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VIRUSES:

FRIENDS OR FOES? THE JOURNEY TO SUBDUE

DR. ROSEMARY AJUMA AUDU

DISTINGUISHED LECTURER

VIRUSES:
FRIENDS OR FOES?
THE JOURNEY TO SUBDUE

A Distinguished Lecture Delivered at the
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Yaba Lagos.
Main Auditorium on
Thursday, 15th November, 2018.

By

DR. ROSEMARY AJUWA AUDU

DEPUTY DIRECTOR OF RESEARCH –
Head, Microbiology Department
Nigerian Institute of Medical Research,
Yaba Lagos

DR. ROSEMARY AJUWA AUDU

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VIRUSES: FRIENDS OR FOES? THE JOURNEY TO SUBDUE

Protocol

The Director General, Professor Babatunde Lawal Salako,

The Directors of Research here present,

The Director of Administration and other Principal Officers of the Institute,

Distinguished Members of the Senior Management Committee,

Colleagues and Friends,

Gentlemen of the Press,

Distinguished Ladies and Gentlemen

Preamble

> *"In the beginning God created the heavens and the earth. So God created mankind in his own image, …. God blessed them and said to them, "Be fruitful and increase in number; fill the earth and subdue it…. God saw all that he had made, and it was very good." Gen 1:1, 27, 28 and 31(NIV)*

I feel highly honoured today, standing before this August assembly of mentors, colleagues, family and friends to present the 8th distinguished lecture in the institute. At the end of the NIMR Scientific Conference last year, I knew the onus lied on me to present the stewardship of my service in this institute, for the past 25years. My presentation will describe my personal experience and contribution to science in my chosen field of research.

Did I say chosen? My journey in the field of microbiology is God ordained. Studying microbiology was not my choice, and specializing in virology was a coincidence. The reason for my unwillingness was the fear of having an unfulfilled career as a microbiologist. My initial impression of virology from my undergraduate days was abstract and I never heard of any good about viruses; it was the ugly path that was obvious. That gave me some concern about the claim that all that God made was very good. Today, I can say boldly that when I decided to follow the path laid for me by the Almighty God, the Father of my Lord Jesus, I found great fulfilment in microbiology as a profession, I discovered that all is not bad about viruses and I have blazed the trail in subduing viruses through quality management system (QMS).

I have been quite privileged to be taught by a very distinguished and passionate virologist in the person of Prof. Sunday Aremu Omilabu, the scientist that saved Nigeria from the Ebola scourge by his prompt diagnosis. Under his tutelage, I have cultured viruses and used several

virological assays and methods which hitherto were abstracts, read only in text books. Working in his laboratory in the early 1990s, was quite explorative for me. In one of our projects, we found there was a challenge at the state and local government levels with maintaining adequate cold chain for vaccines which affected the potency of polio vaccine. This was reported in the national dailies and a staff of the World Health Organization, South West Zone visited to inspect the laboratory where the work was conducted howbeit, in the absence of my supervisor. He left with the impression that the laboratory could not generate reliable results to make such statements. This disturbed me as a young researcher, making me wonder what else we needed to have, to make reports of our research acceptable by the international community. Nevertheless, this report was a prelude to the several challenges we have had with polio immunization programme in the country. Today, we have found the language understood by the international community to generate reports that will gain their confidence.

The study of viruses has traditionally focused on their role as infectious agents and as tools for understanding cell biology. In fact, viruses are even likened to codes that can corrupt systems or destroy data. You will agree with this statement when we mention some common viruses like influenza, HIV, Lassa and Ebola viruses and their devastating effect. This is quite unlike bacteria and other microorganisms which have known beneficial effects in humans and the environment. In my quest to know whether viruses were only foes causing diseases, I have just learned that most viruses are friends and not enemies. Viruses are ubiquitous, in the oceans, our environment, in animals, plants, bacteria, in our body, even in our genomes. They influence our weather, can contribute to control obesity, and can surprisingly be applied against threatening multi-resistant bacteria. The success story of the viruses started more than 3.5 billion years ago in the dawn of life when even cells did not exist. They are the superpower of life. There are more viruses on earth than stars in the sky. Viruses are everywhere. Some of them are incredibly ancient. Many viruses are hundredfold smaller than bacteria, but others are tenfold bigger and they were discovered only recently — the giant viruses, even deep within the permafrost where they were reactivated after 30,000 years.

Mr DG Sir, this lecture will be focusing mainly on viruses, their role in human diseases, reflecting on emerging and re-emerging diseases. It will also highlight the good about viruses and the readiness of the laboratory system to subdue them. I will show the current situation of quality in the laboratory and the future of quality management system. I will attempt to provide my humble contribution to understanding viral diarrhoeal diseases, vaccine preventable diseases, HIV, training of health workers and validation of methods and equipments. I will also

provide my contribution toward controlling of viral hepatitis infections. By the end of the lecture you will be able to determine if viruses are friends or foes.

What Are Viruses?

Viruses are small sub-microscopic infectious agents that replicate only inside a living cell of other organisms. They can infect all types of life forms, from animals, plants to microorganisms including bacteria and archaea [1].

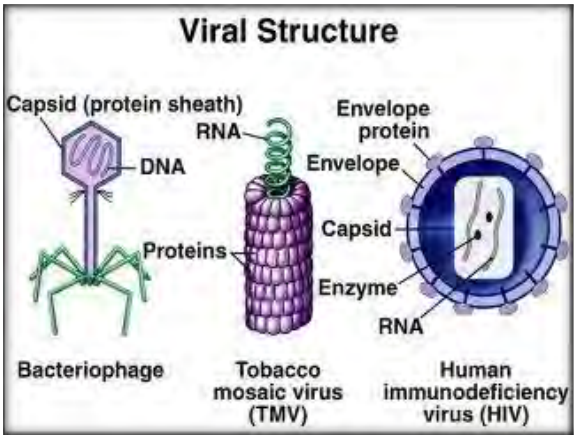

Figure 1: Structure of Viruses
https://goo.gl/X3h7wJ

In 1892, it was described as a non-bacterial pathogen that infects tobacco plants and six years later, the tobacco mosaic virus was discovered [2]. Ever since then, about 5,000 virus species have been described in detail [3], although there are millions of virus types [4]. Viruses are the most abundant type of biological entity and they are found in almost every ecosystem on earth [5, 6]. The shapes of these virus particles range from helical and icosahedral forms to more complex structures (Figure 1). Most viral particles are too small to be seen with an optical microscope.

Figure 2: Influenza virus transmission through coughing and sneezing. *https://goo.gl/q5Kb3A*

Viruses have been described as organisms at the edge of life [7] and as replicators [8] because they possess some and not all qualities of life. Viruses infect a range of host cells which could either be narrow or broad [9]. They spread in several ways, for example, viruses in animals can be carried by blood-sucking insects; influenza viruses are spread by coughing and sneezing (figure 2); norovirus and rotavirus are common causes of viral gastroenteritis transmitted by the faecal-oral route and spread from

person to person through contact, contaminated food or water. HIV is transmitted through sexual contact and by exposure to infected blood.

Strategy of Viral Survival in a host

It is worthy to note that the intention of every viral infection is not actually to kill the host cell infected. Viral diseases are usually unintended consequences of the strategy each virus has devised for survival within the host.

Viruses and their Role in Human Disease

Viruses are primarily known as intracellular pathogens and there are several devastating human, animal and plant diseases attributed to the infection of these agents (figures 3 and 4).

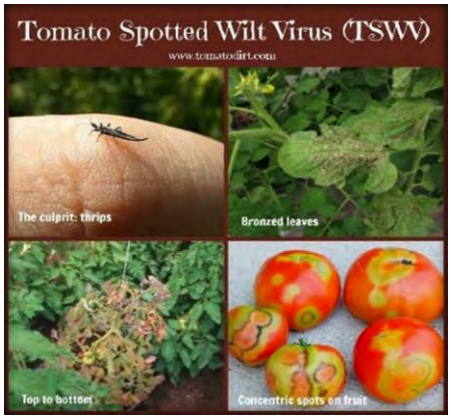

Figure 3: Tomato Diseases of Viral Origin
https://goo.gl/24qooD

Figure 4: Foot and mouth disease of an animal
https://goo.gl/fAPy8y

Examples of common human diseases caused by viruses include chickenpox, cold sores, common cold and influenza. They also cause very severe diseases such as Acquired Immunodeficiency Syndrome (AIDS), Avian influenza, Ebola virus disease, Lassa fever, Severe Acute Respiratory Syndrome (SARS co-V), etc. Some other diseases are being investigated to determine if they have a virus as the causative agent. An example is the connection between human herpesvirus 6 (HHV6) and neurological diseases such as multiple sclerosis and chronic fatigue syndrome [10]. Bornavirus which was known to cause neurological diseases in horses is now being considered to be responsible for psychiatric illness in man [11].

Epidemics and pandemics of viral origin

Viruses have played major roles in both epidemics and pandemics in the past and in the current times they are not relenting in their veracity. While an epidemic is said to occur when a disease affects a greater number of people than is usual for the locality or one that spreads to areas not usually associated with the disease, a pandemic is an outbreak of world-wide proportions. History has it that the native American population were devastated as smallpox was introduced to them by their European colonists, wiping away 70% of their indigenous population. The damage done by this viral disease significantly aided European attempts to displace and conquer the native population [12-14].

The Spanish flu, also known as the 1918 flu pandemic, was an unusually deadly influenza pandemic, and it lasted until 1919. This was a category 5 influenza pandemic caused by the deadly H1N1 influenza virus. The victims of this pandemic were healthy young adults in contrast to most influenza outbreaks which affected majorly children, elderly and weak patients [15]. The pandemic killed as many as 100 million people (figure 5), representing about 5% of the world's population [16].

Figure 5: Effect of Spanish Flu influenza in 1918
https://goo.gl/q2SgMU

HIV/AIDS is a pandemic with more than 70 million people infected with the virus since it was first recognized in 1981 and about 35 million people have died of the infection. HIV is one of the most destructive pandemic recorded in history [17]. Globally, 36.7 million (30.8–42.9 million) people were living with HIV at the end of 2016 [18].

Some other viral epidemic and pandemic diseases

Chikunguya: This is a mosquito-borne viral disease that is transmitted to humans by the bites of infected female mosquitoes (figure 6). Most commonly, the mosquitoes involved are *Aedes aegypti* and *Aedes albopictus*, two species which can also transmit other mosquito-borne viruses, including dengue.

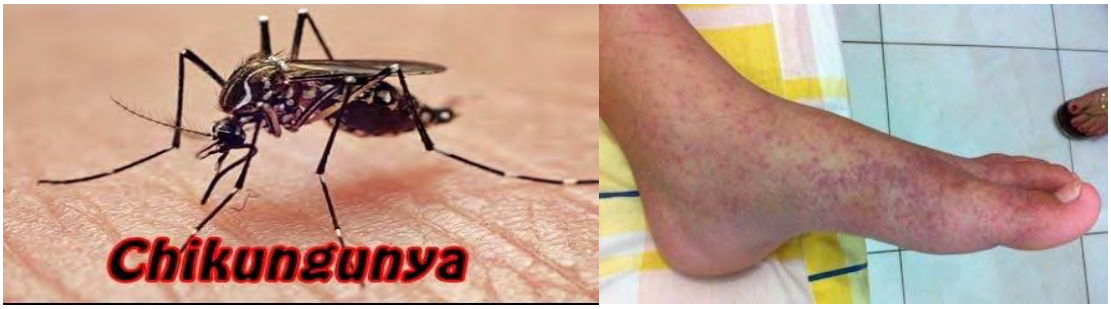

Figure 6: Mosquito biting human body and foot of an infected person
https://goo.gl/sB8AhN

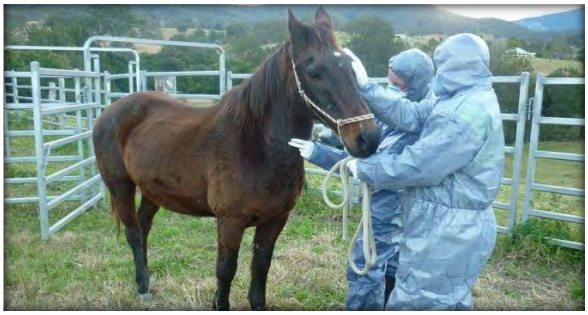

Figure 7: Animal biosecurity officers test a horse for the Hendra virus *https://goo.gl/a61gNp*

Hendra virus infection:

Hendra virus (HeV) causes a rare emerging zoonotic infection that results in severe and often fatal disease in both infected horses and humans (figure 7). The fruit bats are the natural host of the virus.

Monkeypox: is a viral disease first detected in monkeys in Africa in 1958. The symptoms in humans is similar to that seen in smallpox patients (figure 8). The disease is regularly reported in villages of Central and West Africa close to tropical rainforest where there is frequent contact with infected animals. There was a recent outbreak of the disease in Nigeria between September and December 2017. There were a total of 172 suspected cases from 23 states of the country with 61 laboratory confirmed cases [19].

Figure 8: Monkeypox victim

http://bit.do/ezM8R

The virus is usually transmitted to humans from non-human primates such as squirrels or other rodents (e.g. Gambian rats) through contact with the infected animal's blood or a bite. Transmission of the disease can also occur through direct contact with infected patients but there is no evidence to date that person-to-person transmission alone can sustain MPX in the human population.

Yellow fever: This is an acute viral haemorrhagic disease transmitted by infected mosquitoes. The symptoms include fever, headache, jaundice, muscle pain, nausea, vomiting and fatigue (figure 9). A small proportion of patients develop severe symptoms and approximately half of those die within 7 to 10 days. The virus is endemic in Africa where infected mosquitoes of the *Aedes aegypti* specie transmit the virus from person to person. There is an extremely effective vaccine, which is safe and affordable.

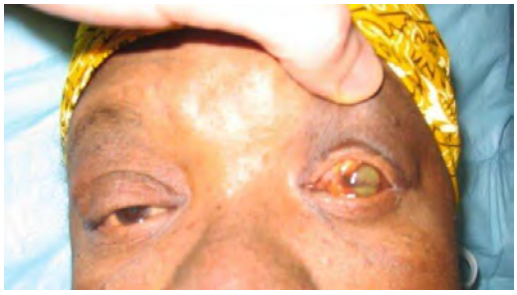

Figure 9: Yellow eyes depicting yellow fever infection http://bit.do/ezM7R

In September 2017, there was an outbreak of yellow fever in Nigeria where a total of 16 states reported suspected cases.

Mr DG Sir, it will interest you to know that NIMR was originally established as a research center for the monitoring and surveillance of yellow fever across the West coast in 1920 [20]. This is where the first yellow fever virus was isolated in monkeys experimentally inoculated with blood collected from yellow fever patients in 1927 (figures 10 and 11).

*Figures 10&11: The West Africa Yellow Fever Commission. Animal Houses of the Yellow Fever Laboratory, Lagos, Nigeria, January 20, 1933. Courtesy of the National Library of Medicine [**Former NIMR Compound**]*

Dengue fever/ Haemorrhagic /Shock Syndrom: This is caused by Dengue virus, a mosquito-borne virus that causes flu-like illness, and occasionally develops into a potentially lethal complication called severe dengue (figure 12). The virus was first isolated from febrile patients in 1964 and 1968 [21] in Nigeria. Dengue virus has caused sporadic outbreaks affecting few patients and its infections are widespread in Nigeria.

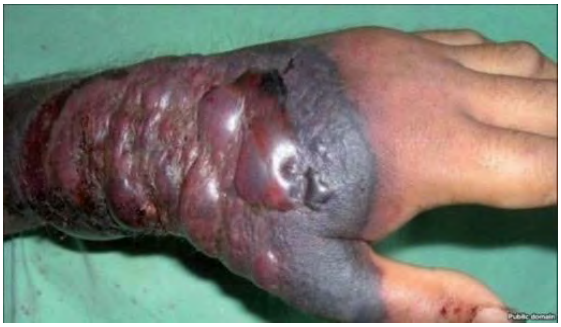

Figure 12:Heamorrhagic manifestation of Dengue heamorrhagic fever
http://bit.do/ezM7a

Zika virus disease: This is caused by a virus transmitted primarily by Aedes mosquitoes. People with Zika virus disease can have symptoms including mild fever, skin rash, conjunctivitis, muscle and joint pain, malaise or headache. These symptoms normally last for 2-7 days. Zika virus has been associated with the cause of microcephaly and Guillain-Barré syndrome (figure 13). Links to other neurological complications are also being investigated.

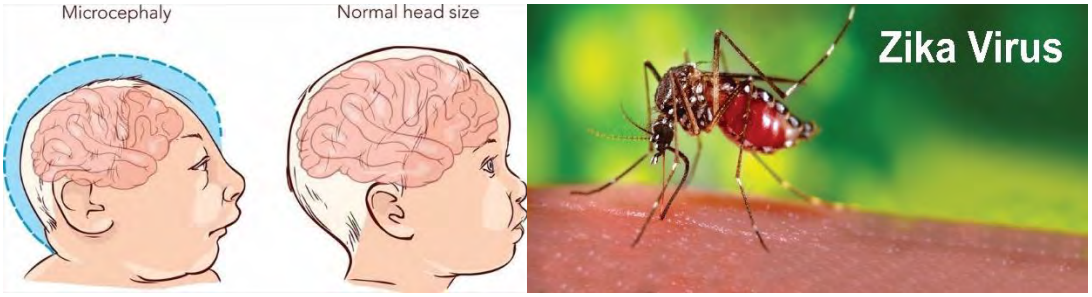

Figure 13: Microcepaly, Normal head size and Infected Aedes *species mosquito*
http://bit.do/ezRGr http://bit.do/ezRGG

Emerging and Re-emerging Viral Diseases

These refer to diseases that either have newly appeared in the population or are rapidly increasing their incidence or expanding their geographic range. Emerging infections can be caused by:

- Previously undetected or unknown infectious agents
- Known agents that have spread to new geographic locations or new populations

- Previously known agents whose role in specific diseases has previously gone unrecognized.
- Re-emergence of agents whose incidence of disease had significantly declined in the past, but whose incidence of disease has reappeared. This class of diseases is known as re-emerging infectious diseases.

Factors responsible for emerging and re-emerging viral diseases

There are many factors involved in the emergence of new infectious diseases or the re-emergence of "old" infectious diseases. Some result from natural processes such as the evolution of pathogens over time, but many are a result of human behavior and practices. It is a known fact that the interaction between human population and our environment has changed, especially in the last century. Factors that have contributed to these changes are population growth, migration from rural areas to cities, international air travel, poverty, wars, and destructive ecological changes due to economic development and land use. With people traveling much more frequently and far greater distances than in the past, living in more densely populated areas, and coming into closer contact with wild animals, the potential for emerging infectious diseases to spread rapidly and cause global epidemics is a major concern.

Mr DG Sir, human behaviour is often a major cause of emerging infectious diseases. More importantly, the main method of control or prevention available are to change human behaviour. It is sometimes emphasized that it is human carelessness, human excesses, human ignorance or human habits of conquest or leisure which contribute directly to the biological niches that viruses are all too capable of exploiting. We must look at ourselves as the engines of viral opportunism. If we will ever conquer the viral world; we must look instead to control the human factors that contribute to emergence. The World Health Organization warned previously that infectious diseases are emerging at a rate that has not been seen before. Since the 1970s, about 40 infectious diseases have been discovered, some of those with viral aetiology include the following:

Crimean-Congo haemorrhagic fever (CCHF): This fever spreads to humans either by tick-bites, or through contact with viraemic animal tissues during and immediately post-slaughter. CCHF outbreaks constitute a threat to public health services because of its epidemic potential,

its high case fatality rates (10-40%), its potential for nosocomial outbreaks and the difficulties in treatment and prevention. CCHF is endemic in all of Africa, the Balkans, the Middle East and in Asia south of the 50° parallel north, the geographic limit of the genus Hyalomma, the principal tick vector (figure 14).

Figure 14: NICD South Africa/R. Swanepoel Ticks of the genus Hyalomma are the principal vector of Crimean-Congo haemorrhagic fever. Female (right), male (left). *https://goo.gl/3gJr6R*

Ebola virus disease: The Ebola virus causes an acute, serious illness which is often fatal if untreated (figure 15). Ebola virus disease (EVD) was first reported in 1976 in 2 simultaneous outbreaks, South Sudan and Democratic Republic of Congo. The 2014–2016 outbreak in West Africa was the largest and most complicated Ebola outbreak since its discovery. The number of cases and deaths in this outbreak was more than all others combined. EBV has the greatest epidemic potential as it spreads between countries, starting in Guinea then moving across land borders to Sierra Leone and Liberia. You very well know that Nigeria was not spared of this disease courtesy Patrick Sawyer an ill Liberian who introduced the virus into the country from an air travel. Fortunately, Prof Omilabu diagnosed the virus promptly in his laboratory which limited the spread of the virus. Notwithstanding, the man who transported the virus into the country died in hospital 5 days later, setting off a chain of transmission that infected a total of 19 people, of whom 7 died. Sexual transmission of the virus has also been established.

Figure 15: Manifestations of Ebola Virus Disease *https://goo.gl/s313tt*

Marburg virus disease: This is a hemorrhagic fever virus and the deadliest virus in the world. As with Ebola, the virus causes convulsions and bleeding of mucous membranes, skin and organs (figure 16).

These viruses are among the most virulent pathogens known to infect humans. Both diseases are rare, but have a capacity to cause dramatic outbreaks with high fatality. The disease was first reported in Germany and Serbia in 1967 in two different large outbreaks. Subsequently, outbreaks and sporadic cases have been reported in Angola, Democratic Republic of the Congo, Kenya, South Africa with Uganda experiencing the latest outbreak in December 2017. Case fatality rates have ranged from 24 to 88%. *Rousettus aegypti*, fruit bats of the Pteropodidae family, are considered to be natural hosts of Marburg virus. The virus is transmitted to people from fruit bats and spreads among humans through human-to-human contact.

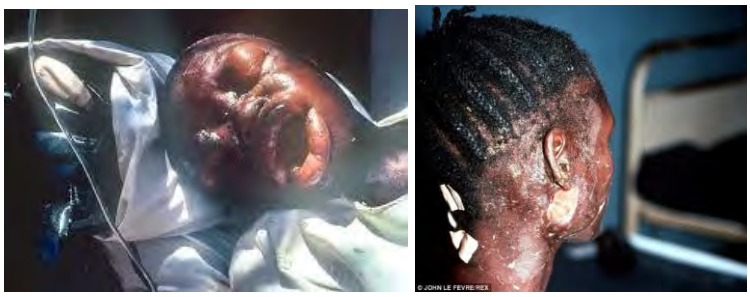

Figure 16: Outbreak of another deadly disease
https://goo.gl/P1zBR2

Lassa fever: This disease is caused by Lassa virus (LASV), a highly infectious pathogen. The infection is endemic in Nigeria and its reservoir is *Mastomys* rats (figures 17 and 18).

figure 17 and 18: Mastomys rats and Breast of Multimamate rat
https://goo.gl/n6S536

The virus is transmitted to man through direct contact with the rodent or contamination of food or household items. Person-to-person transmission occurs and sexual transmission of the virus has been reported. Nosocomial infection has the highest risk among healthcare workers. About 20% of infected persons manifest febrile illness with case fatality rate of 50% during outbreaks. Health care workers are the most vulnerable group as the case fatality among them is usually higher. Between January and August, 2018, there were 2,378 suspected cases reported, 485 laboratory confirmed, 126 deaths with CFR of 26%

in 22 states. The epidemic has subsided but a few cases are still being presented. Lassa fever is endemic in the West African countries of Benin, Ghana, Guinea, Mali, Liberia, Sierra Leone, Togo and Nigeria. As of 22 February 2018, 10 suspected cases who fell ill in Nigeria were reported in Benin, and confirmed cases have been reported from Beninese states that border Nigeria.

Middle East respiratory syndrome coronavirus (MERS-CoV):

MERS-CoV: was first reported in 2012 after genome sequencing a virus isolated from sputum samples from a person who fell ill in a 2012 outbreak of a new flu. As of July 2015, MERS-CoV suspected cases had been reported in over 21 countries, namely: Saudi Arabia, Jordan, Qatar, Egypt, the United Arab Emirates, Kuwait, Turkey, Oman, Algeria, Bangladesh, Indonesia, Austria [23], the United Kingdom, South Korea [24, 25], the United States [26, 27], China [28], Thailand [29], and the Philippines [30]. MERS-CoV is one of several viruses identified by WHO as a likely cause of a future epidemic (figure 19). It was listed for urgent research and development [31, 32].

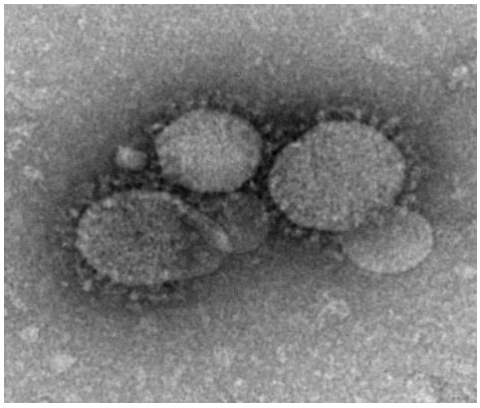

Figure 19: MERS-CoV particles as seen by negative stain electron microscopy. Virions contain characteristic club-like projections emanating from the viral membrane *(Maureen Metcalfe/Cynthia Goldsmith/Azaibi Tamin – https://goo.gl/d846zX*

Severe Acute Respiratory Syndrome (SARS): This disease is caused by SARS coronavirus (SARS-CoV) which was identified in 2003. This animal virus first infected humans in southern China in 2002 and an epidemic erupted which affected 26 countries and resulted in more than 8000 cases in 2003. It is an emerging infectious disease with a high incidence of progression to acute lung injury or acute respiratory distress syndrome (figure 20) with a high rate of mortality.

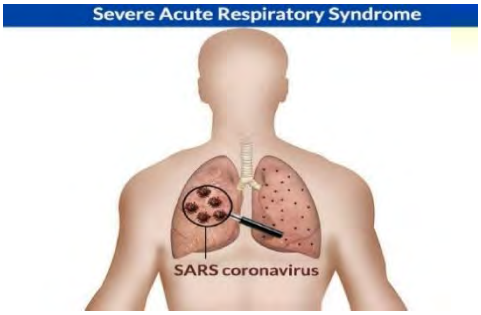

Figure 20: SARS, acute respiratory distress syndrome *https://goo.gl/HtgAHW*

Nipah Virus Infection:

This is a viral infection caused by the Nipah virus, first identified in 1998 during an outbreak in Malaysia. It can both spread between people and from other animals to people. Transmission requires direct contact with an infected source. The virus normally circulates among specific types of fruit bats. Symptoms from infection vary from none to fever, cough, headache, shortness of breath, and confusion. This may worsen into a coma over a day or two. Complications can include inflammation of the brain and seizures following recovery (figure 21). Currently, there is no vaccine or specific treatment. Prevention is by avoiding exposure to bats and sick pigs and not drinking raw date palm sap. About 700 human cases of Nipah virus are estimated to have occurred with a case fatality rate of 50 to 75%. There was an outbreak of the disease in May 2018 which resulted in 18 deaths in India [33, 34].

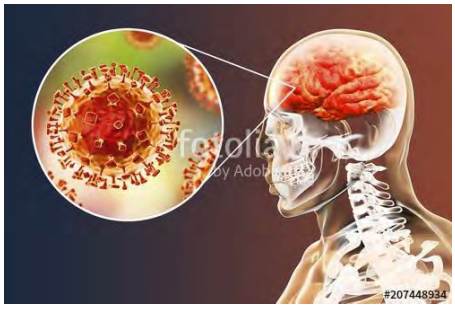

Figure 21: Nipah virus causes fatal encephalitis *https://goo.gl/TPAghZ*

Rift Valley Fever: This is a viral disease that affects animals such as cattle and sheep but can also involve humans (figure 22). The virus is transmitted by mosquitoes and blood feeding flies. The symptoms in man ranges from a mild flu-like illness to severe haemorrhagic fever that can be lethal. The disease can cause significant economic losses due to high mortality rate in young animals and waves of abortions in pregnant females.

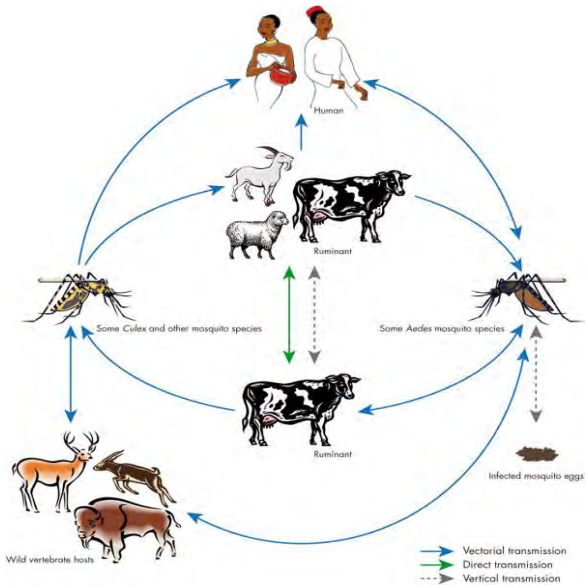

Figure 22: Cycle of Rift Valley fever
https://goo.gl/4LYQE9

The virus was first recognized in 1931 when it caused an epidemic among sheep on a farm in the Rift Valley of Kenya. The virus has caused several other outbreaks in sub-Saharan Africa, North Africa, Saudi Arabia and Yemen.

Bioterrorism

Mr DG Sir, aside from the threats of emerging and re-emerging viruses, the world currently faces the potential of viruses being used as terrorist weapons. Although this issue has received much media attention, the reality is that the deliberate releases of such pathogens may have less medical impact than is generally expected. Many governments of high income countries devoted considerable resources to the development of viruses as weapons of war before deciding that their military usefulness was very limited. The U.S. Centers for Disease Control only recognizes two types of virus as potentially dangerous terrorist weapons [35]. These are smallpox and agents of viral heamorrhagic fevers such as filoviruses and arenaviruses. Emerging viruses such as Nipah virus and hantaviruses are also recognized as possible future threats. However, this is in contrast to a much larger number of bacterial species and toxins. The reason for this is that bacterial pathogens would be much easier for terrorist groups to prepare and disseminate than viruses. The potential threat from bioterrorism is in reality insignificant in relation to the actual number of deaths caused by infections worldwide each year. Nevertheless, this is an issue which governments should be handling with great caution.

All Is Not Bad About Viruses

Mr DG Sir, we have heard lots of the bad things about viruses but here is the good news about viruses too. The word, virus, connotes morbidity and mortality, but that bad reputation is not universally deserved. Viruses, like bacteria, can be important beneficial microbes in human health and in agriculture. For centuries, people have found ways for viruses to be advantageous. For instance, in the 1700s, it was discovered that milkmaids who had a mild version of cowpox were immune to virulent smallpox. Because of this discovery, scientists were able to develop a smallpox vaccine with the Vaccinia virus, which is related to the cowpox virus. It's possible that viruses might be crucial to the development of healthy organs. In 2011, an immunologist conducted an experiment to learn more about the microbiome: a group of about one hundred trillion microbes in our bodies [36]. The microbiome has a lot of important roles, like aiding the development of intestines.

Not all viruses cause disease – some even provide cures! Adeno-associated virus (AAV) can infect humans, but is not known to cause disease [37]. In other words, this virus is good at

getting its genetic information (genes) into human cells. The genes of AAV can be replaced with human genes related to disease. This can cure genetic disease by giving cells a healthy copy of the mutated gene. This revelation helped spark the field of gene therapy. On December 19, 2017, the FDA approved the first directly administered gene therapy in the U.S. to target an inherited genetic disease [38]. This drug, Luxturna, is for patients with a rare form of inherited vision loss that could cause blindness. In this disease, an essential enzyme for normal eye development is missing due to a mutation in the RPE65 gene. Luxturna uses a modified AAV virus to deliver a functional RPE65 gene directly into the retina of the eye. This one-time injection can restore vision long-term.

There are a number of ways in which viruses may produce direct benefits for human health. A few of them are described below:

Gene therapy: Viruses are routinely used in the genetic modification of model organisms for research purposes. The key element of gene therapy is the introduction of functioning genes into the cells of a human patient, to express desired functions or to correct defective or non-operational genes within those cells [39]. The most common target has been cancers, accounting for almost two-thirds of all clinical trials to date (see table 1 below).

Table 1: Characteristics of viral vector systems

Virus	Advantages	Disadvantages
Adenovirus type 5 and others (*Adenoviridae*)	Efficient nuclear entry, high levels of expression possible, specialized vectors available, insert size up to 8kbp (36 kbp in some systems)	May be cytotoxic, immunity to adenovirus may prevent use, narrow host range with some types, safety concerns from previous clinical trials
Adeno-associated virus (*Parvoviridae*)	Nonpathogenic if helper virus not present, infects a broad range of cell types, easy to manipulate ssDNA genome, low immunogenicity, can produce long-lasting expression (vectors necessarily unable to replicate), efficient integration into host genome at defined site	Very limited insert size (5kb), may be high levels of preexisting immunity
Herpesviruses (*Herpesviridae*)	Well characterized, large viruses, wide choice of insertion sites, inserts up to 10 kb (larger in amplicon or episomal vectors	May be pathogenic, cytotoxic, concerns over latency, may transform cells, limited availability of vectors

Vaccinia (*Poxviridae*)	Wide choice of insertion sites, large inserts possible (25 kbp), some systems allow high level expression, wide availability	May be pathogenic for humans, risk of early termination with some inserts, introns can be problematic
Moloney murine leukemia virus, Lentiviruses (*Retroviridae*)	High efficiency of gene transfer, efficient integration into host genome, multiple systems available	Concerns over safety and oncogenicity (including leukemia induction in clinical trials), integration at variable size (8-10 kbp maximum), requirement for actively dividing cells (except Lentiviruses)
Simian virus 40 (*Polyomaviridae*)	Stable high-level expression, low immunogenicity, infects a broad range of cell types, inserts into 18kb possible using pseudovirion (viral particle produced *in vitro* with no viral DNA sequences)	Small genome may restrict insert size, concerns over transformation and possible oncogenicity (if viral sequences present)
RNA viruses (*Coronaviridae, Flaviviridae, Paramyxoviridae, Picornaviridae, Reoviridae, Rhabdoviridae, Togaviridae*)	Capability to target specific cell types, high levels of gene expression	Small genomes restrict insert size, high mutation rate from RNA genome, no defined route of insertion into host genome

Source: Viruses: Biology, Applications, and Control - David R. Harper - Google ...
https://books.google.com › Science › Life Sciences › Microbiology

Once the therapeutic gene is inside the target cell, it is expressed at an appropriate level. Once again, viruses can provide a route to achieve this. Many viruses, such as retroviruses (*Retroviridae)* or adeno-associated virus (AAV; *Parvoviridae*), have a highly efficient integration step in their life cycle, and foreign genes introduced into the viral nucleic acid may use viral mechanisms to become integrated into the cellular DNA in order to permit stable expression. However, this integration is not without its problems for the *Retroviridae,* while the very small size of the AAV genome limits its utility. Other viruses (*Herpesviridae, Polyomaviridae*) may be maintained relatively stable as extrachromosomal genetic elements, while others (*Adenoviridae*) may produce more transient expression.

Any work with recombinant DNA requires careful assessment of the risks and benefits and of the ethical issues involved. Gene therapy, where the intention is to introduce and express recombinant DNA in humans, is one of the most controversial areas. Despite that, more

protocols are being approved worldwide with increasing number of clinical trials. One gene therapy product (Gendicine) has been approved for use against cancer in China.

Cancer prevention and control: A number of viruses are associated with cancer in humans and these have provided the first instances of the prevention of cancers by vaccination. Nevertheless, viruses can also have beneficial applications in the control of cancer. Some viruses are innately able to target and destroy cancer cells, while other methods use molecular approaches based on viral vector systems to create specific therapeutics. Approaches in use or under investigation are listed in table 2 below:

Table 2: Use of viruses in the prevention of control of cancers

Approach	Mode of action	Examples
Prophylactic vaccine*	Stimulation of immune system to prevent a cancer, typically one associated with a virus infection	Existing subunit vaccines for hepatitis B virus, human papillomaviruses
Therapeutic vaccine*	Stimulation of the immune system to control an existing cancer	Experimental approaches under evaluation, e.g. using adenovirus vectors or papillomavirus DNA
Replication-competent virus	Preferential killing of cancer cells by virus	Adenovirus, Newcastle disease paramyxovirus
Modified replication-competent virus	As above, with enhanced killing of cancer cells	Adenovirus with enhanced receptor binding
Nonreplicating virally derived vector	Transfer into cancer cells of a cytotoxic gene	Rexin-G (retrovirus core with cytocidal cyclin G gene)
Virus-directed enzyme prodrug therapy (VDEPT)	Virus-mediated delivery of enzyme combined with systemic administration of prodrug	Recombinant adenovirus expressing herpes simplex enzyme, plus treatment with ganciclovir

**Vaccine may be any of the current types, including live virus, subunit, vector or DNA*
Source: Viruses: Biology, Applications, and Control - David R. Harper - Google ...
https://books.google.com › Science › Life Sciences › Microbiology

Vaccines: Vaccination against viruses that are associated with cancer is the most direct approach to using viruses to prevent cancers. Vaccines for hepatitis B virus (*Hepadnaviridae;* associated with hepatocellular carcinoma) and human papillomavirus (*Papillomaviridae;* associated with cervical cancer) are available and in widespread use. Both use selected proteins

of the virus. Using this approach, vaccines are used to stimulate an immune response in an attempt to control or to eliminate an existing cancer by stimulating specific immunity to the cancerous cells.

Virotherapy: A range of viruses have been used in efforts to produce targeted killing of cancerous cells, and the approach is known as virotherapy. Studies have shown that virus infection of cancer cells may enhance both innate and adaptive immune responses which may be associated with beneficial effects. Virotherapeutic approaches have used either natural, unmodified viruses or genetically modified viruses or virus vectors, either of which can produce selective killing of cancer cells. Typically, viruses are introduced directly into cancerous tissue. A number of RNA viruses have innate anti-cancer activity, producing higher levels of cytotoxicity in cancerous cells. Several types of RNA virus have been investigated as potential therapeutic agents, including reovirus (*Reoviridae*), vesicular stomatitis virus (VSV, *Rhabdoviridae*), and Newcastle disease virus (NDV, *Paramyxoviridae*).

Virus-directed enzyme prodrug therapy (VDEPT): Viruses are also used to insert into target cells an enzyme that can activate an inactive precursor of a cytotoxic drug (a prodrug) that is administered systematically. Therefore, the active, cytotoxic form of the drug is only produced where the relevant enzyme is present and active. For example, an adenovirus expressing thymidine kinase enzyme of herpes simplex virus can be combined with systemic administration of ganciclovir, which is converted by the thymidine kinase to its active form only in cells where this enzyme is present.

Biological Pest Control

The use of biological organisms to control destructive pests is known as biological control, or biocontrol. The application of this measure is being used for the control of agents important to human health. Biological agents can produce long-lasting effects and in some cases are able to spread among target population. They have also been recognized as inherently less toxic than conventional pesticides. Viruses are used for the control of multiple species of insects and have been evaluated for other arthropods such as mites and also for the control of rabbits. Table 3 shows viruses used as pest control agents.

Table 3: Viruses used as pest control agents

Virus type	Number in use	Target
Baculoviruses (various)	13	Caterpillars, sawflies
Oryctes rhinoceros virus	1	Rhinoceros beetle
Myxoma poxvirus	1	Rabbit
Rabbit heamorrhagic disease calicivirus	1	Rabbit

Source: Viruses: Biology, Applications, and Control - David R. Harper - Google ...
https://books.google.com › Science › Life Sciences › Microbiology

Viruses to control insect pests: Baculoviruses (*Baculoviridae*) are a large group of viruses that infect insects and other arthropods. All of them are quite specific in the species that they infect. As part of their natural infectious cycle, baculoviruses are eaten by insect larvae and then they infect the cells of the gut and grow there. The virus then spread from these cells throughout the body of the insect, destroying it and releasing a new generation of virus from the liquefied remains of the killed larva. *Oryctes rhinoceros virus* infects coconut rhinoceros beetle, a destructive tropical pest. The virus results in a generalized infection of larvae, which are killed 9-25 days after infection. It has been in use as a control agent since 1967 and appears to produce long-term control.

Viruses to control rabbits: Viruses have been used to control the devastating numbers of European rabbits infesting much of Australia. The European rabbits that were introduced into Australia in 1859 became very destructive and attempts to control them was unsuccessful until the myxoma virus, was introduced. The virus caused a massively destructive systemic infection killing 90-100% of the European rabbits.

Bacteriophage Therapy

Bacteriophages are viruses that attack and lyse bacteria. They produce two type of enzymes that can be used in therapy: holins and lysins. These enzymes are able to degrade the bacterial cell wall, thus causing their lysis and death. Like other types of viruses, bacteriophages vary a lot in their shape and genetic material (figure 23).

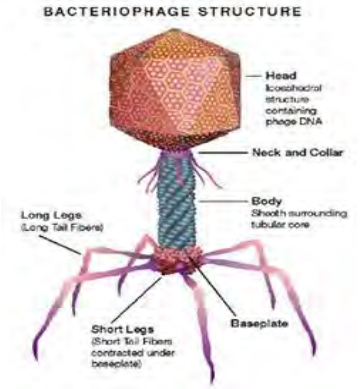

Figure 23: Bacteriophage Structure
https://goo.gl/T6DcW6

Bacteriophages as therapeutic agents: Bacteriophages were discovered before effective antibiotics and was used to control bacterial diseases. In 1919, studies showed successful treatment of typhoid in chickens and dysentery in humans. In 1921, bacteriophages were used against *Staphylococcus* in skin disease [40]. Bacteriophage therapy was widely used around the world in the 1930s and 1940s and it is still being used in Eastern Europe and the former Soviet Union. However, phage therapy was abandoned in the West after antibiotics became widely available [41]. The emergence of antibiotic resistance among pathogenic bacteria is one of the most critical problems of modern medicine, and novel, effective approaches for treating infections caused by multidrug-resistant bacteria are urgently required. In this context, an important approach is to use bacteriophages to eliminate specific bacterial pathogens. Promising results from recent animal studies using phages to treat bacterial infections, together with the urgent need for novel and effective antimicrobials, should bring about a rethink on the value of this therapeutic approach [41].

How Ready Is The Laboratory System?

The Bible tells us in Mark 3:27 that "*No man can enter into a strong man's house, and spoil his goods, except he will first bind the strong man; and then he will spoil his house*." Therefore, you cannot manipulate viruses without adequate fortification. Consequently, there is a need to handle viruses in a quality assured environment before attempting to subdue them. The importance of quality in the laboratory system where these viruses will be tamed is very critical. Quality Management System (QMS) in laboratory medicine adds significant value to patient management and outcomes [42], reduces wastage, minimizes sample rejection and enhances client satisfaction [43], prevents unneeded diagnostic testing, improves turnaround times for accurate diagnosis and reduces the use of inappropriate treatment. Studies have shown that laboratory errors occur at a rate of 32-75% in the pre-analytical phase, 13-32% in the analytical phase and 9-31% in the post analytical phase [44, 45]. In fact, in 2014 during the Ebola disease outbreak, five scientists died working on a single study of the virus. They actually died even before the study was published. In Nigeria, we have lost several health workers in the fight against Ebola and Lassa Fever [46]. These losses and many others may have resulted from lapses in QMS implementation.

Past experience with quality in the laboratory

Mr DG Sir, laboratories form the backbone of health systems, providing health care workers with critical test results for numerous deadly diseases. Nevertheless, in Sub-Saharan Africa, with a huge disease burden majorly of viral origin, laboratories are among the world's most ill-equipped and poorly resourced facilities. It was therefore not surprising that there was a scarcity of accreditation in public laboratories with extremely low level of QMS implementation throughout sub-Saharan Africa [47]. As of 2009, there were 340 diagnostic laboratories in sub Saharan Africa that were accredited and 312 of these (92%) were found in South Africa [48]. Despite the fact that a bulk of laboratory tests are carried out in public laboratories, it was reported that while 282 of the 312(90.4%) laboratories were private, only 28 (9.6%) were public owned. As at 2014 in Nigeria, there were 5, 349 diagnostic laboratories of which only one had ISO certification [49]. This situation resulted in lack of trust of laboratory test results by physicians. A confirmation of this was the increased reports of identical samples having variable results from different laboratories within and in comparison with those outside the country. Worst still is that, laboratory diagnosis were not requesting for suspected viral diseases resulting in the over diagnosis of malaria disease or reports of fever of unknown origin. This became a serious impediment to effective healthcare delivery and disease surveillance in the country. In fact, a vicious cycle was established whereby most physicians in developing countries rely on history-taking and physical examination for patient management, since they have little confidence in laboratory test results even where laboratories exist. As such, inadequate resources are allocated to laboratory services, which in turn translates into less-than-optimal quality assured results, further leading to the neglect of laboratory systems. Whereas, in the real sense, viral disease diagnosis is quite capital intensive requiring uncommon expertise. The vicious cycle is described in figure 24 below [50].

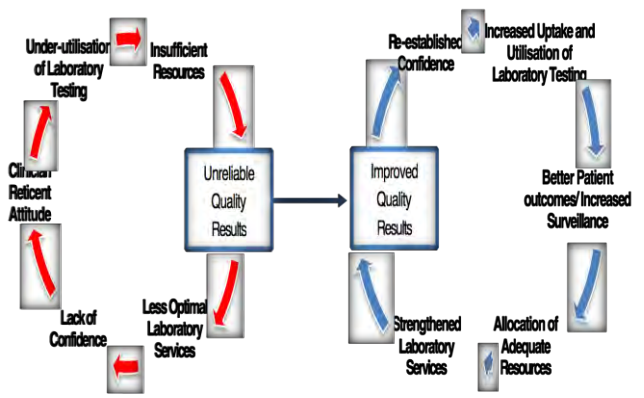

Figure 24: Quality Matters & the Vicious Cycle
Source: Nkengasong JN and Birx D, AJLM 2014

In a study I pioneered to assess the proficiency of laboratories in the diagnosis of HIV, tuberculosis and malaria between 2009 and 2011, we found that ability to correctly diagnose malaria was 2% [51] and tuberculosis was 41.5% [52], and we concluded that there were gross deficiencies in the quality of laboratory services rendered

across Nigeria. In another study we compared positive HIV test results obtained from the approved national HIV rapid testing algorithm with results from a gold standard western blot tests on samples from the same patients. We found that a total of 13 results that had been positive according to the rapid test algorithm were actually negative. This yielded a false-positive rate of 0.6%. We concluded that in order to minimize false-positive diagnoses and the associated potential for long-term psychological and financial impact on the patients, effective measures such as training and retraining of staff should be prioritized (53). On the contrary, in another HIV retesting study I pioneered which involved repeat testing of dried blood spots previously screened negative by the rapid test kits in the national algorithm, we found three infants to be HIV positive by both polymerase chain reaction (PCR) and the fourth generation enzyme immune assay (EIA) [54]. The consequence of these false negative results is that these children if not treated may not live to see their fifth birthday. Time will not permit me to recall all the false test results issued from laboratories. Hence, results issued in laboratories where quality management system principles are not imbibed are not reliable.

As a result of the longtime neglect of laboratories, despite the growing threat from emerging and re-emerging pathogens, very few laboratories have capabilities for diagnosing highly infectious diseases such as viral haemorrhagic fevers, severe acute respiratory syndrome, chikungunya and the highly pathogenic avian influenza virus, including A/H5N1 [55]. Countries often ship specimens to other regions for confirmation, resulting in delayed responses to outbreaks. The establishment of centers of excellence or public health reference laboratories to provide diagnostic services for these highly infectious diseases remains a huge challenge for most countries.

Current Situation of Quality in the Laboratory

An innovative laboratory management tool kit called Strengthening Laboratory Management Toward Accreditation (SLMTA) programme was introduced in 2009, this has changed the landscape of laboratories in developing countries [56]. It is a structured quality improvement programme, that teaches laboratory managers how to implement practical QMS in resource-limited settings using available resources. This programme was created by the US Centers for Disease Control and Prevention, in collaboration with WHO Regional Office for Africa, the Clinton Health Access Initiative, and the American Society for Clinical Pathology. As of May

2018, it has been implemented in over 1,100 laboratories in 52 countries in Africa, Asia, Latin America, the Caribbean, and even Micronesia in the Pacific.

In Nigeria, the drive for QMS commenced in 2010 with the support of the President's Emergency Plan for AIDS Relief (PEPFAR) which emphasized the importance of quality laboratory tests and infused much needed capital into the laboratory systems. Since then, six cohorts of laboratories consisting of 65 laboratories have undergone the SLMTA workshops and five PEPFAR supported public laboratories have been accredited. Others are at different levels of implementation of QMS.

In the quest to increase the frontier of QMS beyond public laboratories in Nigeria, my research team under the leadership of Prof Idigbe, got a grant from International Association of National Public Health Institute (IANPHI) to train a cohort of six laboratories consisting of three public and private laboratories each [57]. These are the only laboratories who have undergone the Strengthening Laboratory Management Towards Accreditation (SLMTA) training without the support of PEPFAR so far in Nigeria and only this cohort has the private sector included. At the fullness of time, they will become internationally accredited private laboratories in Nigeria right after Pathcare, now SYNLAB Laboratory. This grant also brought together, under the same roof, all stakeholders in clinical laboratory practice in Nigeria to be trained as trainers to drive the culture of QMS in the country. This grant also trained three SLMTA master trainers in the country.

The Future of Quality Management System

Reports have it that with the introduction of this quality improvement too- SLMTA, the prospects of implementing sustainable quality assured laboratory medicine seem to be a reality in developing countries [50]. The programme is expanding rapidly and there is a need to sustain the achievements and expand the scope in four strategic ways [58] as described below:

1. **Progression (continued improvement in SLMTA laboratories)**: Quality improvement is a journey and SLMTA provides the tools needed for the journey to provide better diagnostic and patient care. Indigenous capacity building is required for sustainability and there is a need for continual teaching of the principles and techniques of quality improvement. In order not to

return to old habits, continued supervision and audits are very important. More so, it is said that "what gets measured gets done".

2. **Saturation (additional laboratories to implement SLMTA)**: Given the number of medical laboratories in the country and the number enrolled in the programme, there is an urgent need to enroll more laboratories particularly the non PEPFAR and the private laboratories. Therefore, it is important to saturate the laboratories in-country with the knowledge of QMS implementation even if accreditation is not a goal. This will require training of more personnel either using an in or pre-service format. The government needs to be on the driving sit as PEPFAR support will not be forever. There is a need to identify a threshold of laboratories that must be accredited in the country in order to establish this culture and increase confidence in the quality-assured laboratory medicine for evidence-based patient management. To this effect, Standards Organization of Nigeria needs to rise up to its responsibility of enforcing the appropriate standards and midwifing the accreditation process in order to make it more cost effective.

3. **Expansion (implementation in additional countries)**: In 2010, SLMTA implementation commenced in 11 countries and has spread to 52 countries as at May 2018. By December 2017, 54 laboratories within these countries had obtained accreditation. As at December 2014, SLMTA had been implemented in 38% of low-income countries, further expansion beyond PEPFAR supported countries to other low-income countries will require the support of other global partners to fund and assist in implementation [58].

4. **Extension (adapting SLMTA for implementation beyond the laboratory)**: Since the total care of a patient in the healthcare system goes beyond the laboratory, improvements will also need to be made outside the scope of the laboratory. The culture of quality should be adapted to the entire health system to ensure continuous quality patient care.

Therefore, the importance of quality in the diagnosis of infectious diseases cannot be over emphasized as it will impact on patient care, public health surveillance, and biodefense.

My Humble Contribution

Viral Diarrhoeal Diseases

Diarrhoeal disease is the second leading cause of death in children under five years of age. It is both preventable and treatable. Each year diarrhoea kills around 525 000 children under five. A significant proportion of diarrhoeal disease can be prevented through safe drinking-water and adequate sanitation and hygiene. Globally, there are nearly 1.7 billion cases of childhood diarrhoeal disease every year. Diarrhoea is a leading cause of malnutrition in children under five years old and malnourished children have higher risk of death. The incidence of diarrhoea varies by season and age.

Even though safe drinking water and adequate sanitation have been confirmed to prevent diarrhoea, Mr DG sir, from our studies we found high levels of feacal coliforms contaminating foods such as garri [59] and fish [60], the common food of the Nigerian child. Drinking water continues to be a challenge in urban and rural areas in the country despite the fact that we are blessed with several sources of water. Even where water is available, many a times, they are not suitable for human consumption. In a study that examined microbial level of different sources of water (tap, satchet, well and borehole) in Lagos, we found that 79% (19/24) of the samples contained pathogens including *Vibrio cholera* [61].

Investigation Of Different Water Sources As A Possible Cause Of Cholera Outbreak In Lagos In 1997

[1]I. N. Idika, [1]R. A. Audu, [1]K. S. Oyedeji, [1]R. Iyanda and [2]C. A. Egbom
[1]Nigerian Institute of Medical Research,
P.M.B. 2013 Yaba, Lagos, Nigeria
[2]Federal Public Health Laboratory
Yaba, Lagos, Nigeria

Correspondence: I. N. Idika

ABSTRACT

Cholera still remains one of the major causes of high morbidity and mortality in developing countries. In Nigeria this has been attributed to poor health management response to outbreaks. So during a cholera outbreak in Lagos state in October 1997, water

foodborne, nosocomial and person-to-person transmission especially in overcrowded conditions may occur[4]. It spreads rapidly in communities with poor sanitation and unsafe drinking water because it is generally associated with the ingestion of contaminated water, consumption of contaminated shellfish or other

Like a double tragedy, malnutrition which increases the risk of life-threatening diarrhoea in children, continues to prevail in our environment. In an assessment of nutritional status of children in some coastal communities in Lagos, we found that the children were moderately stunted and underweight with a very high prevalence of wasting when compared with

international reference population [62]. Despite the poor nutritional status, these children were burdened with malaria [63, 64]. We also found that some salient factors that predispose children to diarrhoea include: child age particularly 7-12 months when they commence crawling and position in family where children with higher parity, seem to have lower quality of care. Another factor also associated with diarrhoea in children was mothers' occupation as children of traders were found to have higher prevalence of diarrhoea [65]. Therefore, diarrhoea closely linked to malnutrition and malaria continue to take a toll on the Nigerian child. Our children are therefore bedeviled by so many challenges which hampers their total development (figure 25), however, when given a conducive environment they excel like their counterparts anywhere in the world.

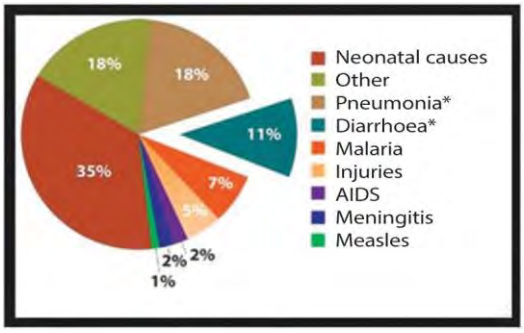

Figure 25: Causes of under 5 mortality
Source: Liu et al, Lancet 2012

Diarrhoea could be caused by pathogens which are either bacterial, parasitic or viral in nature. A study we conducted to find the role of these pathogens in causing diarrhoea revealed that rotavirus was the most prevalent (37.5%) pathogen identified [66].

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Molecular Epidemiology of Rotaviruses in Nigeria: Detection of Unusual Strains with G2P[6] and G8P[1] Specificities

We noted with interest the recent report by Adah and colleagues on the detection of G2P[6] rotavirus strains in Nigeria (1). We have also been conducting epidemiological surveillance of rotavirus strains recovered from young children with diarrheal disease in Lagos, Nigeria (2), and identified similar strains during 2001.

Adah and colleagues identified 12 strains that had the unusual profile of VP7 serotype G2 and VP4 genotype P[6]. Most G2 rotavirus strains bear a specific VP4 genotype of P[4] (4), which is well recognized as belonging to the DS-1 genogroup (7). However, Adah did not extend the characterization of these strains to analyze the potential reassortment of various genes. The DS-1 genogroup can be phenotypically measured on at least three structural genes and one nonstructural gene. These include the VP6 subgroup, VP7 serotype, and VP4 genotype, as well as the migration of the NSP5 gene (7). DS-1 genogroup rotaviruses typically share

(RT-PCR) method with primers *con2* and *con3*. The PCR products were then typed with a cocktail of primers for the different human VP4 genotypes (3).

The VP7 genotypes were examined by the RT-PCR typing method of Gouvea et al. (2, 5). The purified RNA was reverse transcribed, and primers directed to the terminal sequences were used to amplify the entire gene (5). These techniques have been described in detail elsewhere, and similar conditions were used here (2).

In this study, the unusual G2P[6] strains constituted 8 of 21 rotavirus-positive specimens (38%). Analysis of the two other epidemiological markers showed that all eight strains carried a VP6 subgroup I specificity and were characterized by a short RNA electropherotype, although two variations were seen (Fig. 1).

Taken together, these observations indicate that the P[6] VP4 gene may have reassorted onto the DS-1 genogroup

Studies investigating other viral agents of diarrhoea in children and from Lagos and Kwara states showed that rotavirus was the most prevalent, next to it was adenovirus and then

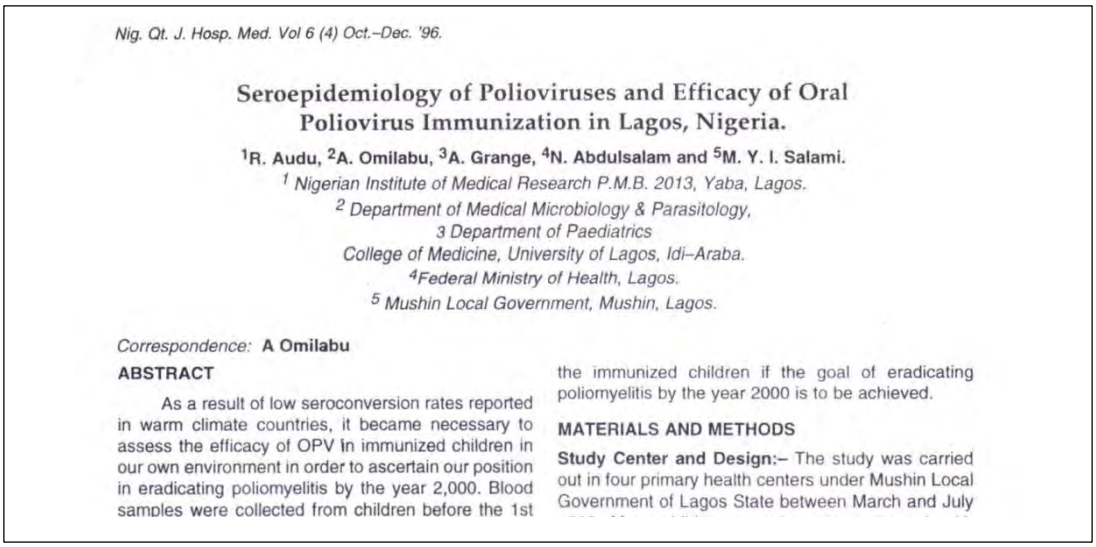
astrovirus [67, 68]. In another study, we recovered fastidious enteric adenoviruses from stool of children and the source of drinking water was found to have significant effect on the frequency of stool per day [69]. Rotavirus infection often times is severe and fatal in children. Treatment of viral diarrhoea is essentially correction of dehydration by Oral Rehydration Salt Solution, hence the use of antibiotics without prescription in the management of diarrhoea should be discouraged. In our collaboration with an African working group on rotavirus network in South Africa, in early 2000, we characterized the rotavirus from stool samples of Nigerian children and found that we had diverse and unusual strains in the country [70, 71]. This is essential for development of effective vaccines which in Nigeria has been included in the childhood immunization programme at 10 weeks of age. This vaccine is expected to avert 160,000 deaths among children in Nigeria annually.

Vaccine Preventable Viral Diseases

Viral infections in humans and animals provoke an immune response that usually eliminates the infecting virus. Vaccines can also produce immune response which confer artificially acquired immunity to the specific viral infection. Vaccination protects children from serious illness and complications of vaccine-preventable diseases which includes amputation of an arm or leg, paralysis of limbs, hearing loss, convulsions, brain damage, and death. In Nigeria, the National Programme on Immunization was established to improve the health of the Nigerian child. However, our studies that evaluated the coverage of infant immunization in a difficult-to-reach area of Lagos metropolis showed a low coverage where only 21% of children were fully immunized while 40% had partial immunization, and 39% had no record of immunization which is provided free of charge [72]. Similarly, only 7.2% of mothers received complete doses of tetanus toxoid immunization in an evaluation of its coverage among mothers of children below one year in the same difficult-to-reach community [73]. Further investigation of the level of antibody transferred to newborns among mothers fully immunized revealed that the antibody level in infants was significantly lower than that obtained in mothers after immunization. We concluded that the late completion of tetanus immunization to time of delivery caused the low level transfer of maternal antibody [74]. Hence, there is a need to improve immunization coverage particularly in hard-to-reach areas and we are advocating that the scheduled timing be adhered to by mothers.

Polio vaccine is used to prevent poliomyelitis. The oral polio vaccine (OPV) produces antibodies in the blood (humoral or serum immunity) to all three serotypes of poliovirus, and

in the event of infection, it protects the individual against polio paralysis by preventing the spread of poliovirus to the nervous system. Mr DG Sir, despite its advantages, polio vaccine has suffered much setback in Nigeria. Factors identified as responsible for persistence of poliomyelitis in Nigeria, are low vaccine efficacy, poor vaccine coverage, suboptimum population immunity, rejection and refusal of vaccine, and lack of awareness [75]. We carried out a two-year study in Lagos where we investigated the potency of live attenuated vaccines, we established that, vaccine potency deteriorates as the distribution channels go down the ladder i.e., from the national cold store level, to the state, then the local government stores and the vaccination centers [76]. Another study we conducted on oral polio vaccine showed that, 87.5% of serotype 1 and 75% of serotype 2 met the WHO standard virus titre but none (0%) of the vaccines met the required WHO titre for serotype 3 [77]. We evaluated the seroepidemiology of polioviruses and efficacy of OPV immunization in Lagos and discovered low seroconversion rates for types 1(75%) and 3 (58%) however, type 2 had the highest (100%) antibody response [78].



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Seroepidemiology of Polioviruses and Efficacy of Oral Poliovirus Immunization in Lagos, Nigeria.

[1]R. Audu, [2]A. Omilabu, [3]A. Grange, [4]N. Abdulsalam and [5]M. Y. I. Salami.

[1] Nigerian Institute of Medical Research P.M.B. 2013, Yaba, Lagos.
[2] Department of Medical Microbiology & Parasitology,
3 Department of Paediatrics
College of Medicine, University of Lagos, Idi–Araba.
[4]Federal Ministry of Health, Lagos.
[5] Mushin Local Government, Mushin, Lagos.

Correspondence: **A Omilabu**

ABSTRACT

As a result of low seroconversion rates reported in warm climate countries, it became necessary to assess the efficacy of OPV in immunized children in our own environment in order to ascertain our position in eradicating poliomyelitis by the year 2,000. Blood samples were collected from children before the 1st the immunized children if the goal of eradicating poliomyelitis by the year 2000 is to be achieved.

MATERIALS AND METHODS

Study Center and Design:– The study was carried out in four primary health centers under Mushin Local Government of Lagos State between March and July

In addition to improving cold chain to maintain vaccine integrity along the distribution line, we also concluded that improved sanitation, hygiene and nutrition would contribute to enhancing the immune response of children vaccinated.

Measles is an infectious disease that spreads rapidly from one infected child to another and is a major cause of blindness, malnutrition and death among children. Measles vaccine prevents measles infection. It has been established that after one dose 85% of children, nine

months of age and 95% over twelve months of age are immune. Nearly all of those who do not develop immunity after a single dose develop it after a second dose. We conducted a study of measles seroconversion status in children vaccinated against measles in Edo state, and observed that 94.7% of the children who were mostly above twelve months of age at the time of immunization developed protective measles antibody which was higher than the 85% pre-vaccination level recorded [79]. However, in another study, we found that when children were vaccinated at nine months of age, only 60% seroconverted with potent measles antibody and it was also observed that only 7% of the vaccines used for that vaccination exercise had virus titre of ≥3.5 Log as recommended by WHO [80]. These findings have been confirmed by a more recent study in Kwara state which also found low potency of the measles vaccine and low seroconversion level [81]. An evaluation of measles cold chain in Lagos state showed that vaccines were potent at the national and state cold stores but lost their potency at the local government and vaccination centers [82]. This was attributed to poor handling of vaccines by staff, therefore cautious efforts should be made to train and retrain staff at these levels of care.

There is an urgent need to improve cold chain for measles vaccines in order to ensure that vaccinated children are immunized thereby reducing the risk of infection after vaccination. We observed that placental transfer of measles antibodies from mother to child was inadequate in 54% of mother-child pair [83]. This might explain why some children come down with measles before the age of nine months. Most times this is caused by either placental insufficiency or pathology due to intra-uterine measles infection. The need for mothers to maintain good health status in pregnancy is very crucial in alleviating this occurrence. This is because babies acquire measles antibody from their mothers across the placenta and this provides protection for the baby, provided they are on breast milk until they are nine months when they are vaccinated.

Safe and effective rabies vaccines are available in Nigeria and are administered to those at risk of infection. Dogs are also vaccinated to prevent transmission of the virus to man. In a study we conducted to determine the presence of the virus among apparently healthy dogs which are in the majority in this environment, we found that 0.9% of the dogs investigated had rabies virus [84]. Bites from unvaccinated dogs cause rabies infection which is almost always fatal. It is therefore advisable that dogs are vaccinated to prevent rabies infection from dog bites.

Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome (HIV/AIDS)

HIV Transmission

HIV infection in Nigeria remains a public health challenge and Nigeria has the second largest HIV epidemic in the world. Although HIV prevalence among adults is much less (2.9%) than other sub-Saharan African countries, due to our very large population, this translates to about 3.6 million people living with HIV in 2016 [85]. Rapid antibody tests are used in Nigeria for HIV diagnosis, but dual testing sometimes produce discordant results. Discordant rapid HIV tests should always heighten suspicion by frontline healthcare workers that early HIV infection is present. It has been reported that discordant rapid tests have value for identifying early HIV infection in high HIV prevalence populations. In a study I carried out with my team, we explored the occurrence of discordant rapid HIV tests using the national algorithm in a low-resource community and found 2.5% rate of discordance with detected HIV-1 RNA. Therefore, we concluded that among adults with sexual risk behavior, discordant rapid tests should trigger strong suspicion of early HIV infection in low HIV prevalence populations [86].

Great progress has been made globally in the prevention of mother-to-child transmission (PMTCT) of HIV especially with the WHO guideline that recommends that all pregnant women living with HIV be provided with lifelong treatment, regardless of CD4 count. Mother-to-child- transmission accounts for 90% of HIV infections in children. Regrettably, Nigeria has the highest number of new HIV infections among children. In a study we conducted within two states in 2006, the rate of transmission of infection was 11% among those that had PMTCT intervention and 30% among those without intervention [87]. In another multicenter study in 2011, we found the average rate of vertical HIV transmission to be 22.5%, but the rate was 9.6% for babies whose mothers had PMTCT service [88].

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Estimation of the rate of mother to child transmission of HIV in Nigeria

*RA Audu, [1]OB Salu, [1]AZ Musa, [1]J Onyewuche, [1]EO Funso-Adebayo, [2]EO Iroha, [2]VC Ezeaka, [2]IMO Adetifa, [3]B Okoeguale and [1]EO Idigbe
[1]Nigerian Institute of Medical Research, PMB 2013, Yaba, [2]Department of Paediatrics, College of Medicine, University of Lagos, PMB 12003, Idi-Araba, Lagos and [3]Federal Staff Clinic, Benin City, Edo State, Nigeria

Summary
Definitive diagnosis of HIV infection in infants <18 months of age who were born to HIV-infected mothers is still posing some difficulty in Nigeria and other developing countries. Within this age definitive diagnosis can only be carried out by antigen based techniques which are indeed not available in these developing countries. This has re-

informations sur ce taux au Nigéria en utilisant les techniques du PCR. Les échantillons de plasma étaient obtenus de 68 enfants des 2 sexes, de moins de 18 mois et des mères infectées dans deux départements de pédiatrie de l'état de Lagos et de Bénin au Nigéria. La présence des ARN du HIV type 1 était déterminé par la technique de l'Amplicor Monitor V 1.5.Les résultats

Despite the current use of effective treatment to prevent transmission of HIV in pregnancy, the rate of mother-to-child transmission has remained high, at an estimated 22% in 2016 due to low access to the PMTCT services [89].

Monitoring HIV Disease Progression

After the diagnosis of HIV is confirmed, the CD4 T-lymphocytes and viral load markers are used in monitoring disease progression. The CD4 count was previously used to determine when to commence antiretroviral therapy (ART) but now all HIV patients are required to start treatment irrespective of their CD4 count. Notwithstanding, it is still useful for opportunistic infection (OI) risk stratification which helps to determine when to start and stop OI prophylaxis or management. It had been established that CD4 count could be comparable between populations however, differences existed between geographical locations hence, it's advisable that the reference value is defined for different populations and locations. As such, we carried out studies to establish the reference values in apparently heathy populations in Nigeria. In a pilot study conducted, we found the range of CD4 count among healthy Nigerians to be between 324 and 1,160 cells/µl [90]. Similarly, in a multicenter study we conducted, the reference range for CD4 was established as 365-1,571 cells/ µl [91]. This is important in clinical decision making in patient management. It was also interesting to note that the CD4/CD8 ratio observed in the study was higher than those observed in many other countries.

This means that Nigerians generally have more competent immune systems. This was quite surprising giving the relatively poor environmental sanitation observed in the country.

In children, the number of circulating T cells increases from infancy until about six months of age. This peak is followed by a gradual decline throughout childhood until adult levels are reached by late childhood. Therefore, it was equally essential to determine the CD4 reference values among children. In a study of T-lymphocyte subsets in apparently healthy Nigerian children, we found that children <3 years had median CD4% lower than WHO normal values but correlated with CDC values [92]. We then concluded that values used by WHO in infants were higher than ours hence recommended that our children be assessed using the CDC reference values which correlated with ours (table 4).

Table 4: Reference ranges of the CD4 and CD8 counts and the CD4/CD8 ratio of study participants[a]

Study group	CD4 (cells/µl)			CD8 (cells/µl)			CD4/CD8 ratio		
	Mean (SD)	Median	Reference range	Mean (SD)	Median	Reference range	Mean (SD)	Median	Reference range
Male	782 (272)	746	351-1,455	422 (184)	417	155-863	2.1 (1.4)	1.9	0.7-51
Female	920 (327)	892	383-1,654	450 (197)	401	133-919	2.4 (2.8)	2	0.8-5.8
Male and female	847 (307)	812	365-1,571	435 (191)		145-884	2.3 (2.2)	1.9	0.7-5.3

- [a]*P* value is 0.0001 for CD4, 0.001 for CD8, and 0.002 for CD4/CD8. *P* values of <0.05 were computed with the Mann-Whitney U test, comparing the distribution of the immunologic parameters between the sexes.

Early in the HIV treatment program in Nigeria, we reviewed the profile of baseline CD4 count and viral load levels in HIV infected treatment naïve patients in Lagos. We found that the patients had a median viral load of 172,641 HIV-1 RNA copies/ml and majority had very low CD4 counts, indicating immunological AIDS [93]. This showed that patients who were accessing treatment at that time had advanced disease. With the current test and treat guidelines, patients do not have to wait to have advanced disease to be treated. Starting ART immediately after a positive HIV test prevents some serious HIV-related illnesses, such as tuberculosis and invasive bacterial diseases. Since ART reduces the amount of the virus in a patient's body, it reduces the risk that it can be transmitted to sexual partners. The challenge however is that only 34% of people living with HIV in Nigeria are aware of their status [94]. Merely 30% of

all people living with HIV were receiving treatment in 2016, meaning that there are still many needless AIDS-related deaths in Nigeria.

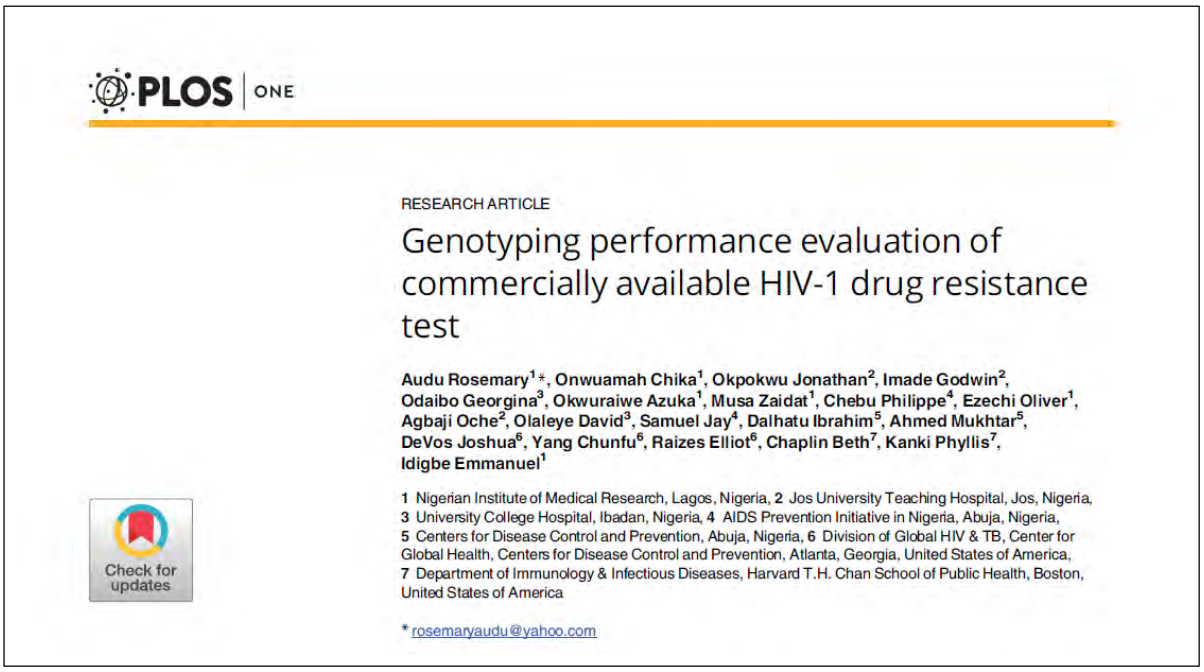
Health Workers Training and Validation of Methods/Equipment

At the Nigerian Institute of Medical Research, we have trained several and different categories of health workers to meet the human resource need especially at the commencement of the HIV treatment program in the country. From an evaluation of some of the training programs, we found that the trainings were of immense benefit to the health workers especially because we had a core group of experienced personnel who served as facilitators but there were suggestions for allocation of more time for the practical component [95]. The need for training and re-training to standardize practice and improve competency of personnel is very key to the success of any program.

At the same time, we validated several methods and diagnostic tools for the monitoring of HIV patients on treatment. Data obtained have informed policy making decisions in the national HIV program. Flowcytometry is the standard method for performing CD4 cell count by immunophenotyping but it is very expensive and there was only one instrument available then in the country. Prior to the commencement of the ART program, we evaluated three methods for CD4 estimation. Given for the few patients on treatment in 2001, the Dynabeads technique which is simple to carry out requiring only a light microscope was recommended for use based on the statistical measure of strength of association observed [96]. As the program matured we evaluated newer technologies and made recommendations for change. For example, we compared the analytical performances of the Amplicor HIV-1 monitor test, a manual technique, with Cobas/Ampliprep Taqman (automated method) and found that the results were comparable [97] hence, the automated method is now in use in the country. Due to the increasing demand for HIV viral load test as a result of the scale up to meet the new testing targets, higher versions of these instruments with higher throughput are being procured for use. No doubt, Nigeria is a large country and will require well-coordinated efforts in equipment donations and boarding efforts to prevent the country from becoming a dumping ground for all types of equipment, the good, the bad and the ugly.

Incomplete suppression of viral replication could result in the development of HIV drug resistance (HIVDR) mutations which compromises the efficacy of ART for patients on treatment and may lead to onward transmission of resistant viruses to newly HIV-infected patients. ATCC HIV-1 drug resistance test kit was designed to detect HIVDR mutations in the

protease and reverse transcriptase genes for all HIV-1 group M subtypes and circulating recombinant forms. The test has been validated for both plasma and dried blood spot specimen types with viral load of ≥1000 copies/ml. We performed an in-country assessment on the kit to determine the genotyping sensitivity and its accuracy in comparison with the ViroSeq assay for detecting HIVDR mutations using plasma samples stored under suboptimal conditions. While ViroSeq system genotyped 46.5% samples with high VL, ATCC genotyped 62.8% samples with lower VL. This study confirmed that ATCC kit showed greater sensitivity in genotyping plasma samples stored in suboptimal conditions experiencing frequent and prolonged power outage [98]. Thus, it is more sensitive particularly for subtypes A and A/G HIV-1 in resource-limited settings.



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RESEARCH ARTICLE

Genotyping performance evaluation of commercially available HIV-1 drug resistance test

Audu Rosemary[1]*, Onwuamah Chika[1], Okpokwu Jonathan[2], Imade Godwin[2], Odaibo Georgina[3], Okwuraiwe Azuka[1], Musa Zaidat[1], Chebu Philippe[4], Ezechi Oliver[1], Agbaji Oche[2], Olaleye David[3], Samuel Jay[4], Dalhatu Ibrahim[5], Ahmed Mukhtar[5], DeVos Joshua[6], Yang Chunfu[6], Raizes Elliot[6], Chaplin Beth[7], Kanki Phyllis[7], Idigbe Emmanuel[1]

1 Nigerian Institute of Medical Research, Lagos, Nigeria, 2 Jos University Teaching Hospital, Jos, Nigeria, 3 University College Hospital, Ibadan, Nigeria, 4 AIDS Prevention Initiative in Nigeria, Abuja, Nigeria, 5 Centers for Disease Control and Prevention, Abuja, Nigeria, 6 Division of Global HIV & TB, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, 7 Department of Immunology & Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, United States of America

* rosemaryaudu@yahoo.com

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Treatment Outcomes

In 2002, the Federal Government commenced the largest ART program in sub-Saharan Africa and it was one of the few countries that used generic drugs since they were relatively cheaper. In a 12-month evaluation of the program, we showed that the clinical and biological results compared favourably to those of patients treated with the branded ARV drugs thereby supporting the use of these generic drugs in resource limited countries [99]. We also evaluated the heamatological and biochemical values of HIV patients at the clinic in the Institute, and we found that the ARV drug combination in use in the country was quite safe [100]. In another

study, we investigated the burden of first line treatment failure and associated risk factors among Nigerians on highly active antiretroviral therapy (HAART) over a 5-year period. We found a first line treatment failure rate of 12.6% which was associated with young age, earlier years of start drugs, low baseline CD4 count and HIV/TB Co-infection [101]. The positive effect of ART on survival of HIV positive patients was established and adherence to drugs and younger age were identified as key prognostic factors.

It has been established that the use of HAART is very effective in controlling the progression of HIV disease and prolonging survival, but these benefits can be compromised by the development of drug resistance. Resistance occurs as a result of mutations that emerge in the viral proteins targeted by antiretroviral agents. Among patients receiving 1L ART, 20% will develop drug resistance at 12 months when viral load is not suppressed. The World Health Organization (WHO) recommends a simplified approach for choosing first-line (1L) and second-line (2L) antiretroviral therapy (ART) for treatment of HIV-1 in adults in resource-limited settings (RLS). The 2L recommendation is based on the expectation that the previously unused NRTI backbone will have preserved activity. However, when failure is detected, which occurs when viral load (VL) monitoring is performed only every 12 months, as currently recommended in WHO guidelines, the accumulation of drug resistance mutations (DRM) may result in compromise of the 2L NRTI backbone.

In a CDC sponsored study we conducted, the patterns of drug resistance mutations (DRM) from patients failing zidovudine (AZT)-containing versus tenofovir (TDF)-containing ART were assessed to evaluate the predicted susceptibility to second-line (2L) nucleoside reverse-transcriptase inhibitor (NRTI) backbone options. We found that at time of 2L switch, 28.2% of patients on AZT-containing regimens had developed resistance to TDF, whereas only 6.8% of patients on TDF-containing 1L had mutations compromising susceptibility to AZT. Similarly, patients on 1L AZT had 9.9 times higher odds of having a compromised 2L NRTI option than patients on 1L TDF [102]. This therefore is a further evidence to support the use of TDF as the preferred 1L NRTI because it allows for preservation of the recommended 2L NRTI option. A review of the genotypic data in this cohort also showed that contrary to widespread thought that thymidine analogue mutations (TAMs) do not develop in the presence of K65R, our study found that nearly one-third of patients with K65R developed TAM-2s [103]. Although this does not appear to confer a significant resistance cost to future 2L ART options, we have provided new data that build on previous studies, suggesting that some widely accepted dogma might not hold true in all settings.

Treatment of individuals who do not maintain viral suppression on their first antiretroviral regimen can be challenging in resource-limited settings due to uncertainty about viral resistance and limited data about virological response to the remaining available medications. Some have already been exposed to nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors, thereby resulting in drug resistance patterns that can be variable and complex. More than half of people in low- and middle-income countries may not maintain viral suppression on second-line antiretroviral therapy (ART). We evaluated the rate of accumulation of major protease (PR) mutations following 2L failure in our setting. Among patients failing 2L ART for over 24 months, almost two-thirds developed regimen-compromising PR resistance with a median of five International AIDS Society PR mutations. On the average, patients developed 0.6 PR mutations for every 6 months they were maintained on a failing 2L regimen [104]. The majority of patients had preserved susceptibility to Darunavir/ritonavir (DRV/r) just as reported in another study [105], suggesting that 3L therapy may be an option. Therefore, there is a need to increase access to this 3L drugs and the Nigerian government should take the lead in providing them due to the current donor fatigue being experienced.

In 2016, 36.7 million people were living with HIV globally, 30% did not know their HIV status, and there were about 1.8 million new infections [94]. There is a global challenge to reach the 90-90-90 goal. This is an ambitious plan to ensure that by 2020, 90% of the world population living with HIV know their status, 90% of those who know their status are placed on drugs and 90% of those on drugs have achieved viral suppression. The ultimate goal is that by 2030, the world would see an end to the AIDS epidemic. So the question is, what improvements have been made across board towards achieving the 90-90-90 goal? Globally, it is estimated that 70% of all people living with HIV knew their HIV status in 2016, 77% of these were accessing antiretroviral therapy, and viral suppression occurred in 82% of people on treatment. This translates to a progress of 70 – 77 – 82 as a global average: not bad. With countries like Botswana which have already achieved the 90-90-90 targets, this tells us that the global attainment of all three 90s by 2020 is both feasible and achievable [94].

Mr DG Sir, despite the 90-90-90 target, Nigeria still has a long way to go. We have the second largest burden of HIV in the world after South Africa, with a population of 3.2 million people living with the virus, but only an estimated 1.1 million of these know their status (34%). Though 88% of those who know their status are on treatment and 81% of those have achieved

viral suppression. This gives us a performance score of 34-88-81, revealing that only 30% of people living with HIV in Nigeria are on treatment. This means that we still have a long way to go in ending AIDS in Nigeria despite the 90-90-90 target. Though there has been long-standing controversy on the validity of the estimates from Nigeria, the current ongoing National AIDS Indicator and Impact Survey will help resolve this.

While we wait for the data from this survey, we must continue to aggressively work towards ensuring that 90% of those who are living with HIV know their status, and improve on linkage of newly detected positives to sites where they can access treatment. This must also be coupled with increased capacity for offering treatment especially in underserved areas and locations. The prospect of achieving this goal may have increased with new deals on more effective and cheaper dolutegravir, a medication used for HIV treatment and the establishment of mega laboratories for viral load testing, as the laboratory in the institute is also being expanded (figures 26 a-e). Most importantly, we must hold our government accountable to increase its funding to tackle AIDS and quit relying only on external donor funding. 90-90-90 is possible in Nigeria. Together we can end AIDS.

Figures 26 a-e: HIV-1 viral load system transformation in NIMR

26a - Cobas Amplicor V1.5 (manual extractions)

26b - Cobas Amplicor (Semi automated)

26c - Cobas Taqman 48 Analyzer (Automated Real Time Amplification)

26 d - Cobas Taqman 96 Analyzer (Automated Real Time Amplification)

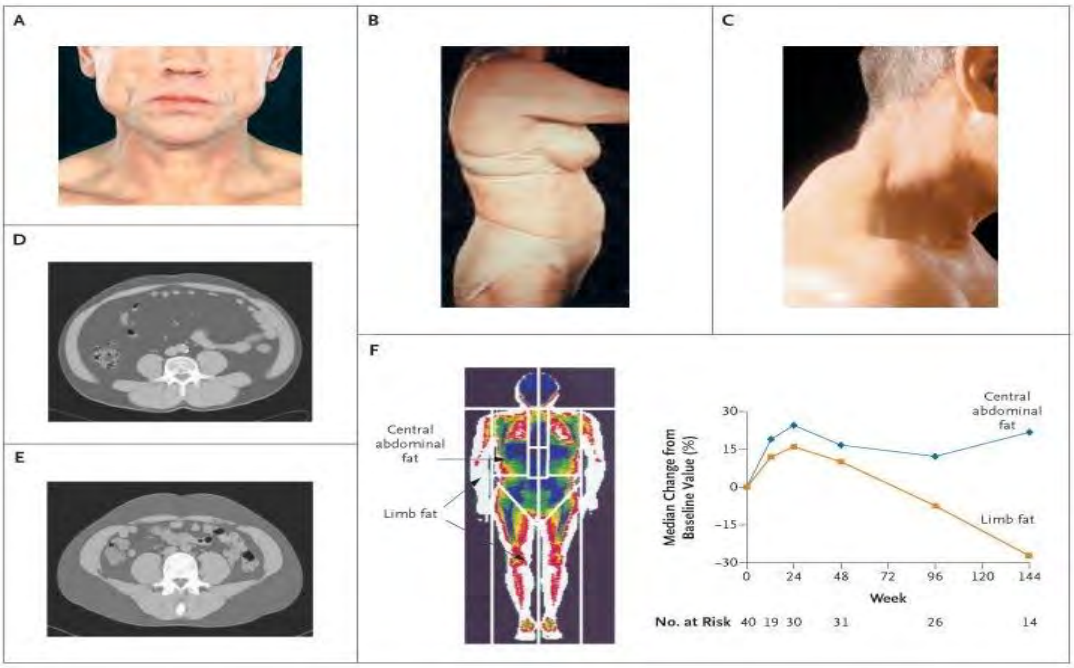
26e - New Systems for Viral Load Mega Laboratories (Cobas 6800 and 8800)

Effects of HIV and ART on the System

Mr DG Sir, the availability of potent antiretroviral therapy has changed the "face" of HIV, or AIDS, epidemic. However, patients experience a variety of unusual physical changes, including changes in the appearance of the face, that are associated with their HIV disease and its treatment. Patients with HIV/AIDS frequently present alterations in lipid metabolism (figure 27) due to infection with HIV itself, including elevated serum concentrations of triglycerides and low levels of total cholesterol.

Figure 27: Lipoatrophy and Fat Accumulation in HIV-Infected Adults.

Panel A shows a patient with facial lipoatrophy; Panel B, a patient with abdominal fat accumulation and breast hypertrophy; and Panel C, a patient with a dorsocervical fat pad (or "buffalo hump"). Panel D shows a single-cut abdominal CT scan at the mid-L4 vertebral level; the scan reveals a reduced amount of subcutaneous adipose tissue (scan area, 9 cm^2) and an increased amount of visceral adipose tissue (106 cm^2) in a patient with lipodystrophy. By contrast, a scan from a patient without lipodystrophy reveals 53 cm^2 of visceral adipose tissue and 144 cm^2 of subcutaneous adipose tissue (Panel E). Panel F (left) shows a whole-body dual-energy x-ray absorptiometry study, with standardized regions of interest for analysis of body composition. Panel F (right) shows prospective data reflecting changes in limb and truncal fat over time among adults commencing their first antiretroviral regimen. (The images in Panels A and C are from Carr and Cooper[8]; the graph in Panel F [right] is adapted from Mallon et al.,[9] with the permission of the publisher.)



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We conducted some studies to evaluate the effect of the disease in our environment. Among a cohort of treatment naïve HIV patients in Lagos, we confirmed that the presence of HIV infection affected some of the lipid profile parameters of the patients tested, they had altered cholesterol and high density lipoprotein [106]. Elevations in serum total cholesterol and triglyceride levels, along with dyslipidemia that typically occur in patients with HIV infection, may predispose patients to complications such as premature atherosclerosis, particularly coronary heart disease, and pancreatitis. In another study where we examined the level of total serum proteins and globulins in a cohort of HIV patients, we found that hypergammaglobulinaemian was common in HIV infected Nigerians [107]. An investigation of osmotic fragility and Na^+-K^+ ATPase activity of the erythrocytes of HIV/AIDS patients indicated that fragility was potentiated, the latter was not altered [108]. This means that the HIV patients were susceptible to osmotic stress leading to reduced nutrient intake resulting in malnutrition. Blood chemistry and platelet serotonin uptake was also evaluated among HIV patients and we found that serum glutamic oxaloacetic transaminase activity is significantly increased in HIV patients which explains the polymyositis effect seen in advanced disease whereby the patients progressive proximal muscle weakness involving upper lower extremities, with increased difficulty in rising from chair, climbing stairs and using their arms [109].

We also investigated the urinary creatinine levels in HIV/AIDS patients in comparison with apparently healthy controls. We found a significant reduction in the urinary creatinine level of HIV/AIDS patients when compared to healthy controls indicating that HIV infection may contribute to renal disease in Nigeria [110]. The prevalence of asymptomatic bacteriuria and its risk factors in HIV positive pregnant women was determined in a cohort study. The prevalence of bacteriuria was18.1% with *Escheria coli* and *Proteus mirabilis* being the most common bacterial isolates. The risk factors included previous urinary tract infection, high viral load, low CD4 count and maternal hemoglobin [111]. Similarly, in another study of HIV-positive women we found that they experienced more menstrual abnormalities of amenorrhea, oligomenorrhea and irregular periods compared to HIV negative controls [112]. Women at greater risk of menstrual abnormalities are with CD4 count of <200 cells/ul, body mass index <20 and those ARV-drug-naïve.

In order to understand the impact of antiretroviral drugs on the reproductive function of HIV infected persons, we conducted a study on two generation of mouse to determine the effect of either zidovudine or nevirapine on their reproductive capability. We found that there was foetal

loss when only the fathers received ART but when both parents received treatment, fertility was enhanced [113]. This confirms that couple disclosure of status is very important and in this era of test and treat, the couple will access treatment together with the resultant benefits.

HIV and Co-infections

The immunodeficiency caused by chronic HIV infection increases the risk of co-infection with pathogens that are controlled by innate and adaptive cellular immune responses and some that are controlled by phagocytic antibody responses. Furthermore, administration of HAART in the setting of HIV co-infection does not always restore the pathogen-specific immune response to normal levels. There are five leading infectious diseases that continue to cause significant morbidity and mortality in HIV-infected individuals globally: tuberculosis (TB), cryptococcosis, hepatitis B virus (HBV), hepatitis C virus (HCV), and malaria. Understanding the complex interaction between HIV, these co-infections, and the host immune response is central to developing new strategies for optimal treatment and prevention.

Mr DG Sir, the World Health Organization (WHO) has estimated that approximately 14 million people worldwide have HIV and Mycobacterium tuberculosis co-infection and that TB is the most common opportunistic infection in individuals with HIV infection, accounting for about 26% of AIDS-related deaths. In 2015, there were an estimated 10.4 million new TB cases, 1.2 million (11%) of these were among people also living with HIV [114]. The two infections are strongly linked. Whereas individuals with healthy immune systems may not fall ill from TB infection, people living with HIV with a low CD4 count are at greater risk of TB infection. In fact, the risk of developing active TB is estimated to be 26 and 31 times greater in people living with HIV than in those who are HIV-negative [115]. It is known that HIV causes anaemia just as well as TB. I and my team conducted a study to determine the impact of HIV/TB co-infection on HIV induced anaemia and we found that the co-infections did not worsen anaemia in the studied subjects [116]. In another study, we evaluated the possible impact of co-infections of tuberculosis and malaria on the CD4 cell counts in HIV infected subjects. We observed that the median CD4 counts in all groups of subjects were similar and significantly lower than those of healthy controls. This confirmed that co-infections with TB and malaria did not have any impact on the CD4 cell of HIV infected subjects [117]. There are multiple ways that HIV can alter the immune response to *M. tuberculosis*. $CD4^+$ T-cell depletion appears to be particularly important in the failure to generate or the loss of a cellular immune response to *M. tuberculosis*

in patients with HIV infection. The rate of TB reactivation in HIV-infected patients increased with declining $CD4^+$ T-cell counts, and patients with CD4+ T-cell counts <200cells/µl were particularly susceptible to disseminated TB, presumably reflecting the poor granuloma formation in patients with this degree of immunodeficiency.

Between 5-25% of HIV-infected patients are co-infected with HBV. The consequences include higher rates of chronicity after acute HBV infection, higher level of HBV replication and rates of reactivation, less spontaneous clearance, higher rates of occult HBV (i.e. detectable HBV DNA positivity in the absence of HBsAg seropositivity), more rapid progression to cirrhosis and HCC, higher liver-related mortality, and decreased treatment response compared with persons without HIV coinfection [118]. Similarly, persons with HIV/HCV coinfection generally have more rapid progression of liver fibrosis, especially those with a CD4 cell count of <200 cells/µl. Furthermore, even among patients in whom ART leads to successful control of HIV infection (i.e. undetectable HIV viral load), the risk of hepatic decompensation among co-infected patients is higher than among patients with HCV monoinfection [119]. For these reasons, all persons with HIV/HCV coinfection should be considered for HCV treatment.

We conducted a study to determine the prevalence and factors associated with HBV and HCV infection in pregnant HIV infected Nigerians. We found the prevalence of HIV/HBV and HIV/HCV co-infections to be 4.2% and 1.5% respectively. We also had a prevalence of 0.08% for triple infection of HIV/HBV/HCV. The co-infections with HBV and HCV were associated with induced abortion but blood transfusion and elevated transaminase was associated with only HBV [120]. A 12-month laboratory based investigation was carried out to assess the three monthly trend of virological and immunological parameters of triple infection of HIV-1 co-infected with HBV and HCV as compared with HIV-1 mono-infection. The median HIV-1 viral load declined within the time points studied and CD4 count also increased within the same period. The pattern in the mono and triple infections were similar with no statistical difference [121]. Therefore, we concluded that co-infections with HBV and HCV do not alter immunological and virological response to treatment at 12 months of therapy. However, side-effects such as depression or weight loss as well as severe anaemia, thrombocytopenia and neutropenia have been reported.

Management of viral hepatitis in patients with HIV disease is quite challenging and complex. With effective HIV treatment, people with HIV/HBV co-infection are living longer. In a randomized open label study with participants allocated into treatment groups, we assessed

effect of selenium as adjunct to HAART in the management of HIV/HBV co-infection. The result showed that the rate of HBV clearance was significantly higher among patients on HAART-plus-Selenium at 18 months when compared with rate of clearance among those on HAART-only [122]. Therefore, selenium may be beneficial as adjunct to HAART in HIV/HBV management.

Viral Hepatitis B and C Infections

Hepatitis is an inflammation of the liver. The condition can be self-limiting or can progress to fibrosis (scarring), cirrhosis or liver cancer. Hepatitis viruses are the most common cause of hepatitis in the world but other infections, toxic substances (e.g. alcohol, certain drugs), and autoimmune diseases can also cause hepatitis. There are 5 main hepatitis viruses, referred to as types A, B, C, D and E. These 5 types are of greatest concern because of the burden of illness and death they cause and the potential for outbreaks and epidemic spread. In particular, types B and C lead to chronic disease in hundreds of millions of people and, together, are the most common cause of liver cirrhosis and cancer. Hepatitis D virus (HDV) is a defective virus as it depends on the helper function of HBV for its replication and expression. HDV causes Type D Hepatitis, and has no independent existence but can survive and replicate as long as HBV infection persists in the host body.

Occult Hepatitis Infection

Blood transfusion is an important route for the transmission of infection especially when donated blood is not screened for hepatitis B virus (HBV) infection. Screening of donated blood for Hepatitis B surface Antigen (HBsAg) was introduced in the 1970s. This greatly reduced HBV transmission due to blood transfusions as blood found to be HBsAg positive was not transfused. In many developing countries including Nigeria, screening of blood donors or blood donated, for HBsAg alone, is still the only practice on which the prevention of HBV transmission during blood transfusion is based. The presence of HBV infection in some individuals negative for HBsAg but having detectable HBV DNA in the liver or blood have been reported. Occurrence of HBV transmission resulting from transfusion of blood tested and found to be HBsAg negative has been documented. Occult HBV infection (OBI) is a term that denotes HBV infections in which HBsAg cannot be detected and the presence of infection is only established by amplifying and detecting HBV DNA. One significant importance of OBI is the risk of transmission of HBV from individuals with OBI to recipients. Hence, we investigated the prevalence of occult HBV among blood donors, and using ELISA to screen

HBsAg negative sample we had a prevalence of 1% while using HBV DNA, we found a prevalence of 5.4% OBI confirming that OBI infection existed in blood donors in Nigeria [123]. Therefore, the use of HBsAg alone for screening prospective donors will not eliminate the risk of HBV transmission in blood transfusion or stem cell transplantation.

Prevention of Mother-To-Child-Transmission of HBV

What few people thought possible little more than a decade ago is now reality: scientific and operational advances are greatly reducing the number of deaths from HIV. The number of infant HIV infections has decreased by 58% between 2001 and 2013 and mother-to-child transmission (MTCT) of HIV might well be eliminated globally in the next few years [124]. In contrast, the prevention and management of HBV infection lags well behind, especially in sub-Saharan Africa. Early HBV transmission is the main route by which HBV infection is perpetuated in high-prevalence communities. Therefore, we conducted a study to determine the prevalence of perinatal transmission of HBV and the maternal characteristics influencing it in Nigeria. Using HBV DNA, intrauterine infections were detected in 72% of newborns and high maternal HBV-DNA level was strongly associated with increased neonatal HBV-DNA titre [125]. About 90% of children infected through vertical transmission develop chronic hepatitis B. Meanwhile, 96% of adults will clear primary HBV infection. Therefore, interrupting early transmission is the key to breaking the cycle of ongoing HBV infection. In spite of incorporation of HBV vaccines into the Expanded Program on Immunization, children continue to be infected with HBV through MTCT. The addition of birth dose of HBV vaccine is a cost-effective method to reduce MTCT. Furthermore, better protection against HBV MTCT can be achieved by treatment of pregnant women with high HBV viral loads using tenofovir. This drug is already widely used in HIV PMTCT programs. It is therefore suggested that HIV PMTCT be expanded to deliver care for HBV-infected pregnant women. With the adoption of birth-dose vaccination policy and expansion of PMTCT program, elimination of HBV MTCT in Africa and Nigeria in particular is achievable [126].

Virological Evaluation of HBV and HCV Infections

HBV DNA concentrations quantified by real-time polymerase chain reaction (PCR) correlate with disease progression and are used to differentiate active HBeAg-negative disease from inactive chronic infection, and for decisions to treat and for subsequent monitoring. Serial measures over a few months or longer are preferable, but there remains a lack of consensus

regarding the level below which HBV DNA concentrations are indicative of "inactive" disease, or the threshold above which treatment should be initiated [118]. HBV DNA concentrations are also used for optimal monitoring of response to antiviral therapy, and a rise may indicate the emergence of resistant variants. WHO standards are available for expression of HBV DNA concentrations (IU/mL) to ensure comparability. The same assay should be used in the same patient to evaluate the efficacy of antiviral therapy. Access to HBV DNA testing remains very poor in resource-limited settings. I led my team to carry out a study to determine the pattern of HBV viral load levels of patients assessing management in Nigeria. In the cohort of patients evaluated, we found that HBV viral load ranged between 4,145 and 68,011,800 DNA copies/ml which is indicative of very high viral titre [127]. High viral load is a risk factor for hepatocellular carcinoma.

HBV DNA quantification is important for decisions on initiating antiviral therapy and monitoring individuals on antiviral therapy. However, HBV DNA viral load assays may not be widely available in LMICs including Nigeria. Access could be facilitated by utilizing the same platforms currently in use for HIV viral load monitoring and through access to point-of-care assays for HBV DNA. In facilities where HBV DNA viral load measurements are possible, reporting should be standardized to IU/mL (I IU/ mL ≈ 5.3 copies/mL).

Approximately 15–45% of persons who are infected with HCV will spontaneously clear the infection [119]. These persons are HCV seropositive but are no longer infected with HCV. A nucleic acid test (NAT) for HCV RNA, which detects the presence of the virus, is needed to distinguish persons with chronic HCV infection from those who have cleared the infection. It is therefore the standard of care to carry out a NAT for HCV RNA for persons who are found to be anti-HCV antibody positive. A NAT for HCV RNA is also important prior to commencing and during treatment to assess the response to treatment. HCV is a small, positive single stranded, RNA-enveloped virus with seven major genotypes and sixty-seven subtypes. The distribution of the genotypes varies according to geographical locations. Until recently, it was necessary to determine the genotype of the infecting virus before commencing treatment with peg-IFN and ribavirin because the dose and duration of treatment varied depending on the genotype identified in an individual. Our team conducted a study to provide information on the prevalent HCV genotypes and viral load titers in Nigerian patients. The median viral titre in the study population was 141,166 IU/mL. Genotypes 1,2,3,4 and 6 were found (figure 28), however, genotype 1 was the most prevalent and is one of the most difficult to treat HCV genotypes [128]. Currently, direct-acting antivirals (DAAs) are used for treatment of HCV with

>90% cure rate, and the 2016 Hepatitis treatment guidelines provide recommendations as to the preferred and alternative drugs [119] but; there is still some variation in recommended regimen. Therefore, there is a need to know the genotypes for effective treatment options. However, determination of genotypes may no longer be necessary as the pangenotypic drugs become more readily available and accessible. Although use of newer DAAs has transformed the treatment of HCV infection globally, access to these drugs is just being pioneered in Nigeria. Hence, knowledge of pre-treatment viral load (VL) and genotype remain relevant in determining treatment success in certain clinical situations using the more available older DAAs and pegylated interferon therapy.

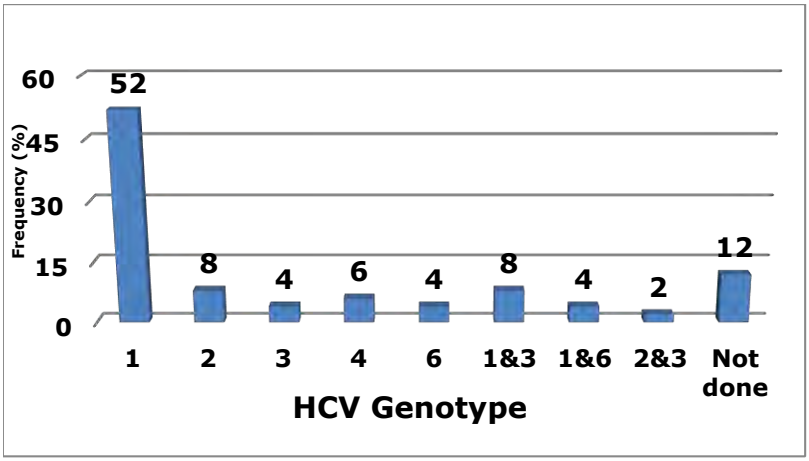

Figure 28: Bar Chart of HCV Genotypes in a Nigerian Cohort

There was indirect evidence showing that NAT for HCV RNA is underutilized in populations in which it is indicated [119]. The implications of not conducting an immediate NAT for HCV RNA include labelling persons as being infected with HCV when, in fact, they had spontaneously cleared the infection. Such individuals could unnecessarily face stigma and discrimination, including difficulties with employment and procuring health services. Knowing whether someone has chronic HCV infection allows health workers to provide prevention messages to protect the infected individual (e.g. alcohol reduction counselling) as well as the health of their family or contacts by informing them of methods to reduce the risk of transmission of HCV. Knowing someone's HCV status provides an opportunity to link him or her with appropriate care.

A potential harm of knowing one's HCV infection status is the psychological stress related to having a life-threatening infection, particularly if HCV treatment is not accessible. Despite this, the benefits of immediate testing versus delayed testing outweighs the potential harms. Patients with resolved HCV infection following spontaneous clearance would be reassured and those who learn of their infection can take steps to protect their health and that of others.

Hepatitis D Infection

Hepatitis D infection is a liver disease in both acute and chronic forms caused by hepatitis D virus (HDV) that requires HBV for its replication. Hepatitis D infection cannot occur in the absence of hepatitis B virus. HDV is transmitted through contact with the blood or other body fluids of an infected person. Vertical transmission from mother to child is rare. At least 5% of people with chronic HBV infection are co-infected with HDV, resulting in a total of 15 – 20 million persons infected with HDV worldwide. However, this is a broad global estimation since many countries do not report the prevalence of HDV. We therefore conducted a study to determine the prevalence of HDV among chronic HBV infected individuals with or without liver disease. The prevalence of 2% was found in both asymptomatic and chronic liver disease [129]. HDV-HBV co-infection is considered the most severe form of chronic viral hepatitis due to more rapid progression towards liver-related death and hepatocellular carcinoma. Worldwide, the overall number of HDV infection has decreased since 1980s. This trend is mainly due to a successful global HBV vaccination programme. Currently, treatment success rates are generally low. Hepatitis D infection can be prevented by hepatitis B immunization.

Viral Haemorrhagic Fevers

Mr DG Sir, early this year, through your able leadership my team ~~and I~~ veered into the world of viral haemorrhagic fevers (VHF) in collaboration with the Centre for Human and Zoonotic Virology, College of Medicine, University of Lagos led by Prof Omilabu and Federal Medical Center, Owo, Ondo State (figure 29). We are seeking to unravel the characteristics of the Lassa fever virus responsible for the unprecedented epidemic this year. We are also evaluating the current medical practice to determine the drivers of the recurrent epidemic and proffer strategies to combat the disease significantly in order to alleviate the unacceptably high case fatality rate.

Figure 29 a: Courtesy visit to Ondo State Commissioner of Health by NIMR and UNILAG Lassa Fever Research Team

Figure 29 b: NIMR, UNILAG and FMC Owo Lassa Fever Research Team on the field

The total number of suspected cases between January and August 2018 alone is 2434, with 492 confirmed, 10 probable cases and 130 deaths recorded [130]. The case fatality rate is 26.4% stimulating the Nigerian Center for Disease Control to solicit support from World Health Organization and other partners to control the outbreak. Healthcare workers are a particularly vulnerable group especially when barrier nursing and infection control practices are not maintained. Factors implicated in past hospital outbreaks resulting in high nosocomial infections and deaths include use of parenteral injections and surgery without due attention to good clinical practice [131]. Preliminary results obtained so far shows that sexual transmission of the virus may be contributing to the endemic nature of the disease as we found some persons who had recovered from the disease still excreting the virus. The study is still on-going as we are exploring the role of other VHFs such as dengue, yellow fever and rift valley viruses in the large number of unconfirmed Lassa fever cases.

Quality Management System Implementation in NIMR

Mr DG Sir, NIMR has taken the lead in the implementation and roll out of QMS in the country (figure 30). Prior to the roll-out of the SLMTA programme globally, in the year 2008, the former Human Virology Laboratory (now Center for Human Virology and Genomics) under my humble self, was the first and only clinical diagnostic laboratory to be in conformity to the requirements of NIS ISO 9001:2008 [49].

Figure 30: Staff of Human Virology writing an examination after a training on QMS

Page 1 of 5 Lessons from the Field

Experience of quality management system in a clinical laboratory in Nigeria

Authors:
Rosemary A. Audu[1]
Ugochukwu Sylvester-Ikondu[1]
Chika K. Onwuamah[1]
Olumuyiwa B. Salu[1]
Fehintola A. Ige[1]
Emily Meshack[1]
Maureen Aniedobe[1]
Olufemi S. Amoo[1]
Azuka P. Okwuraiwe[1]
Florence Okhiku[1]
Chika L. Okoli[1]
Emmanuel O. Fasela[1]
Ebenezer. O. Odewale[1]
Roseline O. Aleshinloye[1]
Micheal Olatunji[1]
Emmanuel O. Idigbe[1]

Affiliations:

Issues: Quality-management systems (QMS) are uncommon in clinical laboratories in Nigeria, and until recently, none of the nation's 5 349 clinical laboratories have been able to attain the certifications necessary to begin the process of attaining international accreditation. Nigeria's Human Virology Laboratory (HVL), however, began implementation of a QMS in 2006, and in 2008 it was determined that the laboratory conformed to the requirements of ISO 9001:2000 (now 2008), making it the first diagnostic laboratory to be certified in Nigeria. The HVL has now applied for the World Health Organization (WHO) accreditation preparedness scheme. The experience of the QMS implementation process and the lessons learned therein are shared here.

Description: In 2005, two personnel from the HVL spent time studying quality systems in a certified clinical laboratory in Dakar, Senegal. Following this peer-to-peer technical assistance, several training sessions were undertaken by HVL staff, a baseline assessment was conducted, and processes were established. The HVL has monitored its quality indicators and conducted internal and external audits; these analyses (from 2007 to 2009) are presented herein.

Lessons learned: Although there was improvement in the pre-analytical and analytical

The laboratory embarked on a journey toward international accreditation through the SLMTA programme and emerged the first public medical diagnostic laboratory in Nigeria to be ISO 15189 accredited (figures 31 and 32). Not resting on our oars, the laboratory alongside others both in Nigeria and globally, applied to the World Health Organization to be assessed for listing as a WHO pre-qualification laboratory. After two assessments by auditors from WHO, Geneva, the laboratory again under my able leadership in February 2018, was listed as a WHO prequalification laboratory. This makes it the only laboratory from West Africa and one of sixteen from thirteen countries globally to be so recognized.

Page 1 of 7 Original Research

Improving quality in national reference laboratories: The role of SLMTA and mentorship

Authors:
Rosemary A. Audu[1]
Catherine C. Onubogu[2]
Nkiru N. Nwokoye[2]
Eke Ofuche[3]
Shirematee Baboolal[4]
Odafen Oke[5]
Elizabeth T. Luman[6]
Emmanuel O. Idigbe[2]

Affiliations:
[1]Human Virology Laboratory, Nigerian Institute of Medical Research, Nigeria

[2]National Tuberculosis Reference Laboratory, Nigerian Institute of Medical Research, Nigeria

Background: The Nigerian Institute of Medical Research houses two reference laboratories: the virology and tuberculosis laboratories. Both were enrolled in the Strengthening Laboratory Management Toward Accreditation (SLMTA) programme.

Objective: To describe the impact of SLMTA and discuss factors affecting the results, with an emphasis on mentorship.

Methods: The SLMTA programme was implemented from April 2010 through November 2012. Participants attended three workshops and executed quality improvement projects; laboratory auditors evaluated performance using a standard checklist. The virology laboratory did not receive mentorship; however, the tuberculosis laboratory had an international mentor who visited the laboratory four times during the programme, spending two to four weeks embedded within the laboratory during each visit.

Results: There was an overall improvement in the performance of both laboratories, with the virology laboratory increasing 13% (from 80% at baseline to 93% at exit audit) and the tuberculosis laboratory increasing 29% (from 66% to 95%). These scores were maintained nine

Figures 31 (a- b): SANAS assessor scrutinizing our documents and records

Figure 32 (a-b): Staff of CHVG with SANAS Assessors after successful audit

Mr DG Sir, we have found that quality is the only language that the international community understands in other to adjudge results from a laboratory as accurate and reliable. We have blazed the trail in QMS in NIMR through persistent hard work with the cooperation of my amiable and dedicated staff members. We were privileged to have had top management who believed in us and gave us all the support we needed to stir the boat of QMS in the institute. Of worthy note is my boss and mentor, Prof Emmanuel Oni Idigbe, in fact he conceived the idea and gave us all the support. I'm glad that we did not let him down. However, this took a journey of 16 years, equivalent of a primary, secondary and tertiary education in Nigeria (6-3-3-4). In a study to evaluate the role of mentorship in the implementation of QMS, we found that mentorship is one of the factors that enhances substantial improvement in the laboratory [132]. We are currently stirring the boat of QMS in the Microbiology department as we are expanding our scope of accreditation to include the Center for Tuberculosis Research within the next couple of months.

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DECREE NO. 56 OF 1971

Certificate

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With NIS ISO 9001 : 2008

For **NIGERIA INSTITUTE OF MEDICAL RESEARCH (HUMAN VIROLOGY LABORATORY)**

(HUMAN VIROLOGY DIAGNOSTIC AND MONITORING ASSAYS FOR HIV AIDS AND OTHER PATHOLOGICAL CONDITIONS AND PHYSIOLOGICAL DISORDERS)

Validity for a period of **3 years** From date of issue.

Dec 31, 2013

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South African National Accreditation System

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MEDICAL TESTING LABORATORY
CHEMISTRY, MOLECULAR DIAGNOSTICS AND SEROLOGY

The facility is accredited in accordance with the recognised International Standard

ISO 15189:2012

The accreditation demonstrates technical competency for a defined scope and the operation of a quality management system

While this certificate remains valid, the accredited facility named above is authorised to use the relevant accreditation symbol to issue facility reports and/or certificates

Mr R Josias
Chief Executive Officer

Effective Date: 25 August 2017
Certificate Expires: 24 August 2021

SLIPTA

WHO AFRO Stepwise Laboratory Quality Improvement Process Towards Accreditation

Certificate of Recognition issued to:

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has been audited per the WHO AFRO SLIPTA Checklist and has met the requirements for the following star recognition level:

★ ★ ★ ★

Valid: September 5, 2013 – September 4, 2015

The African Society for Laboratory Medicine accepts no liability for the laboratory testing conducted in facilities enrolled in SLIPTA.

The laboratory has achieved a star ranking on the SLIPTA Tier of Recognition of Laboratory Quality Management and is not a certificate of accreditation.

Tsehaynesh Messele, Ph.D.
Chief Executive Officer, ASLM

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Your reference: 1912-190-9

Centre for Human Virology and Genomics
Nigeria Institute of Medical Research
Attention: Dr Rosemary Audu
Yaba PMB 2013, Lagos
Nigeria

10 September 2018

Dear Dr Audu,

Listing of Centre for Human Virology and Genomics, Nigeria Institute of Medical Research as a WHO Prequalification Evaluating Laboratory

This is with reference to the letter received by the World Health Organization (WHO) on 6 January 2017 expressing your laboratory's interest to be assessed and listed as a WHO Prequalification Evaluating Laboratory and to conduct performance evaluations of in vitro diagnostics (IVDs) submitted for WHO Prequalification assessment.

Following successful Stage 1 and Stage 2 audits of your Laboratory's Quality Management System, WHO is glad to inform you that the Human Virology Laboratory was listed on 15 February 2018 for the following analytes: **HIV antigen/antibodies, HIV Nucleic acids, HB surface Antigen, HBV Nucleic acids, HCV antibodies and HCV Nucleic acids** as indicated at: http://www.who.int/diagnostics_laboratory/alternative/en/

In order to adequately conduct the performance evaluation of products submitted for prequalification assessment, you are expected to acquire, characterize and store required testing panels as specified in the respective WHO Performance evaluation Protocols available at http://www.who.int/diagnostics_laboratory/evaluations/alter/protocols/en/. In addition, you are expected to obtain local government and ethical clearances to conduct the evaluations and work with manufacturers to obtain import licenses for the products under evaluation.

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Centre for Human Virology and Genomics
Nigeria

Page 2 of 2
10 September 2018

Your laboratory will be re-assessed at regular intervals. As a WHO Prequalification Evaluating Laboratory, your laboratory shall continue to have up-to-date respective International Organization for Standardization (ISO) accreditation and submit to WHO relevant annual External Quality Assessment (EQA) reports.

Yours sincerely,

Mr Deus Mubangizi
Coordinator
Prequalification Team
Regulation of Medicines and other Health Technologies

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RECOMMENDATIONS

Mr DG Sir, emerging and re-emerging viral infectious diseases have increased over the past few decades therefore, to control these infections requires three pronged approach involving:

1. Monitoring for early warning signs through review of hospital cases, self-screening for medical signs, seroprevalence, vector and animal health control. This will guaranty prompt detection of these infectious agents thereby curtailing their spread.
2. Intervention by anticipating epidemics, vector reduction, treatment of patients, vaccination, communication and information. Amongst others, improved communication and information will provide a more open route for reporting that could push governments toward greater transparency.
3. Research for accumulation of knowledge of virus replication cycle, ecosystems and virus transmission, methods for early detection, pathophysiology, etc. This is quite essential if we seek to develop therapeutics and vaccines to control these infectious agents as we cannot fight effectively against a health threat that we do not know well.

To minimize the health and socioeconomic impacts of emerging viral diseases, major challenges must be overcome in the national and international capacity for early detection, rapid and accurate etiological identification response and effective control.

CONCLUSION

Now let me find out from you, are viruses friends or foes?

Mr DG Sir, all is not bad about viruses, yet as foreign as they may seem, the boundary between man and viruses is strangely becoming unclear. We should manipulate and subdue them in order to domesticate them for our benefit. However, in doing that, laboratories must follow specific rules of quality management system to prevent them from escaping and harming man. ~~So~~ Indeed, all that God made was good.

ACKNOWLDGMENTS

The DG Sir, I thank the Almighty God for giving me all that pertains to life and godliness. He has directed my path in the journey of life and has given me the grace to achieve all that I have accomplished so far. The Lord indeed has been my helper, my defender, my shield and buckler. I owe all I am to Him who loves me so much.

I am forever grateful to my parents of blessed memory, Mr and Mrs John Adaji Okolo, for their sacrificial love for me and for giving me a good foundation. My father an educationist, ensured I always had the best of teachers and my mother ensured I embraced reading as a culture. They went all length to provide me and my siblings with quality education which was the legacy they left behind for us. For this and many more, I remain forever grateful.

In my journey of life, God has brought my way, great men and women who have left remarkable prints upon my life and for whom I remain ever grateful. These include my secondary school teachers: Mrs Grace Anago, Ms Akpabio and Mrs Ogunlowo; my university lecturer and supervisor in ABU Zaria, Prof Lucy Ogbadu; my postgraduate lecturers at the University of Lagos, Prof 'Tolu Odugbemi, Prof Fagbenro-Beyioku (of blessed memory) and Prof A.O. Coker. This includes my indefatigable and distinguished supervisor, Prof Sunday Aremu Omilabu. Over the past 26 years, I have known him to be a virologist per excellence, who controls viruses mainly when others have closed from work. He knows the viruses and

they know him and he calls them by name; while others are running away from the viruses, he is chasing them. Indeed, I'm proud to specially recognize Prof Omilabu on this special occasion of mine, for mentoring me, encouraging me in this fiery world of viruses and still finding time to review this lecture notes of mine, despite his very busy schedule. Others who have also impacted me greatly and to whom I remain grateful include Prof F. Ogunsola, Prof A.S. Akanmu, Prof Funmi Lesi, Prof Grace Otinwa, all of University of Lagos and Prof Patricia Lar of University of Jos. I also appreciate Dr Bola Oyefolu of the Lagos State University, for his comradeship in the field of virology.

Working in NIMR has been a thrill for me especially, as I have worked nowhere else. God has used great men here to mentor me and I remain thankful to them. They include Prof Oni Idigbe, my first head of department and onetime director general of this institute, who believed in me and gave me daunting tasks but with his support, I was able to deliver on most assignments. Others are Dr Adesina Adeiga, Dr Daniel Olukoya, Dr Abraham Alabi and Dr Afolabi. I learnt on-the-job administration from Mr Sola Olagundoye, former director of finance and accounts, Mr Francis Osagiede, former director internal audit, and Barr Obi, former director of administration. I appreciate Dr Catherine Onubogu, Dr Francisca Nwaokorie, Dr Judith Giwa-Amu, Prof Kola Oyedeji and Dr Biodun Ogunjimi who though are no longer in the institute, have continued to be a source of great encouragement to me.

I appreciate members of several research teams that I have worked with in NIMR particularly, the HIV drug resistance team, the viral hepatitis team and of recent, is the Lassa Fever research team. Worthy of mention are Dr Ezechi and Dr Musa. As a training coordinator for several years in the institute, I have enjoyed a good working relationship with several staff members cutting across the entire institute, that I will not attempt to list their names in order not to leave out any. I appreciate your contributions toward the success of all our trainings.

I am grateful to all the staff of the Center for Human Virology and Genomics with whom I have travelled my sixteen years of the hard and rough way of quality management system. It was a journey of commitment, dedication and discipline and today God has crowned our efforts with good success. These young men and women are indeed a rare gem and I will encourage you to continue to run the good race of quality management system no matter the terrain you find yourself, you will always be outstanding in that path. I also appreciate all staff of Microbiology department for embracing quality management system, I assure you that though

many will not understand why you do certain things, at the end, you will be the admiration of all. Keep on implementing quality!!!

Below are pictures of me and staff of Microbiology Department consisting of Diarrhoea Immunology Parasitology Research Division, Center for Human Virology and Genomics and Center for Tuberculosis Research.

Staff of Diarrhoea, Immunology and Parasitology Research Division posing after a departmental meeting

Staff of Center for Human Virology and Genomics rejoicing after obtaining ISO 9001:2008 certification

Staff of Center for Tuberculosis Research posing after a mentorship visit

I have been very privileged to have had a long history of favour with the leadership of this institute. They have believed in me, given me opportunities and provided resources to function. Surely, the outcome has been resounding success. I therefore want to thank the past director generals of this great institution who have impacted my life and career in the persons of Prof Lateef Salako, Prof Oni Idibge and Prof Innocent Ujah. I also appreciate Prof Babatunde Lawal Salako, the current director general, for his visionary leadership. I pray we shall achieve greater heights even in his tenure.

The support from the Harvard and APIN Family in my years as head of the former Human Virology Laboratory is tremendous. I therefore appreciate Prof Phyllis Kanki and her team from Harvard School of Public Health, Boston for the tri-country conferences we had, it was at the first of such conferences at Gaborone, Botswana that the idea of embracing quality management system was borne. I also appreciate Dr Prosper Okonkwo, Dr Jay Samuel, Mr Eke Ofuche and all the APIN staff for their unwavering support while NIMR was under their watchful care. I appreciate the many friends that I made from within and outside the country in this great family. I also appreciate our current implementing partners, FHI 360 for their unflinching support of the Center for Human Virology and Genomics and Institute of Human Virology Nigeria for their wonderful partnership with our Center for Tuberculosis Research.

I must acknowledge all my mentees in particular my PhD scholars: Dr Olumuyiwa Salu, Dr Chika Onwuamah, Dr Azuka Okwuraiwe and those in making: Mr Sam Amoo, Mrs Fehintola Ige and Mr Joseph Shaibu. I have learned a lot from you and I thank you all for your contributions towards my progress. I specially thank Dr Salu for his support toward the preparation of this lecture and for reviewing it.

Many people have impacted my life that I may not be able to mention all by name, I appreciate you all. I thank my Old Girls of Federal Government Girls' College Bakori set 85 and the alumni of Microbiology Students of Ahmadu Bello University Zaria set 89. I appreciate my recent mentees from the National Association of Microbiology Students.

I also thank members of Children Evangelism Ministry and staff and partners of Calvary Ministries. I particularly appreciate all the children in my bible and teens clubs (past and present), you have been the source of my inspiration. I thank members of the Foursquare Gospel Church family for being a strong pillar in my journey of life. The spiritual nourishment I derived from there has kept me going. I specially appreciate my pastors Rev and Rev (Mrs) Emmanuel O.O.O. Adejoro for their love and support.

I thank my siblings and their families for their constant support and encouragement, namely Dr and Mrs Jacob Afolayan, Barr and Dr (Mrs) Fred Okolo, Dr and Barr (Mrs) Clement Okolo, Mr and Mrs Koye Phillips, Ms Joy Okolo, Ms Faith Okolo, Mr and Mrs Charles Okonma and Mr David Okolo. Your love and cooperation is well appreciated.

I appreciate the wonderful love and support of my children, Solomon, Steven and Silas. My home has been a boys' hostel and my children call me the minister of labour and productivity. Their wonderful support in ensuring the smooth running of the home even in my absence is commendable. I thank my many other children who at one time or the other have lived with me particularly Demola Adegbite, Tobi Aigoro, Emmanuel Ogunlisi, Ezra Omokheni, Ezekiel Omokheni, Emmanuel Ojo, Benjamin Babatope and our lovely family friend Adebanke Adenusi.

Mr DG Sir, permit me to pay tribute to my wonderful husband of 29 years, Mr Ibrahim Solomon Audu. You have truly been a great pillar of support to me. You kept true to your vow of 31 years ago, that you want a wife who is intelligent and a scholar. You have provided such a conducive environment and support that made me press on in my career. You stood by me in all my late hours of work and many trips despite your equally busy schedules. I feel really

overwhelmed with your support and encouragement and I have come to a conclusion that there is no me without you.

Finally, I say to God be the glory for the great things He has done. He so loved me that He pulled my feet from the miry clay and planted it upon the solid Rock where I stand. I encourage all to anchor unto Him, who alone can keep us standing when the tide of life is fierce.

Mr DG Sir, distinguished ladies and gentlemen, I thank you all for honouring me with your presence today.

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CITATION OF DR ROSEMARY AJUMA AUDU

Dr. Rosemary Ajuma Audu was born on 30th September 1968 in Kaduna to the family of Mr John Adaji Okolo and Mrs Serah Titi Okolo. She attended Army Children School, 1 Div. Artillery and proceeded to Federal Government Girls' College, Bakori from 1980 – 1985. She was admitted into the School of Basic Studies, Zaria from 1985 – 1986, then gained admission into Ahmadu Bello University, Zaria where she studied Microbiology and graduated with a Bachelor of Science (Hons.) Degree in 1989. She was posted to Rivers State for National Youth Service Corp. After orientation, on the ground of marriage, she redeployed to Lagos for her primary assignment at the Department of Microbiology and Parasitology, College of Medicine, University of Lagos between 1989 -1990. She returned to the department where she enrolled for a Master of Science Degree between 1991-1992 and subsequently, a PhD in Medical Microbiology, Virology sub-specialty, from 1996-2000.

Upon completion of her master's degree, she was employed in NIMR in January 1993 as a Junior Research Fellow. She rose through the rank until she got to her present position as a Deputy Director Research in 2013. Since joining the institute, she has held the following academic appointments:

- Head, Diarrhoea Unit, Microbiology Department: 1996-2001
- Head, Human Virology Laboratory: 2001- 2017
- Head, Microbiology Department: 2017 – To date

She has also served in several committees within the institute as:

- Member, Scientific Seminar Committee: 2000 – 2013
- Member, Housing Committee: 2004 – 2008
- Member, Grant Writing Committee: 2010 – 2016
- Member, Senior Management Committee: 2011 – To Date
- Member and Chair, Scientific Conference Committee: 2012 – 2016
- Member, Institutional Review Board: 2012 – To Date
- Member, Scientific Committee: 2016 – To Date
- Chair, Training Committee: 2004-2010; 2016 - To Date

Dr. Audu has served at the national level in the following capacities:

- Team Member, Development of Guidelines for the Use of Antiretroviral Drugs in Nigeria, Federal Ministry of Health: 2005
- Member, Paediatric HIV and AIDS Technical Working Group – Federal Ministry of Health: 2006 – 2010
- Member, Laboratory Quality Assurance Technical Working Group: 2006
- Member, Technical Advisory Committee for Laboratory and Blood Safety Component of National AIDS/STIs Control Programme: 2006 – 2010
- Member, National Task Team on Anti-Retroviral Therapy: 2010 – 2016
- Member, National Laboratory Quality Assurance Team: 2010 – 2014
- Member, Steering Committee on Trainings under the Consolidated GFATM Round 5, 8 & 9 Grants: 2011
- Quality Auditor for the African Society for Laboratory Medicine (ASLM): 2014 – To Date
- Member, National Task Team on Viral Hepatitis: 2016 – To Date

Dr. Rosemary Audu as the founding head of the Center for Human Virology and Genomics, set up the quality management system in the laboratory and under her leadership the laboratory became the first and only ISO 9001:2008 certified diagnostic laboratory in the country. The laboratory was also enrolled in the Strengthening Laboratory Management Toward Accreditation (SLMTA) program and had a Four Star rating in the WHO AFRO Stepwise Laboratory Quality Improvement Process Towards Accreditation (SLIPTA) program. As a result of her passion and commitment to standards, the Center for Human Virology and Genomics became the first public clinical laboratory to be ISO 15189:2012 accredited in the country and the only center in West Africa, listed as WHO prequalification laboratory for in-vitro diagnostics.

Dr. Audu has attended several trainings on quality management system, she is a SLMTA trainer and an ASLM and ISO 15189:2012 certified assessor involved with training and auditing of laboratories in the country. She set up the first viral load testing facilities in the country for HIV, HCV and HBV as well as the first genotyping facilities for HIV and HCV. She is currently the head of Microbiology Department of the Institute which is comprised of three divisions namely: Center for Human Virology and Genomics, Center for Tuberculosis Research and the Diarrhoea, Immunology and Parasitology Research. She is saddled with the responsibility of

implementing quality management system in her department and also expanding the scope of the accreditation to include the Center for Tuberculosis Research.

Dr Audu is a past Fogarty fellow and a Heymann fellow of IANPHI leadership academy. As a research scientist, she has mentored 16 postgraduate students including 5 PhD candidates within and outside the Institute. She is also a trainer, a post graduate examiner and a reviewer for several national and international journals. She has over 70 articles in national and international peer-reviewed journals and 12 monographs. She has attended 70 national and international conferences with 27 oral presentations.

Dr Rosemary Audu loves listening to music and ministering to children and teenagers. She is happily married to her heartthrob, Mr Ibrahim Solomon Audu and their marriage is blessed with three promising young men: Solomon (Jnr), Steven and Silas.