan, K. S. B., Chowdhury, A., Mazumdar, R. M., Hossain, M. N., Islam, S., & Chowdhury, M. A. (2013). Nucleic acid amplification: Alternative methods of polymerase chain reaction. *Journal of Pharmacy & bioallied sciences*, *5*(4), 245.

Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball LA. (2005). *Virus Taxonomy; Seventh Report of the International Committee of Viruses.* San Diego: Academic Press.

Flatt, J. W., & Butcher, S. J. (2019). Adenovirus flow in host cell networks. *Open biology*, *9*(2), 190012.

Green, N.M.; Wrigley, N.G.; Russell, W.C.; Martin, S.R.; McLachlan, A.D. Evidence for a repeating cross-f sheet structure in the adenovirus fibre. EMBO J. 1983, 2, 1357–1365

Green, M. R., & Sambrook, J. (2019). Nested polymerase chain reaction (PCR). *Cold Spring Harbor Protocols*, *2019*(2), PDB-prot095182.

Goldsmith, C. S., & Miller, S. E. (2009). Modern uses of electron microscopy for the detection of viruses. *Clinical microbiology reviews*, *22*(4), 552-563.

Guerrant RL, Van Gilder T, Steiner TS et al. Practice guidelines for the management of infectious diarrhoea. Clin. Infect. Dis. 32(3), 331–351 (2001)

Harper, D. (2011). *Viruses: biology, applications, and control*. Garland Science.

Hársi, C.M., Rolim, D.P., Gomes, S.A. (1995). Adenovirus genome types isolated from stools of children with gastroenteritis in São Paulo, Brazil. *Journal of Clinical Microbiology.*45:127-134.

Hazelton, P. R., & Gelderblom, H. R. (2003). Electron microscopy for rapid diagnosis of emerging infectious agents. *Emerging infectious diseases*, *9*(3), 294.

Herrmann, J. E., Perron-Henry, D. M., & Blacklow, N. R. (1987). *Antigen Detection with Monoclonal Antibodies for the Diagnosis of Adenovirus Gastroenteritis. Journal of Infectious Diseases, 155(6), 1167–1171*

Hierholzer, J. C. (1992). Adenoviruses in the immunocompromised host. *Clinical microbiology reviews*, *5*(3), 262-274.

Hilleman, M.R., Werner, J.H., 1954. Recovery of the new agent from patients with acute

respiratory illness. Proc. Soc. Exp. Biol. Med. 85, 183–188.

Huang, S., Kamata, T., Takada, Y., Ruggeri, Z.M., Nemerow, G.R. (1996). Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells. *Journal of Virology*, 70: 4502-4508.

Jones, M.S II., Harrach, B., Ganac, R.D. (2007). New adenovirus species were found in a patient presenting with gastroenteritis. *Journal of Virology*, 81(11), 5978–5984

Kadri, K. (2019). Polymerase chain reaction (PCR): principle and applications. *Synthetic Biology-New Interdisciplinary Science*.

Kapikian, A.Z., Hoshino Y., Chanock R.M. (2001) Rotaviruses. In Field's Virology (4th edition). Knipe DM, Howley PM, Griffin DE (eds). Lippincott, Williams and Wilkins: Philadelphia; 1787-1833.

Kaufman, H. E. (2011). Adenovirus advances: new diagnostic and therapeutic options. *Current opinion in ophthalmology*, *22*(4), 290-293.

Khanal, S., Ghimire, P., & Dhamoon, A. S. (2018). The repertoire of adenovirus in human disease: the innocuous to the deadly. *Biomedicines*, *6*(1), 30.

Kojaoghlanian, T., Flomenberg, P., Horwitz, M.S., (2003). The impact of adenovirus

infection on the immunocompromised host. *Review of Medical. Virology*. 13, 155–171

Kosulin, K., Geiger E., Vecsei A. (2016). Persistence and reactivation of human adenoviruses in the gastrointestinal tract. Clin Microbiol Infect 22:381, e1–e381.e8

Lee, S. V., & Bahaman, A. R. (2012). The discriminatory power of agarose gel electrophoresis in DNA fragments analysis. *Gel Electrophoresis-Principles and Basics*.

Leite, J. P. G., Pereira, H. G., Azeredo, R. S., & Schatzmayr, H. G. (1985). Adenoviruses in faeces of children with acute gastroenteritis in Rio de Janeiro, Brazil. *Journal of Medical Virology*, *15*(2), 203-209.

Lenaerts, L., De Clercq, E., & Naesens, L. (2008). Clinical features and treatment of adenovirus infections. *Reviews in medical virology*, *18*(6), 357-374

Lewis, P. F., Schmidt, M. A., Lu, X., Erdman, D. D., Campbell, M., Thomas, A., ... & Gilbert, D. (2009). A community-based outbreak of severe respiratory illness caused by human adenovirus serotype 14. *The Journal of infectious diseases*, *199*(10), 1427-1434.

Li, L., Phan T.G., Nguyen T. A. (2005) Molecular epidemiology of adenovirus infection among a pediatric population with diarrhoea in Asia. Microbiol Immunol. 49(2), 121–128.

Lijuan, L., Huaqing, Z., Liyun, S., Lingfeng, C., Menghua, X., Niuniu, D., and Jin, X. (2017). Detection and molecular characterization of human Adenovirus infections among hospitalized children with Acute Diarrhea in Shanghai, China, 2006-2011. *Canadian Journal of Infectious Diseases and Medical Microbiology*

Liying, L., Yuan, Q., Zhang, Y., Jie, D., Liping, J.,and Huijin, D. (2014). Adenoviruses associated with acute diarrhea in children in Beijing, China. PLoS ONE 9(2): e88791.

Lopman, B. A., Steele, D., Kirkwood, C. D., & Parashar, U. D. (2016). The vast and varied global burden of norovirus: prospects for prevention and control. *PLoS medicine*, *13*(4), e1001999.

Lynch, J. P, Fishbein M., Echavarria M. (2011). Adenovirus. *Seminars in Respiratory and Critical Care Medicine.* 32, No. 4

Maizel Jr, J. V. (1971). Polyacrylamide gel electrophoresis of viral proteins. In *Methods in virology* (Vol. 5, pp. 179-246). Elsevier.

Mead, P.S., Slutsker L., Dietz V., (1999). Food-related illness and death in the United States. *Emergency Infectious Disease*. 5(5), 607–625

Moore, P., Steele A.D., Lecatsas G., Alexander J.J. (1998). Characterization of gastroenteritis-associated adenoviruses in South Africa. *South Africa Medical Journal*, 88, 1587-1592.

Nedeljko, R., Zoran, L., Biljana, V., Vladimir, R., Dušica, S. (2015). Acute diarrhea in children. *Srpski arhiv za celokupno lekarstvo.* 43(11-12), 755-762

Nevins, J. R., & Darnell, J. E. (1978). Groups of adenovirus type 2 mRNA's derived from a large primary transcript: probable nuclear origin and possible common 3'ends. *Journal of virology*, *25*(3), 811-823.

Oh, D.Y., Gerhard, G., and Eckart, S. (2003). Viral Agents of Acute Gastroenteritis in German Children: Prevalence and Molecular Diversity. *Journal of Medical Virology,* 71, 82–93.

Okoh, A. I., Sibanda, T., & Gusha, S. S. (2010). Inadequately treated wastewater is a source of human enteric viruses in the environment. *International journal of environmental research and public health*, *7*(6), 2620-2637.

Opere, W. M. (2019). Detection of Pathogenic Human Adenoviruses and Enteroviruses in Water Samples Collected from Lake Victoria Along Homa Bay Town, Homa Bay. Kenyatta University.

Pierson, T. C., Sánchez, M. D., Puffer, B. A., Ahmed, A. A., Geiss, B. J., Valentine, L. E., ... & Doms, R. W. (2006). A rapid and quantitative assay for measuring antibody-mediated neutralization of West Nile virus infection. *Virology*, *346*(1), 53-65.

Pombo, A., Ferreira, J., Bridge, E., & Carmo-Fonseca, M. (1994). Adenovirus replication and transcription sites are spatially separated in the nucleus of infected cells. *The EMBO journal*, *13*(21), 5075-5085.

Pscheidt, V. M., Gregianini, T. S., Martins, L. G., & Veiga, A. B. G. D. (2021). Epidemiology of human adenovirus associated with respiratory infection in southern Brazil. *Reviews in medical virology*, *31*(4), e2189.

Raaij, M.J., Mitraki, A., Lavigne, G., and Cusack, S. (1999) A triple beta-spiral in the adenovirus fibre shaft reveals a new structural motif for a fibrous protein. *Nature* 401, 935–938,

Rajan, A.., Persson, B.D., Frangsmyr, L., Olofsson, A., Sandblad, L., Heino, J., Takada, Y., Mold, A.P., Schnapp, L.M., and Gall, J., (2018). Enteric Species F Human Adenoviruses use Laminin-Binding Integrins as Co-Receptors for Infection of Ht-29 Cells. Sci. Rep. 8, 10019

Rampersad, S., & Tennant, P. (2018). Replication and expression strategies of viruses. *Viruses*, 55.

Rowe, W.P., Huebner, R.J., Gilmore, L.K., Parrott, R.H., Ward, T.G., (1953). Isolation of a

cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exp. Biol. Med. 84, 570–573.

Rux, J.J., Burnett, R.M. (2004). Adenovirus structure. *Human Gene Therapy*, 15, 1167–1176,

Ryu, W. S. (2017). Virus life cycle. *Molecular Virology of Human Pathogenic Viruses*, 31.

Saeed, M. O. M. (2015). *The mortality rate of diarrhoea disease in children in age 0 up to 5 years in al-bulk hospital, Omdurman 2014* (Doctoral dissertation).

Sauerbrei A, Sehr K, Brandsta¨dt A, Heim A, Reimer K, Wutzler P. (2004). Sensitivity of human adenoviruses to different groups of chemical biocides. *Journal of Hospital Infections*, 57(1), 59–66

Sinclair, R. G., Jones, E. L., & Gerba, C. P. (2009). Viruses in recreational water-borne disease outbreaks: A review. *Journal of applied microbiology*, *107*(6), 1769-1780.

Singh, S., Kumar, R., & Agrawal, B. (2019). *Adenoviral Vector-Based Vaccines and Gene Therapies: Current Status and Future Prospects. Adenoviruses.*

Shieh, W. J. (2021). Human adenovirus infections in pediatric population-an update on clinic-pathologic correlation. *biomedical journal*.

Shinozaki, T. A. T. S. U. H. I. K. O., Araki, K. A. Z. U. K. O., Ushijima, H. I. R. O. S. H. I., & Fujii, R. Y. O. C. H. I. (1987). Antibody response to enteric adenovirus types 40 and 41 in sera from people in various age groups. *Journal of clinical microbiology*, *25*(9), 1679-1682.

Tiemessen, C. T., Erasmus, M. J., Kidd, A. H., & Wegerhoff, F. O. (1989). Infection by enteric adenoviruses, rotaviruses, and other agents in a rural African environment. *Journal of medical virology*, *28*(3), 176-182

Uhnoo, I., Wadell, G., Svensson, L., Johansson, M.E. (1984). Importance of enteric Adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. *Journal of Clinical Microbiology*.20:365-372.

Uhnoo, Ingrid; Svensson, Lennart; Wadell, Göran (1990). *Enteric adenoviruses. Baillière's Clinical Gastroenterology, 4(3), 627–642.*

Weigele, P. R., Scanlon, E., & King, J. (2003). Homotrimeric, β-stranded viral adhesins and tail proteins. *Journal of Bacteriology*, *185*(14), 4022-4030.

Wickham, T.J., Mathias, P., Cheresh, D.A., Nemerow, G.R. (1993). Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. *Cell*, 73: 309-319.

Wigginton, K. R., Ye, Y., & Ellenberg, R. M. (2015). Emerging investigators series: the source and fate of pandemic viruses in the urban water cycle. *Environmental Science: Water Research & Technology*, *1*(6), 735-746.

Williams, D. B., & Carter, C. B. (1996). The transmission electron microscope. In *Transmission electron microscopy* (pp. 3-17). Springer, Boston, MA.

Zhang, Y., Bergelson, J.M. (2005). Adenovirus receptors**.** *Journal of Virology*, 79: 12125-12131

APPENDICES

Appendix 1

Measuring cylinder, Micropipette, Pipette tips, Vortex mixer, Centrifuge, Eppendorf tubes, Spin column, Agarose tank, Conical flask, Measuring scale, Falcon tube, Powdered gloves, Nitrite gloves, Thermocycler, Hot plate stirrer, Microwave, PCR strip tubes

Appendix 2

Buffer AVL (Viral lysis buffer), Buffer AVE, Buffer AW1 (Wash buffer 1), Buffer AW2 (Wash buffer 2), AVL cRNA (Carrier RNA), Master mix, PCR grade water, Ethidium Bromide, Agarose gel powder, Tris Borate Ethylene-Diamine-Tetra-Acetic-Acid (EDTA), Primers (AdV1, AdV2, AdV3 and AdV4), Template (DNA), QIAamp Viral RNA Mini Kits (QIAGEN, Germany) and Jena Bioscience Kit.

Appendix 3

Normal saline solution was prepared in a conical flask using 500 ml of distilled water and one tablet of normal saline and stirred using a hot plate stirrer. Eppendorf tubes were labelled 1v to 100v and 700 µl of normal saline was pipetted into the Eppendorf tube. Total samples =100

Appendix 4

The formula and procedure required for the primer dilution and DNA extraction are as follows:

$$\mathbf{n} \times 0.56\text{ml} = \mathbf{y}\text{ ml}$$

$$\mathbf{y}\text{ ml} \times 10\ \mu\text{l/ml} = \mathbf{z}\ \mu\text{l}$$

where: n= the number of samples to be processed simultaneously

y= calculated volume of Buffer AVL

z= volume of carrier RNA-Buffer AVE to add to Buffer AVL

100 samples x 0.56mls of buffer AVL = 56 ml (buffer AVL)

56 ml was converted to microliter i.e., 56 x 1000= 56,000 µl of buffer AVL

56 ml x 10 microliter per ml= 560 microliter (carrier RNA)

Therefore 560 µl of carrier RNA was added to 56,000 µl of buffer AVL.

Appendix 5

The calculation of the working solution for the primers:

AdV1, AdV2, AdV3 and AdV4 primer: nmoles =25,

25 x 10= 250 µl

Therefore add 250 µl of PCR-grade water to the lyophilized primers to get a 100 µm concentration of AdV1 and AdV2 primer. Pulse centrifuge for 15 secs.

$$C_1V_1=C_2V_2$$

C_1= 100 µm, V_1=? C_2 = 20 µm, V_2= 100 µl

100 X V1=20 X100

V1=20 µl

Appendix 6

The calculation for the 1st round and 2nd round rection table is as follows:

$$C_1V_1=C_2V_2$$

C_1= 5, V_1=? C_2= 1 V_2= 25 µl

5 X V1=1 X 25

V1= 5 µl

Calculation of the primers for the master mix cocktail:

$$C_1V_1=C_2V_2$$

C1= 20 µm V1= 1 µl C2=? V2=25 µl

20 X 1 = C2 X 25

C2= 0.8 µm

Appendix 7

Below is the required calculation for the agarose gel needed for the analysis:

1.8% of agarose gel was prepared

Total volume of gel slabs = 45mls

Therefore 1.8÷100 x 45÷1=0.81g of agarose powder.

Appendix 8

The samples positive for Adenovirus infection and the serial number assigned to it:

SERIAL NUMBER	SAMPLE CODE
11V	SH 9
14V	SH 83
16V	KH 2
21V	OF 3
25V	SH 43
27V	FMC 013
30V	FH 12
35V	KH 3
40V	SH 68
42V	SH 61
43V	KH 1
49V	SH 86
53V	SH 143
55V	SH 248
61V	SH 256
63V	SH 260
65V	SH 244
69V	SH 187
72V	SH 185
76V	SH 245
80V	SH 238
81V	SH 186
82V	SH 139
88V	SH 265
89V	SH 196
91V	SH 136
92V	GH 4
93V	SH 45
94V	SH 19

Appendix 9

Abbreviations:

µm- Micrometer

µl- Microliter

PCR- Polymerase Chain Reaction

EAd- Enteric Adenovirus

HAdV- Human Adenovirus

DNA- Deoxyribonucleic Acid

RNA-Ribonucleic Acid

TBE-Tris Borate Ethylene diamine tetra acetic acid

HIV- Human Immunodeficiency Virus

UV- Ultra Violet