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TOPIC:

HIV FELLOW CO-TRAVELLERS AND PARTNERS-IN-"CRIME": SUCCOUR FROM MEDICINAL PLANTS AND THE SEA

By

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An Inaugural Lecture Delivered at the University of Lagos Main Auditorium on Wednesday 21st September, 2016

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PREAMBLE

Mr. Vice Chancellor Sir, it gives me immense pleasure to welcome you all to this inaugural lecture. I give honour and adoration to the Almighty God for preserving my life and for making this day a reality. I want to express my profound appreciation to the Vice Chancellor for giving me the opportunity to present this inaugural lecture at this time. This lecture which ought to have been given about seven years ago as a new Professor was delayed because of some exciting development in my research activities. However, I have discovered that there will be no end to new discoveries and dimensions in research endeavours and I may end up getting carried away and not presenting an inaugural at all if I failed to give myself a break. Today's lecture is the 16th inaugural lecture in the 2015/2016 session and the 2nd from the Faculty of Science for the period.

Mr. Vice Chancellor Sir, I am a product of the best three Nigerian Universities, having taken my first degree from

University of Lagos (The University of First Choice and the Nation's Pride) my Masters from O.A.U (Ife) (Great Ife) and my Ph.D. from University of Ibadan. I have also had the opportunity of serving in three other higher institutions of learning namely, Yaba College of Technology (for almost a decade), Federal University of Technology, Minna and Delta State University, Abraka where I was the pioneering Head of the Department of Microbiology between 1993 and 1996. Coining a title for today's inaugural lecture was a bit challenging for me considering the fact that my research efforts cut across basic and applied Microbiology.

INTRODUCTION

What is Microbiology?

Microbiology is the study of microscopic organisms (called microorganisms) and the various chemical reactions they bring about in nature. Microorganisms impact both positively and negatively on human life.

The existence of microbes (microorganisms) was not established until Antony van Leeuwenhoek of Delft Holland in 1674 demonstrated their presence with the aid of simple (one lens) microscope. Leeuwenhoek's microscope was extremely crude by today's standard but by careful manipulation and focusing, he was able to see organisms as small as bacteria from specimens taken from scurf of the root of decayed tooth as well as from rain water. He described major groups of bacteria (spheres, rods and spirals), protozoa, algae, yeast, erythrocytes, and spermatozoa and reported his findings in a series of letters to the Royal Society of London for almost 50years until his death in 1723.

The observation of Leeuwenhoek was confirmed by other workers but progress in understanding the nature and relevance of these tiny organisms was slow. It was not until the 19th century that improved microscopes became available. This led to improved knowledge about details of the cell.

Microbiology as a science did not developed until the latter part of the 19th century. The long delay occurred because in addition to microscopy, certain basic technique for study of microorganisms had to be developed. Also, in the same 19th century, answer to two critical questions had to be proffered which led to the development of these techniques thereby laying the foundation of modern Microbiology. The two questions were:

(i) Do animals and plants as well as other living organisms evolved spontaneously?
(ii) What is the nature of contagious disease?

Spontaneous Generation of Organisms:

The theory of spontaneous generation states that the microbes arise automatically in decomposing matter. Aristotle (384-322 B.C.) emphasised that animals might evolve spontaneously from soil, plants or other unlike animals. The theory of spontaneous generation persisted until Italian physician Franseso Redi (1626-1697) demonstrated that the generation of maggots by decaying meat resulted from the presence of fly eggs and was not spontaneously generated as previously believed.

Louise Pasteur's work on disproving spontaneous generation led to the development of methods for control of the growth of microorganisms. By means of his famous goose-necked flask experiments, Louise Pasteur, a French Chemist-turned-Microbiologist was able to prove that putrefaction or fermentation of food and broth was not a spontaneous process, arising from non-living organism but rather due to living entities contacted from air and the environment. The English physicist John Tyndall (1820-1893) dealt a final blow to spontaneous generation in 1872 by demonstrating that dust did indeed carry germs and that if dust was absent broth remained sterile even if directly exposed to air.

The "Germ" Theory of Disease

In medieval period, most believed that illness or disease was due to causes such as supernatural forces (spiritual attack), poisonous vapour called miasmas and the imbalance between

the four humours thought to be present in the body. These were blood, phlegm, yellow bile and black bile. However, support for the germ theory of disease began to accumulate in the early nineteenth century and Agostino Bacsi (1773-1856) first showed that microorganism could cause disease when he demonstrated in 1835 that silkworm disease was caused by fungal infection.

Indirect evidence that microorganisms were agents of human disease came from the work of English Surgeon Joseph Lister (1827-1912) who developed a system of antiseptic surgery designed to prevent microorganisms from infecting wounds. Instruments were heat sterilised and phenol was used on surgical dressings and at times sprayed over the surgical areas. The first direct demonstration of the role of bacteria in causing disease came from the study of anthrax by German physician Robert Koch (1843-1910) who later developed a set of criteria that provided an experimental framework for the study of infectious microorganisms. He also developed the first methods for the growth of pure cultures of microorganisms.
The golden era of Microbiology was established between 1860 and 1910 because of improvement and new discovery in area of instrumentation and methodology. Moreover, various members of the German school isolated, in addition to the tubercle bacillus, the cholera vibrio, typhoid bacillus, diphtheria, bacillus, pneumococcus, staphylococcus, streptococcus, meningococcus, gonococcus and tetanus bacillus.

The discovery of yet smaller disease causing entities, viruses was made possible when Chamberland (1851-1908), one of Pasteur's associates, constructed a porcelain bacterial filter in 1884 and the first virus pathogen to be studied was the tobacco mosaic disease virus.

The science of Microbiology is highly interdisciplinary embracing Biochemistry, Molecular Biology, Physiology, Genetics, Ecology, Chemistry, Geology, Computer Science and Engineering. Indeed, a microbiologist has been aptly described as a "Jack of all trade and a servant to none".

Microbiology has both basic and applied aspects. The applied branches of Microbiology include the following: Medical Microbiology, Pharmaceutical Microbiology, Aero-Microbiology, Exo-Microbiology, Beverage Microbiology, Food Microbiology, Industrial Microbiology, Environmental Microbiology, Geochemical Microbiology, Soil and Agricultural Microbiology.

Table 1: Some important events in the development of microbiology: 300 years of microbiology

YEAR	SCIENTIST	OBSERVATIONS/DISCOVERY
Fourth Century BC	**Aristotle**	Living things do not need parents, spontaneous generation apparently occurs.
Mid 1500s	**Fracastoro**	"Contagion" passes among individuals, objects and air.
Mid 1600s	**Kircher**	"Microscopic worms" are present in blood of plague victims.
Mid 1600s	**Francisco Redi**	Proposed that fly larvae arise by spontaneous generation.
Late 1600s	**Van Leeuwenhoek**	Invents his microscope and observed that microscopic organisms are present in numerous environments.
Mid 1700s	**John Needham**	Microorganisms in broth arise by spontaneous generation.
Mid 1700s	**Lazzaro Spallanzani**	Heat destroys microorganisms in broth.
Late 1700s	**Edward Jenner**	Recovers from cowpox do not contract smallpox.
Mid 1800s	**John Snow**	Water is involved in disease transmission.
1861	**Louis Pasteur**	Introduced the terms aerobic and anaerobic in describing the growth of yeast at the expense of sugar in the presence or absence of oxygen.
1867	**Louis Pasteur**	Devised the process of destroying bacteria known as pasteurisation.
1876	**Robert Koch**	Koch demonstrated that anthrax is caused by Bacillus anthracis.
1877	**John Tyndall**	Explains fractional sterilisation and clarifies the role of heat resistant factors (spores) in putrefaction.
1879	**Albert Neisser**	Identifies *Neisseria gonorrhoeae*, the pathogen that causes gonorrhoea

1881	**Paul Ehrlich**	Refines the use of the dye methylene blue in bacteriological staining and uses it to stain the tubercule bacillus.
1883	**Robert Koch**	The definite proof of the germ theory of disease.
1884	**Ilya Ilich Metchnikoff**	Demonstrates phagocytosis.
1884	**Hans Christian J. Gram**	Develops a dye system for identifying bacteria [the Gram stain].
1884	**Charles Chamberland**	Develops an unglazed porcelain filter that retains bacteria.
1892	**Dmitri Ivanowski**	Demonstrates filterability of a pathogenic agent.
1894	**Alexandre Yersin**	Isolates *Yersinia (Pasteurella) pestis.*
1903	**William Leishman**	Observes *Leishmania donovani.*
1915	**Frederick Twort**	The first discovery of bacteriophage.
1917	**Felix d'Herrelle**	Describes bacterial viruses and coins the name "bacteriophage."
1924	**AlbertCalmette & Camille Guerin**	Introduce a living non-virulent strain of tuberculosis (BCG) to immunise against the disease.
1928	**Frederick Griffith**	Discovers transformation in bacteria.
1929	**Alexander Fleming**	Discovers Penicillin.
1940	**Howard Florey & Ernest Chain**	Purifies Penicillin.
1944	**Selman Waksman**	Discover streptomycin.
1952	**Alfred Hershey & Mart ha Chase**	Suggest that only DNA is needed for viral replication.
1953	**James Watson & Franc is Crick**	Describes double-helix structure of DNA.
1975	**David Baltimore, Renato Dulbecco, and Howard**	Interaction between tumour viruses and the genetic material of the cell.
1983	**Barre-Sinoussi Robert Gallo?**	Discovery of HIV Virus
1983	**Barbara McClintock**	Discovery of mobile genetic elements.

1993	**Kary Mullis**	Invention of the polymerase chain reaction (PCR).
1997	**Stanley B. Prusiner**	Discovery of prions.
2005	**Barry J. Marshall & J. Robin Warren**	Discovery of the bacterium Helicobacter pylori and its role in gastritis and peptic ulcer disease.

(Adapted from Thomas, 1998)

The Present and Future of Microbiology: (Molecular Biotechnology)

Since the early 1970s, when recombinant DNA technology was first developed, there has been an explosion of knowledge in biological sciences and especially in Microbiology. Microbes are veritable tools in shaping the world of Molecular biotechnology. With the advent of Polymerase Chain Reaction (PCR), chemical DNA synthesis, genomics, proteomics and metabolomics, our understanding of and ability to manipulate the biological world have grown exponentially. Presently, it is not uncommon for researchers to engineer organisms by modifying both the activity and the regulation of existing genes while at the same time introducing new pathways. Today, molecular biotechnology has given us several hundred new therapeutic agents, with many more in the pipeline as well as dozens of transgenic plants.

The use of DNA has become a cornerstone of modern forensic, paternity testing and ancestry determination. Several new recombinant vaccines have been developed with many more in the horizon. The list goes on and on. It has been estimated that worldwide, there are currently several thousand biotechnology companies employing tens of thousands of scientists. When the exciting science being done at universities, government laboratories and research institutes around the world is factored in, the rate of change and discovery in biological sciences is outstanding. I pray that Nigeria is not left out of this modern endeavour.

Mr. Vice-Chancellor Sir, my research work for two and half decades has been focused largely on the following areas of Microbiology:

- HIV co-infection and associated pathogens in immuno compromised
- Bacterial drug resistance and virulence
- Antimicrobial properties of medicinal plants and crude drugs
- Search for Bioactive compounds (mainly antitumour and antimicrobials) from marine Algae and Actinomycetes

In this lecture, I will discuss bacterial and viral organisms causing blood stream infections in immuno-compromised HIV patients and others as co-travellers and "partners-in-crime" of the deadly virus. I will also inundate my listeners with my modest contributions in the use of medicinal plants in combating microbial infections. Finally, I will also give my audience an insight into my discoveries in the exciting world of marine drug research.

HIV Co-infection in immunocompromised

In 1983, scientists (Barre-Sinoussi *et al.*, 1983; Gallo *et al.*, 1983), discovered the virus (HIV-1) and firmly associated it with AIDS in 1984 (Gallo *et al.*, 1984; Montagnier *et al.*, 1984). The virus was at first named human T-cell lymphotropic virus-type III/lymphadenopathy-associated virus (HTLV-III/LAV) by an international scientific committee. This name was later changed to human immunodeficiency virus (HIV). A related virus called HIV-2 was subsequently discovered in West Africa (Clavel *et al.*, 1986).

Epidemiology of HIV

HIV currently afflicts more than 36.9 million people worldwide. Since the start of the epidemic, about 78 million people have become infected with the virus and 39 million people have died of AIDS related illnesses. By the end of 2014, there were about 2 million new HIV infections (UNAIDS, 2014). In Sub-Saharan Africa, there were about 25.8 million people living with HIV in 2014 and 790,000 people died of AIDS related illnesses, while there were an estimated 1.4 million new HIV infections. Women accounted for more than half the total number of people living with HIV in Sub-Saharan Africa, while the region

accounts for 66% of global total of new infections in 2014 (UNAIDS, 2014).

Nigeria, with a national HIV prevalence of 3.4% (approx. 3.3 million living with HIV) ranks second among the countries with the highest burden of HIV in the world, only after South Africa. There were about 220,393 new infections, while 210,031 people died in the country from AIDS related cases by the end of 2014 (UNAIDS, 2014; NACA, 2014). There are wide variations in HIV prevalence within the country's six geo-political zones, with the highest prevalence in the South-South zone (5.5%), while the South-East zone has the lowest prevalence (1.8%). Four states of the federation namely: Rivers (15.2%), Taraba (10.5%), Kaduna (9.2%) and Nassarawa (8.1%) has the highest prevalence, while Ekiti State has the lowest prevalence. HIV prevalence is highest among people aged 33-39 years (4.4%), while those aged 40-44 (2.9%) and 15-19 (2.9%) years has the lowest prevalence. Prevalence is high among female (3.5%) than males (3.3%). Across the country, urban prevalence is higher than rural in all six geopolitical zones. Among the most-at-risk-populations, the brothel-based female sex workers (BBFSW) has the highest prevalence (27.4%), followed by non-brothel based female sex workers (NBBFSW) (21.7%), and men who have sex with men (MSM) (7.2%). MSMs and Injection Drug Users (IDUs) and their partners contribute about 10% and 9% respectively of annual new infections (NACA, 2014).

Bloodstream Infections (BSI): Mr. Vice-Chancellor Sir, *the HIV virus has been aptly described as a typical member of Liverpool football fan club; it does not walk alone* because it always co-exists with other opportunistic pathogens in the blood. Bloodstream infections can be grouped under four types namely: bacteremia, fungemia, viremia, and parasitemia.

Types of Bloodstream Infections:

Bacteremia constitutes a significant public-health problem and presents an important cause of morbidity and mortality in HIV infected patients. Gram-negative organisms, particularly *Salmonella enterica*, rival or exceed Gram-positive organisms

in importance in several published reports on bloodstream infections in both adults and children from African countries (Akpede *et al.*, 1991; Gordon *et al.*, 2001; Ayoola *et al.*, 2003; Archibald *et al.*, 2003). Disseminated infections with Salmonella Typhimurium, S. Enteritidis, S. Arizona, S. Dublin and other non-typhoidal *Salmonella* serotypes have also been recognised early in the HIV epidemic. Adeleye *et al.* (2008a) in a first report on BSI in this environment isolated six non-typhoidal Salmonellae comprising of four Salmonella Typhimurium and two Salmonella Enteritidis from 201 blood samples of HIV positive patients in Lagos (Table 2).

Coagulase-negative Staphylococci (CoNS) which had long been thought to be clinically insignificant when isolated in the diagnostic laboratory have emerged as important opportunistic pathogen in the clinical setting and cause diseases if the external barrier of the skin or mucous membranes have been breached especially in immunocompromised patients.

In my own findings (Adeleye *et al.*, 2010), this organisms accounted for 58% of total bacterial isolates causing blood stream infection in adult HIV patients in Lagos some of whom were on antiretroviral (Table 2).

Table 2: CD4 Counts, Antiretroviral Treatment Status, and Bacteria Isolated from 26 HIV Positive Patients Attending Lagos University Teaching Hospital (Some patients were already on ART). (Adeleye *et al.*, 2010)

Sl. no.	Bacterial isolate	CD4 count cells/µL of blood	ART status	Sex of patients
1	*Staphylococcus scuri*	224	+	F
2	*Staphylococcus cohni urealiticum*	221	+	F
3	*Staphylococcus epidermidis*	191	+	M
4	*Salmonella* Typhimurium	55	-	M
5	*Staphylococcus warneri*	145	+	F
6	*Staphylococcus epidermidis*	288	+	M
7	*Staphylococcus epidermidis*	34	-	M
8	*Staphylococcus xylosus*	62	-	F
9	*Staphylococcus chromogenes*	10	-	F
10	*Staphylococcus cohni cohni*	18	-	F
11	*Staphylococcus epidermidis*	460	+	F
12	*Salmonella* Enteritidis	15	-	F
13	*Salmonella* Enteritidis	146	-	M
14	*Staphylococcus cohni urealiticum*	60	-	F
15	*Staphylococcus epidermidis*	6	-	F
16	*Staphylococcus epidermidis*	604	+	F
17	*Staphylococcus warneri*	46	-	M
18	*Salmonella* Typhimurium	392	+	F
19	*Salmonella* Typhimurium	14	-	M
20	*Staphylococcus epidermidis*	7	-	M
21	*Salmonella* Typhimurium	47	-	M
22	*Escherichia coli*	360	+	F
23	*Pseudomonas flourescence*	228	+	F
24	*Ochrobactrum anthropi*	220	+	F
25	*Moraxella* sp.	110	-	F
26	*Chryseobacterium meningospecticum*	88	-	M

ART=Antiretroviral therapy; F=Female; HIV=Human immunodeficiency virus; M=Male

Similarly, encapsulated bacteria, including *Streptococcus pneumonia* and *Haemophilus influenzae* are two of the most common bacterial pathogens in HIV-infected persons (**Adeleye** *et al.*, 2008b, Reddy *et al.*, 2010; Meremo *et al.*, 2012). *Staphylococcus aureus*, *E. coli*, *Enterococcus* spp., *Brucella* spp. and *Mycobacterium tuberculosis* have also been reported as frequently isolated bloodstream pathogens among HIV patients (Reddy *et al.*, 2010, Crump *et al.*, 2011; Meremo *et al.*, 2012). *Pseudomonas aeruginosa* has also emerged as one of the most common cause of Gram-negative bacteraemia and pneumonia in HIV-infected hospitalised patients and its incidence in AIDS patients appears to be on the rise with many studies demonstrating an annual increase in cases (Hart, 2000; Kohli *et al.*, 2006, Ogunsola *et al.*, 2009). This is in agreement with our own observation (Adeleye *et al.*, 2012). The isolation and positive association of *Klebsiella pneumonia* with bacterial pneumonia has also been reported in

some studies (Kohli *et al.*, 2006; Ogunsola et al., 2009). *Treponema palladium*, a spirochete and etiologic agent of syphilis, also an important biological factor associated with HIV infection (Fleming and Wasserheit, 2002; Caceres *et al.*, 2008; Beyrer *et al.*, 2012).

Viremia is also a common occurrence among HIV patients. Viruses that have been reported in BSIs include: hepatitis B (HBV) and C (HCV) viruses, *Herpes simplex* virus (HSV), Cytomegalovirus (CMV) and Rubella virus (RV). Because of the shared route of transmission, HIV patients are at risk of co-infection with either hepatitis B or C viruses, or both. Similarly, HIV/HSV co-infection is also common among HIV positive population because HSV is sexually transmitted. The association of CMV and rubella viruses with HIV had to do with the immunocompromised state of the HIV patient, which allows these viruses to establish infections as opportunistic pathogens.

Mr. Vice-Chancellor Sir, in order to minimise the effect of antiretroviral therapy on the outcome of our investigation on blood stream infection, I initiated a study profiling both bacterial, viral and parasitic co-infections in 300 Antiretroviral treatment (ART) naïve adult HIV positive patients in Lagos which was partly funded by University of Lagos Central Research Fund CRC/2012/8. The results of the study showed that the prevalence of bacteremia was 11%, HBsAg was 8.33%, anti-HCV antibody was 11.67% and HBsAg/anti-HCV antibody was 0.67% (Table 3). The overall prevalence of hepatitis in the study was 20.67%. Among 80 HIV patients, the prevalence of anti-HSV IgM and IgG was 26.25% and 83.75%, anti-CMV IgM and IgG was 25% and 75%, and anti-RV IgM and IgG was 1.25% and 73.75% respectively. Anti-*Toxo* IgM and IgG were detected in 2.5% and 100% of the patients respectively, while anti-*Treponema* antibody was detected in 20% of the patients.

The prevalence of bacteremia, anti-HCV, anti-HSV IgM, anti-CMV IgM and anti-*Toxo* IgM were higher among female than male patients. In contrast, HBsAg and anti-RV IgM were more

prevalent in male than female patients. The prevalence of anti-*Treponema* antibody was 50% each for both male and female patients. A total of 33 (11%) bacteria were recovered from the blood cultures of the 300 HIV patients. Coagulase-negative staphylococci (CNS) were the predominant isolates, accounting for 18 (54.55%) of the isolates including: *Staphylococcus xylosus* (11), *Staphylococcus warneri* (2), *Staphylococcus cohnii* spp *cohnii* (2), *Staphylococcus epidermidis* (1) and *Staphylococcus capitis* (2), *Staphylococcus aureus* accounted for 8 (24.24%) of the isolates, while 7 Gram negative rods comprising of 4 *Pseudomonas aeruginosa* and *3 Klebsiella pneumoniae* where also isolate. Comparatively, there is little difference in type and status of bacteria causing blood stream infections in both ART experienced and naïve HIV patients in Lagos as CoNS was still the dominant bacteria in both groups. Incidences of BSIs were more common among HIV patients aged between 28-37 years. However, the seroprevalence of HBsAg (36%) and anti-RV IgM (100%) were highest among patients aged 38-47 and 48-57 years respectively.

Table 3: An Overview of Bloodstream Infections among the 300, and 80 HIV Patients Evaluated (All patients have not commenced ART) (Adeleye *et al.* 2012) (Opara *et al.*, 2013) (Olajumoke *et al.* 2014) (Opara *et al.*, 2015)

Infection	No. studied	Total No. positive (%)	No. Positive male (%)	No. Positive female (%)	P value
Bacteremia	300	33 (11)	10 (30.3)	23 (69.7)	< 0.05
HBsAg	300	25(8.33)	19 (76)	6 (24)	< 0.05
Anti-HCV	300	35 (11.67)	14 (40)	21 (60)	< 0.05
HBsAg/anti-HCV	300	2 (0.67)	1 (50)	1 (50)	> 0.05
Anti-HSV IgM	80	21 (26.25)	7 (33.33)	14 (66.67)	> 0.05
Anti-HSV IgG	80	67 (83.75)	24 (35.82)	43 (64.18)	< 0.05
Anti-CMV IgM	80	20 (25)	8 (40)	12 (60)	> 0.05
Anti-CMV IgG	80	60 (75)	21 (35)	39 (65)	> 0.05
Anti-RV IgM	80	1 (1.25)	1 (100)	0 (0)	> 0.05
Anti-RV IgG	80	59 (73.75)	22 (37.29)	37 (62.71)	> 0.05
Anti-Toxo IgM	80	2 (2.5)	0 (0)	2 (100)	> 0.05
Anti-Toxo IgG	80	80 (100)	28 (35)	52 (65)	< 0.05
Anti-Treponema	80	16 (20)	8 (50)	8 (50)	< 0.05

FIRST REPORT OF TORQUE TENO VIRUS (TTV) IN NIGERIA

Mr. Vice Chancellor Sir, I am privileged to report the isolation of Torque teno virus for the first time in Nigeria from the blood stream of HIV positive patients in Lagos. Torque teno virus (TTV), also called Transfusion Transmitted Virus, was first reported in a Japanese patient in 1997 by Nishizawa. It is a single stranded circular DNA of 3.8kb. Other variants discovered are TTMDV (Torque Teno Midi Virus) and TTMV (Torque Teno Mini Virus). They differ in genomic length and genetic identity. It is often found in patients with liver diseases although for the most part, TTV infection is believed to be asymptomatic. Despite that, the link between TTV infection and a given pathology has not been conclusively proven, the hypothesis of relationship between viral load and the immune status of the host was suggested. Moreover, although initially suspected to be transmitted only by blood transfusion the global dispersion of the virus in population and its detection in

various biological samples (plasma, saliva, faeces, etc.) suggest combined modes and in particular the spread by saliva droplets, as well as sexual transmission.

In an ongoing study initiated by me and in collaboration with Prof. Pascal Bessong of University of Venda South Africa, One hundred and thirty blood samples from HIV positive patients and same number from blood donors were screened for TTV and its variants. The result showed that 87(66.9%) HIV blood samples were co-infected with TTV, while 32(24.6%) was recorded for blood donors (Table 4). The PCR result of digested product is shown in plate 1 (Elesinnla *et al.*, 2016). The complete TTV genone has been succefully amplified and cloned in plasmid vector and inserted into a bacterium, *Escherichia coli.* To the best of our knowledge this is the first report of cloning of TTV genone into a vector in Africa.

Table 4: Frequency of TTV in Different Population (Elesinla *et al.*, 2016)

	HIV Positive	Blood Donors
Total Number of Samples	130	130
Number Positive	87(66.9%)	32(24.6%)
Number Negative	43(33.1%)	98(75.4)

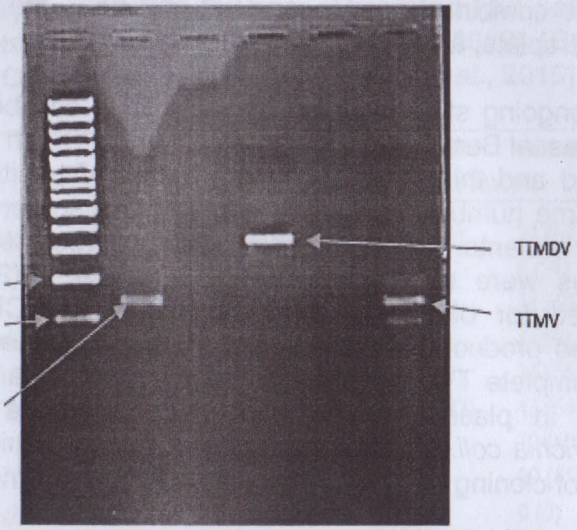


Plate 1: Agarose gel result for TTV, TTMV AND TTMDV (Elesinnla *et al.*, 2016)

Bacteria as Agents of Diseases and their Antibiotic Resistance

As soon as the germ theory of disease was established, there were frantic efforts worldwide to isolate and identify the causative agents of various diseases. Notable researchers like Robert Koch (1843-1910) were at the fore-front of these pioneering efforts. Among the bacterial agents discovered were *Salmonella*, *Shigella*, *Escherichia coli*, *Campylobacter* spp., and *Aeromonas*, which are causative agents of diarrhoea. Infectious enteric diseases including acute diarrhoea are of great public health importance in developed and developing countries. Antibiotics have been at the fore-front of treatment regimen in combating infectious diseases including diarrhoeal diseases among farm animals and humans. This has led to the emergence of multiple drug resistance.

Mr. Vice Chancellor Sir, between 1986 and 1989 I investigated 1,000 diarrhoeic stool samples from three general government

hospitals in Ibadan (namely The University of Ibadan College hospital, State hospitals Adeoyo and Ring Road) as well as Veterinary Farm at the University of Ibadan. The stool samples were cultured on MacConkey, Deoxycholate citrate agar plates and selenite F broth and incubated at 37°C for 24 hours. Non-lactose fermenting (NLF) colonies resembling *Salmonella* and *Shigella* were purified on fresh MacConkey agar plates and transferred to KIA (Kliger Iron Agar) slants and confirmed to be *Salmonella* and *Shigella* biochemically and serologically using commercially prepare antisera by Well-come Nigeria Limited. Thereafter, antibiotic susceptibility testing was performed according to "Kirby and Bauer" methods (1966) using antibiotic discs (Oxoid) with the following drug contents: Cotrimoxazole 25µg, Colistin Sulphate (50µg) Nitrofurantoin (200 µg) Streptomycin (10 µg), Chloramphenicol (10 µg) Tetracycline (10 µg), Ampicillin (10 µg) and Nalidixic acid (30 µg) *Escherichia coli* NCIC 10418 was used as control.

The minimum inhibitory concentration (MIC) for Ampicilin, Streptomycin, Tetracycline and Chloramphenicol was determined for each of resistant strain according to Errison and Sherris (1971) method. Plasmid DNA was isolated by the technique of Birnboin and Dolly (1979). It was detected by electrophoresis in 0.8% agarose stab gels in Tris-borate buffer. Gels were later stained with 0.5% ethidium bromide and photographed with UV light illumination. The molecular size of the plasmid DNA was estimated by comparison with plasmid of known size from *E. coli* strain V517 (**Adeleye**, 1990).

Overall, fifty three bacterial isolates comprising of thirty *Shigella* spp. (23 *S. flexneri*, 4 *S. dysenteriae* and 3*S. boydii*) and twenty three *Salmonella* spp. (8S. Typhi and 15 non-typhoid strains) were identified in both animals and human samples. This study established the possibility of cross-infection from both sources. Antibiogram showed that all were sensitive to Nalidixic acid. The *Shigella* strains were highly resistant to Colistin Sulphate, Streptomycin and Teteracycline while all the non-typhoid *Salmonella* were resistant to Colistin sulphate (Table 5).

Table 5: Number of Percentage of *Salmonella* and *Shigella* Strains Resistant to Antibiotics (Adeleye and Adetosoye, 1993 a,b)

Drugs (15)*	*S. typhi* (8)* *Shigella* spp. (30)*	*Salmonella* spp.	
Co-trimoxazole	3 (37.5%)	6 (40.0%)	24 (80.0%)
Colistic sulphate	7 (87.5%)	15(100.0%)	28 (93.0%)
Nitrofuratoin	2 (25.0%)	10(66.6%)	18 (93.0%)
Streptomycin	7 (87.5%)	9 (60.0%)	28 (93.0%)
Chloramphenicol	2 (25.0%)	4 (27.0%)	21 (70.0%)
Tetracycline	4 (50.0%)	7 (47.0%)	27 (90.0%)
Ampicillin	3 (37.5%)	10 (66.6%)	21 (70.0%)
Nalidix acid	0 (0.0%)	0 (0.0%)	0 (0.0%)

*No. of strains isolated and tested.

On the whole, 11 resistance patterns were recognised and none of the isolates was sensitive to all the antibiotics tested (Table 6). Thirty two of the fifty three isolates were found to harbour one or more plasmids whose molecular weight ranged between 1.5 and 55.5 kb. Some strains harboured as many as ten plasmids. Plasmid profile analysis distinguished more strains than did antimicrobial susceptibility patterns indicating presence of a large number of *Salmonella* and *Shigella* isolates in Ibadan environment. It also suggests a multiple source of infection with Ibadan city (**Adeleye** and Adetosoye 1993a).

Table 6: Antibiotic Resistance Patterns and Number of Different Plasmid Profile in *Salmonella* and *Shigella* Strains (Adeleye and Adetosoye, 1993a)

Resistance pattern							*Salmonella*	*Shigella*	No. of different plasmid profile(per strain)
Sxt	Ct	Fd	Am	S	C	Te	1	10	6(1*;5+)
Sxt	Ct	Fd	Am	S	Te		2	3	3(1;2)
Sxt	Ct	Fd	S	C	Te		1	1	1(1;0)
Sxt	Ct	Am	S	Te	C		_	4	3(0;3)
Sxt	Ct	S	Te	C			_	4	3(0;3)
Ct	Fd	Am	S	Te			_	7	6(0;6)
Sxt	Ct	Am	S	Te			6	_	5(5;0)
Ct	Fd	Am	S				2	_	0(0;0)
Ct	Fd	S					4	_	1(1;0)
Ct	S						4	_	3(3;0)
	Ct						3	1	1(0;1)

Sxt-Cotrimoxazole; Ct-Colistin sulphate; Fd-Nitrofurantoin; S-Streptomycin; C-Chloramphenicol; Te-Tetracycline; Am-Ampicillin
**Salmonella*
+Shigella

I also carried out conjugation experiments to investigate whether the observed resistance patterns were transferable. Selected multiple resistant strains were used as donors and *E. coli* K12 525 Nal+ was used as sensitive recipient. Single colonies of donor and recipient strains were cultured overnight in Brain heart Infusion broth (BHF oxoid). One drop each was inoculated unto fresh 5ml BHF broth and incubated at 37°C for 3.5 hours with gentle shaking. One ml of the donor and 2.5ml of recipient cultures were mixed and conjugated for 18 hours at 37°C without shaking. Transconjugation were selected on MacConkey agar supplemented with Nalixidic acid per ml and one of the following drugs: Ampicilin (Ap), Streptomycin (Sm), Teteracycline (Tc) and Chloramphenicol (Cm) at concentration of 25 µg ml. After incubation at 37°C for 27 hours, plates were inspected for colonies of resistant *E. coli*. Five to ten colonies were picked and purified on fresh MacConkey agar plates, confirmed to be *E. coli* and tested for resistance patterns (Walton, 1966).

All the *Salmonella* spp. and *Shigella* spp. screened conjugatively transferred single or multiple resistance to the *E. coli* recipient (Table 7). The auto transferability of resistance (R) plasmid observed has very grave implications. It means that antibiotic resistance in these bacterial pathogens is plasmid borne and can be transferred promiscuously to other bacterial isolates in the intestine thereby leading to epidemic spread of drug resistance in the environment. Thus, there is need for judicious use of antibiotics in order to prevent the spread of multi-drug resistance (**Adeleye**, 1992a).

Table 7: Incidence of Conjugally Transmissible drug Resistance among Strains of *Salmonella, Shigella* and *E. coli* (Adeleye, 1992a)

Resistance Patterns	Total No. Tested	Biotype/Serotypes of Tested Strains	No. Exhibiting Transmissible Resistance	Resistance Phenotypes of Transconjugation
			Salmonella	
Ap Sm Cm Tc	2	*S. typhi (2)*	2	Ap Sm Tc (1) Ap Sm Cm (1)
Ap Sm Tc	2	*S. typhi (1) Salmonella spp (2)*	2	Ap Tc (2)
Ap Sm Cm	6	*S. typhi (5) Salmonella spp (1)*	6	Ap Sm Cm (3), Ap Sm (1)
Ap Cm	2	*Salmonella spp (2)*	2	Ap Cm (2)
Ap Tc	1	*S. typhi*	1	Ap Cm (1), Ap (1) Ap Tc (1)
		Shigella		
		S. flexneri (18)		
Ap Sm Cm Tc	23	*S. dysenteriae (2) S. boydii (1)*	23	Ap Sm Cm Tc (2) Ap Tc (5) Ap Cm (2) Ap Sm (6) Ap (8)
	3	*S. dysenteriae (2) S. boydii*	3	
Ap Sm Tc	2	*S. flexneri*	2	Ap Sm Tc (1), Ap Tc (1)
			E. coli	
Ap Sm Cm	5	055(2) 026(2) 0124(1)	4	Ap (1) Ap Cm (2)
Ap Sm Cm Tc	1	044(1)	1	Ap Sm Cm Tc (1)
	10	055(5) 026 (2)	7	Ap Sm Cm (1) Ap Cm Tc (2)
Ap Sm Cm		0142(2)		Ap Sm Cm (1)
Ap Sm Tc	3	0125(1) 0111(2) 055(1)	3	Ap Cm Tc (4), Ap Cm (3)
Ap Sm Tc	6	0128(3) 055(2) 0111(1)	5	Ap Sm Tc (3)
Ap Tc				Ap Tc (2), Ap (3)

Ap = Ampicillin; Sm = Streptomycin,
Cm = Chloramphenicol; Tc = Tetracycline

Surveillance of antimicrobial resistance is indispensable for empirical treatment of infections and preventing the spread of antimicrobial resistant organisms.

The antibiotic susceptibility of aerobic bacterial pathogens associated with wound infections was also investigated.

Bacterial aetiology of non-surgical wounds was investigated using standard procedures. One hundred swab samples grew *Staphylococcus aureus* (98), *Staph epidermidis* (55) *Escherichia coli* (36), *Pseudumonas aeruginosa* (28), *Klebsiella pneumonia* (23) *Enterococcus* spp. (15) mainly Ciprofloxacin, vancomycin and rifampicin were the most active antibiotics against methicillin resistant *S.aureus* (MRSA). Multidrug resistance (MDR) was encountered in 72 of the isolated organisms (**Adeleye** and Ogunwale, 2004).

In proactive manner, the antibiotic susceptibility of potentially pathogenic halophytic vibrio was also investigated. Forty four potentially pathogenic halophilic vibrio species isolated from sea foods in Lagos were screened. Antimicrobial susceptibility tests showed that all the isolates (100%) were resistant to Amoxicillin, Augumentin, Chloramphenicol and Nitrofurantoin. Multiple resistance to Gentamycine, Nitrofurantoin, Tetracycline, Augumentine, Chloramphenicol, Amoxycilin, Ofloxacin, Cotrimoxazole, Ceftriazone and Ciprofloxacin, was also observed. Indeed resistance to all the antibiotics assayed occurred in 8 (18%) of the total isolates. Thus, infection caused by Vibrio spp. contaminating sea foods in this environment will be difficult to treat because of their high antibiotic resistance nature (**Adeleye** *et al.*, 2008c).

Tuberculosis (TB) continues to be a major public health threat, with an estimated 8.7 million new cases and 1.4 million deaths (WHO, 2012). WHO estimated that in 2008, between 390,000 and 510,000 people developed Multi Drug Resistant Tuberculosis (MDR-TB) worldwide with 69,000 occurring in Africa and 11,000 in Nigeria.

Mr. Vice Chancellor Sir, in an ongoing collaborative research (with NIMR) to identify the phylogenetic diversity of Mycobacterium tuberculosis complex and evaluate the mutational hotspots conferring drug resistance, the use of a novel high-through-put tuberculosis-spoligo-rifampicin-isoniazid-typing (TB-SPRINT) was employed. A number of observation come to the fore regarding the multiple drug

resistance status (MRD) of the TB isolates (Kunle-Ope *et al.* 2015a).

Out of the 250 TB isolates evaluated to four anti-TB drugs (Streptomycin, Isonazid, Rifampicin and Ethanbutol) 110 (44%) were resistant to Streptomycin, 9 (3.6%) to Isonazide, 20 (8%) to Refampicin, 8 (3.2%) were resistant to 2 or 3 anti-TB drugs while 69 (27.6%) were resistant to both Isonazid + Rifampicin (MDR-TB).

The latter (69 MDR-TB) were further evaluated for susceptibility to four second line anti-TB drugs (Ofloxacin, Capreomycin, Kanamycin and Ethionamide). 4 (5.8%) were resistant to Ofloxacin and classified as Pre-Extensively Drug Resistant Tuberculosis (Pre XDR-TB), 3 (4.35%) were resistant to both Capreomycin and Ethionamide (Pre XDR-TB) while 14 (20.29%) were resistant to Ethionamide. In a related study, same authors encountered MDR-TB in more than half of previously treated TB cases while 11.8% was recorded in new cases (Kunle-Ope *et al.* 2015b). Earlier, **Adeleye** *et al.*, (2008b) investigated the prevalence and Cotrimoxazole resistance of *Streptococcus pneumoniae* isolated from sputum of 100 HIV positive patients attending the Nigerian Institute of Medical Research clinic using standard microbiological methods. Eleven of the sputum specimen grew *S. pneumoniae*. Antimicrobial susceptibility tests showed that all the isolates were resistant to Cotrimoxazole. It was advocated that continuous surveillance of the bacterium (*S. pneumoniae*) in sputum samples of HIV positive subjects was necessary in order to regulate treatment regimen, in view of the fact that Cotrimoxazole was the drug recommended by WHO for respiratory infections in HIV patients. Some of my other contributions on antibiotic resistance are:

1. **ADELEYE**, I. A. and OGBECHI, I. (2005).
2. **ADELEYE**, ADELEYE, CAROLINE IDIKA AND ASOWATA OSARETIN (2008).
3. **ADELEYE**, 4. ANIFOWOSHE, A., OPARA, M., and **ADELEYE**, I. A. (2012).

Mr. Vice Chancellor Sir, There is paucity of information on the molecular mechanisms of bacterial antibiotic resistance in many parts of Africa, including Nigeria. Available knowledge in this field indicates that drug resistance can involve alteration or mutation in the DNA of the bacteria. Multidrug resistance can also be conferred through horizontal transfer of resistance genes through mobile genetic elements (plasmids, transposons, gene cassettes and integron). The first mechanism was demonstrated by me in the study of non-typhoidal *Salmonella* isolates from HIV patients and it accounted for the resistance to Ampicillin, Chloramphenicol, Co-trimoxazole and Tetracyclines (**Adeleye** *et al.*, 2008a). Similarly we, **Adeleye** *et al.*, (2011a), observed non-plasmid mediated MDR in vibrio and Aeromonas spp. isolated from sea foods in Lagos.

Plasmid mediated resistance involves the transfer of part or all of the resistance gene promiscuously among isolates as demonstrated in my earlier mentioned conjugation experiment (**Adeleye**, 1992a). Among the families of antibiotics frequently prescribed is the β-lactam. This group includes penicillins, Cephalosporins, Carbepenems and Monobactams. All of them possess the β-lactam ring which is part of the core structure.

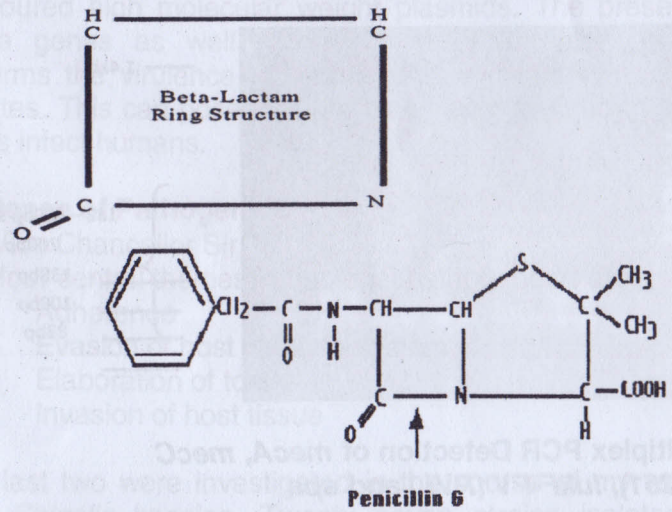


Figure 1: β-lactam Ring

However, many microorganisms develop resistance to these groups of antibiotics by expressing one of the many β-lactam genes which are borne either in the chromosome or on many of the plasmid. More than 1,000 different β-lactamase enzymes have been documented in various species of bacteria (Ehman *et al.*, 2012). The first plasmid mediated β-lactamase in Gram negative, TEM-1 was described in the early 1960s (Data and Kontomichalou, 1965). In our recent study we observed low carriage of the gene (TEM-1) coding for this enzyme in 245 MDR enteric isolates from veterinary sources in Lagos (Ogwu *et al.*, 2015) (**Adeleye** *et al.*, 2016a). The observed high prevalence of MDR in the isolates may be due to the presence of other types of β-lactamase encoding genes including SHV, OXa, CTX, KPC, BlaOXA.

In a related study, Adeleye *et al.*, (2016b) determined the presence of genes coding for methicillin resistance (mec A, mec C), vancomycin resistance (van A clusters), Panthon – Valentine- Leucocidin (PVL) toxin as well as Spa (staphylococcal protein A) in *Staphylococcus aureus* strains from veterinary animals in Lagos. Eleven of 16 isolates displayed the presence Spa gene (in some cases two to three amplifications of the gene) (Plate 2).

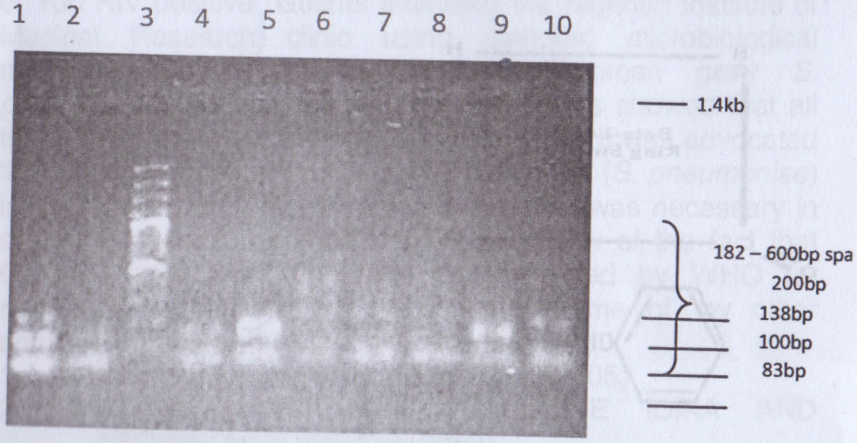


Plate 2: Multiplex PCR Detection of *mecA*, *mecC* (*mecALGA251*), *lukF-PV (PVL)* and *spa*

Lane 1: Isolate with lab code S40with *spa 220bp,mecC and pvl*amplification)
Lane 2: Isolates with lab code G2 with spa and pvl amplification only
Lanes 3: 1.4kbp ladder.
Lane 4: Isolate with lab code S29 (*spa* of 182bp and pvlamplification)
Lane 5: Isolate with lab code S31 (*spa* of 182bp *and pvl*amplification)
Lane 6: Isolate with lab code S16 (two spa variant and pvlamplification only)
Lane 7: Isolate with lab code C16 (two spa variant and pvl amplification only)
Lane 8: Isolate with lab code C44 (spa and pvl amplification only)
Lane 9: Isolate with lab code C41 (spa and pvl amplification only)
Lane10: control MSSA. (spa and pvlamplification only)

One isolate displayed band for Mec C and eleven isolates demonstrated the presence pvl toxin gene but none of the isolates had van A gene clusters. Plasmid characterisation of the multi antibiotic resistant (MAR) isolates showed that they harboured high molecular weight plasmids. The presence of these genes as well as high molecular weight plasmids confirms the virulence and molecular resistant nature of the isolates. This can pose a treatment challenge if the organisms cross infect humans.

Virulence of Pathogenic Bacteria

Mr. Vice Chancellor Sir,
The four central themes in bacterial pathogenesis are:
(i) Adherence
(ii) Evasion of host immune system
(iii) Elaboration of toxins
(iv) Invasion of host tissue

The last two were investigated in the course of my research with *Shigella* species. Twenty seven strains isolated from diarrhoeic piglets and humans were investigated for the

production of heat labile and heat stable eneterotoxin using the Rabbit Illeal loop Ligation Test (RILT). Ten New Zealand rabbits weighing between 1.2 and 2.1 kg were used. They were deprived of food but were allowed to drink water *ad lib*, 24 hours prior to surgery (**Adeleye** and Adetosoye, 1998).

Surgical Procedure: The rabbit was anaesthesised for surgery by injecting pentabarbitol through the ear vein to effect. The technique of preparing intestinal segments was similar to that described by Moon and Whipp (1970). A little incision of about 8 cm long was made on the linea alba. The small intestine was carefully brought out and the duodenum was identified. About 10 ml of warm NaCl solution was injected into the intestine. Ligatures were made with catgut carefully so as to avoid disruption of mesenteric capillaries. The intestine was ligated to form series of approximate 10 cm segments interrupted by 5 cm sutures. An average of five ligated ileal loops were made in each rabbit.

Assay for Heat Labile (LT) and Heat Stable (ST) Enterotoxin: Twenty four hour old broth culture of *Shigella* isolate was centrifuged at 18,000 rpm for 30 min at 10°C. The filtrate was divided into two portions. One portion was heated at 65 °C for 30 min (Heat Stable-ST toxin) while the other portion was left unheated (Heat Labile-LT toxin). Using a 10 ml syringe, 1ml of the untreated (Heat Liabile, LT) toxin was injected into each of the alternate 10 cm loops. The interrupted 5 cm loops were kept uninoculated. The first and last ligated intestinal loops contained the control (LT) toxins i.e. *V. cholera* 1939 and ordinary nutrient broth respectively. The intestine was later replaced into the abdominal cavity and the incision closed in two layers by continuous suture. The rabbits were kept in a warm place for 18 hours. Later it was killed by injecting pentabarbitol. The incision was opened and the intestine brought out. The volume of fluid in each ligated loop was measured in milliliters using a graduated syringe.

The length of the loop was measured in centimetres with a ruler. A ratio of $\frac{Volume}{Length}$ was determined and values of 0.8 and above was regarded as positive LT (Adetosoye *et al.*, 1984).

For the heat stable (ST) enterotoxin assay a similar process described above was carried out with fresh rabbits but 1 ml of preheated enterotoxin (held at 65°C for 30mins) was used. Introduction of enterotoxins of *Shigella* into the lumen of the ligated segments of small intestine in living rabbits resulted in an accumulation of exudates to a varying degree causing dramatic dilation of the segments in six of the twenty seven rabbits assayed for heat labile enterotoxin. These were 4 *S. flexneri,* 1 *S. dysenteriae* and 1 *S. boydii* (Table 8). None of the heat stable toxins produced fluid accumulation thereby indicating that heat labile enterotoxin is one of the virulence factors produced by *Shigella* spp. in its pathogenicity (Table 8) (**Adeleye** and Adetosoye, 1998). *This is a proof of concept: a smoking gun.*

Table 8: Enterotoxingenic Activity of Crude Culture Enterotoxin Filtrate Preparations of *Shigella* spp. in Rabbit Ligated Ileal Loop: (Heat labile enterotoxin) (Adeleye and Adetosoye, 1998)

Strain Number	Loop Number	Identification	$\frac{V}{L}$	Enterotoxin Production (+ve Control)
+ 0g. 19339	1	V. cholera	1.1	
Y002	3	*S. flexneri* 1	0.5	–
Y003	4	*S. flexneri* 6	1.01	+ve
Y004	22	*S. flexneri* 2	0.4	–
Y005	31	*S. dysenteriae* 1	0.4	–
Y006	17	*S. boydii*	0.5	–
Y016	5	*S. flexneri* 1	0.7	–
Y017	15	*S. flexneri* 2	0.4	–
Y018	28	*S. flexneri* 3	0.6	–
Y019	9	*S. flexneri* 2	0.8	+ve
Y020	7	*S. flexneri* 6	0.2	–
Y021	32	*S. boydii*	0.4	–
Y023	10	*S. flexneri* 6	0.4	–
Y024	14	*S. flexneri* 5	0.4	–
Y026	13	*S. dysenteriae* 1	0.2	–
Y027	8	*S. dysenteriae*	0.5	–
Y029	6	*S. dysenteriae* 1	0.8	+ve
Y030	16	*S. boydii*	1.1	+ve
Y032	18	*S. flexneri* 2	0.4	–
Y033	26	*S. flexneri* 2	0.8	+ve
Y034	33	*S. flexneri* 2	0.4	–
Y037	35	*S. flexneri* 4	0.2	–
Y038	36	*S. dysenteriae* 1	0.2	–

Y044	19	*S. flexneri* 1	0.2	–
Y045	29	*S. flexneri* 6	0.6	–
Y046	20	*S. flexneri* 2	0.3	–
Y048	27	*S. flexneri* 4	0.3	–
Y049	30	*S. flexneri* 6	1.0	+ve
-B	31	Broth	0.2	–

Sereny Test of Invasiveness

Using another animal model, we investigated the invasiveness of 30 *Shigella* isolates from darrhoeic human and piglets. The sereny test (Sereny, 1957) was employed. This procedure is used for screening the ability of *Shigella* to penetrate epithelial cells and cause ulcerate lesions. An essential feature of this reaction is the penetration of corneal cells by virulent bacteria resulting in the ulceration of the corneal leading to purulent keratoconjuctivitis. Twelve of the isolates (40%) were invasive and caused purulent keratoconjuctivitis in the experimental guinea pigs eyes 24-96 after inoculation of the purified bacterial suspension into the eyes of the guinea pig (Table 9). The experiment confirmed the ability of the *Shigella* isolate to cause infection by invasive process (Adeleye and Adetosoye, 1992b).

Table 9: Invasiveness (by Sereny test) of *Shigella* spp. Isolated from Diarrhoeic Humans (Adeleye and Adetosoye, 1992b)

		RESULT AFTER			
Isolate No	Identification	24hr	48hr	72hr	96hr
Y001	*S. flexneri* 3	-	+	+++	+++
Y002	*S. flexneri* 1	-	-	-	-
Y003	*S. flexneri* 6	+	++	+++	+++
Y004	*S. flexneri* 2	-	-	-	-
Y005	*S. dysenteriae* 1	-	+	++	+++
Y006	*S. boydii*	-	-	-	-
Y007	*S. boydii*	-	+	++	+++
Y008	*S. dysenteriae* 1	-	-	-	-
Y009	*S. boydii*	-	-	-	-
Y010	*S. dysenteriae* 1	-	-	-	-
Y011	*S. flexneri* 2	-	-	-	-
Y012	*S. flexneri* 2	-	-	-	-
Y013	*S. flexneri* 4	-	-	-	-
Y014	*S. flexneri* 6	-	-	-	-
Y015	*S. flexneri* 3	-	+	++	+++
Y016	*S. flexneri* 1	-	+	++	+++
Y017	*S. flexneri* 6	-	-	-	-
Y018	*S. flexneri* 2	-	-	-	-
Y019	*S. flexneri* 6	-	-	-	-
Y020	*S. flexneri* 6	-	+	++	+++
Y021	*S. flexneri* 2	-	+	++	+++
Y022	*S. flexneri* 2	+	+	++	+++

Y023	*S. flexneri* 1	-	-	-	-
Y024	*S. flexneri* 6	-	-	-	-
Y025	*S. flexneri*	-	-	-	-
Y026	*S. flexneri* 1	-	+	++	+++
Y027	*S. flexneri* 2	-	-	-	-
Y028	*S. flexneri* 3	-	+	++	+++
Y029	*S. flexneri* 3	-	-	-	-
Y030	*S. dysenteriae* 1	+	+	+	++

KEY: + = Positive Sereny test i.e production of muco-purulent discharge/reddening of the eyes
-= Negative Sereny test

In a related study, we investigated the pathogenicity of *Vibro* spp. contaminating sea foods in Lagos (**Adeleye** *et al.*, 2010a). Twenty-five sea foods samples (shrimps, craps and cuttle fish) collected from seven fishing companies and some local fishermen were cultured for the presence of *Vibrio* spp. a total of 44 Vibrios belonging to five different species were isolated. The most predominant spp. were *V.alginolyticus* (31.8%) followed by *V. harveyi* (27.3%), *V. mimicus* (22.7%), *V.parahaemelyticus* (11.4%) and *V. cholerae* (6.8%).

Table 10: Occurrence of Vibrio Species in the Seafood Samples from Seven Fishing Companies and Catches from Local Fisherman at Agboyi-Odo and Oworonsoki in Lagos (Adeleye *et al.* 2010a)

	NUMBER OF ISOLATES									
ISOLATES	C1	C2	C3	C4	C5	C6	C7	A	O	TOTAL
Vibrio alginolyticus	2	0	3	1	1	1	1	3	2	14(31.8%)
Vibrio parahaemolyticus	1	3	0	0	0	1	0	0	0	5(11.4%)
Vibrio mimicus	0	2	1	1	0	1	1	3	1	10(22.7%)
Vibrio harveyi	2	1	2	0	2	1	1	2	1	12(27.3%)
Vibrio cholerae	0	0	0	0	0	0	0	1	2	3(6.8%)
TOTAL	5	6	6	2	3	4	3	9	6	44(100%)

- C=Company: A=Agboyi-Odo: O=Oworonsoki

We also investigated the pathogencity of selected strains using tests for haemolysis and enterotoxin production.

Haemolysis was determined by subculturing on fresh blood agar plates and observing lyses of blood cells after 24 hr incubation at 37°C. Enterotoxin production (which is assessment of virulence) was determined using the infant mouse and suckling mouse assays. (Merson *et al.*, 1980, Kothary and Richardson, 1987) Intestinal fluid accumulation was also determined using the method of Baselski *et al.* (1977) All the strains (100%) of *V. parahaemolyticus, V. mimicus* and *V. cholera* lysed human red blood cells while 5(30%) of *V. alginolyticus* and 6(50%) of *V. harveyi* were also positive for haemolysin production. None of the isolates (0%) elicited fluid accumulation in the experiment mice intestines. However, histological sections showed that *V. parahaemolyticus V.cholerae* and 2 strains of *V.mimicus*, 1 strain each of *V. alginolyticus* and *V. harveyi* caused erosion of epithelial linings of the intestine of experimental mice.

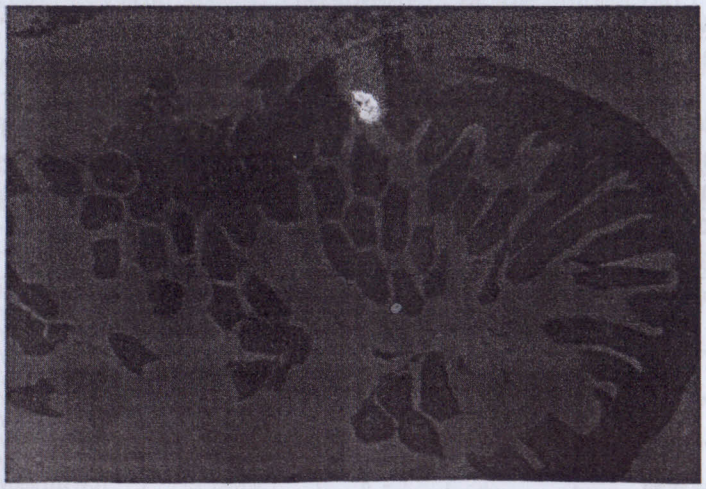


Plate 3: Histological Section of the Intestine of an Infant Mouse Fed with *Vibrio parahaemolyticus* (VP1) (Adeleye *et al.*, 2010a).

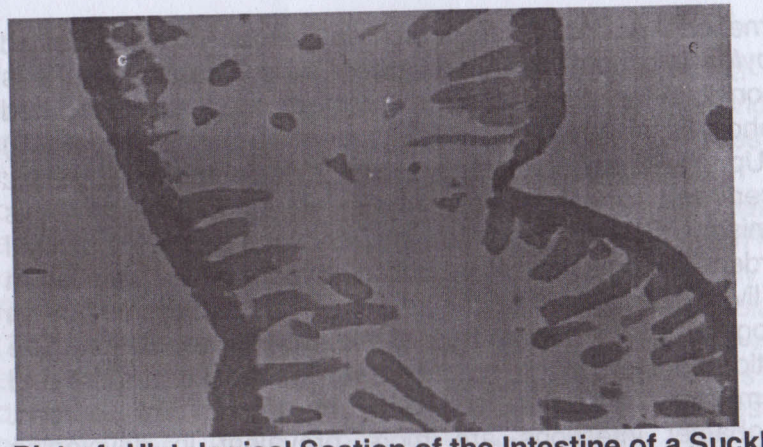


Plate 4: Histological Section of the Intestine of a Suckling Mouse Fed with *Vibrio cholerae* (VC1) (Adeleye *et al.*, 2010a).

This indicates that the organism can cause infections in humans by invasion of the epithelial linings of susceptible hosts and not by production of enterotoxin (**Adeleye** *et al.*, 2010).

Mr. Vice Chancellor Sir, with the advent of modern assay procedures involving the use of biotechnology, investigation of virulence factors in bacterial organism have taken new dimension. Animals are no longer subjected to the trauma of surgery or eye inoculations. Besides, the animal right advocates have campaigned vigorously against the use of animal models in many experiments that bring harm to the animals. Instead, Genes responsible for virulence in bacterial organisms and their expression are directly sought for using molecular methods in-vitro.

Helicobacter pylori vac A and cag A Virulence Genes and their Protein Expression

Helicobacter pylori (HP) is a Gram-negative spiral or helical shaped bacillus that colonises the gastric epithelial surface and can withstand the stomach's acidic environment by microaerophylic growth capability and high urease activity (IARC Working Group 1994, Moss and Sood, 1997). The

International Agency for Research on Cancer (IARC) classified *H. pylori* infection in humans under type I carcinogen. It is responsible for disease state such as mucosa associated lymphoid tissue lymphoma (MALT), peptic ulcer disease (PUD) etc. Up to 95% of duodenal ulcer and 75% gastric ulcer, gastric cancer and colorectal carcinoma, is attributable to this organism. Other diseases include non-gastro intestinal disorders like myocardial infarction, hyperemesis gravidarum and liver cirrhosis. It is one of the most successful human pathogens infecting over half of the world's population. The infection begins early in childhood via the fecal oral route and transmission appears to be from person to person. Helicobacter infections are more prevalent in developing countries due to poor sanitation, overcrowded living conditions and lack of clean water supplies (Graham, 1989).

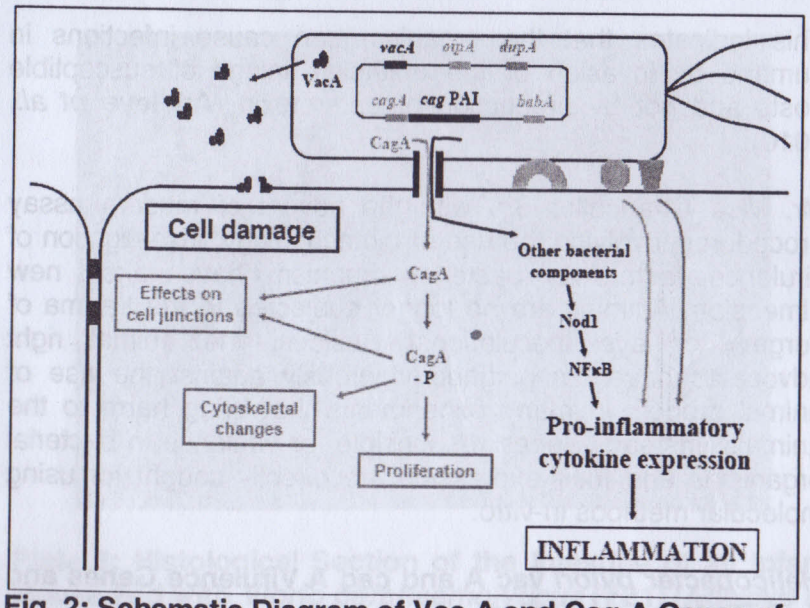


Fig. 2: Schematic Diagram of Vac A and Cag A Genes of *Helicobacter Pylori*

The vaculating cytotoxin and cytotoxin associated protein, encoded *Vac* A and *Cag* A (Fig. 2) respectively are important virulence determinant of *Helicobacter pylori.*

In a collaboratory study, we investigated one hundred and twenty dyspeptic patients receiving upper diagnostic endoscopy in three teaching hospitals in South Western Nigeria (Seriki *et al.*, 2015). Paired samples (antrum and corpus) of gastric biopsies were collected from each patient and PCR (Polymerase Chain Reaction) for *H. pylori* 16SrRNA, *cag* A, *vac* A and Western blotting were carried out. By the PCR, the *cag* A and *vac* A gene were detectable in all isolates (240/240) and also all the isolates (240/240) had the vacA alleles of s1m1 while none of the isolates (0/240) were s1m2 or s2m2. *Vac* A and *cag* A protein expression by western blotting was 97.5% (234/240) and 99.5% (239/240) respectively (Fig 3, Table 11, Plate 5). Our H. pylori isolates were *cag* A, *vac* A (s1m1) positive and these genes are associated with the occurrence of peptic ulceration and chronic gastritis.

However, endoscopic diagnosis indicated normal mucosal architecture in most of the patients samplex!

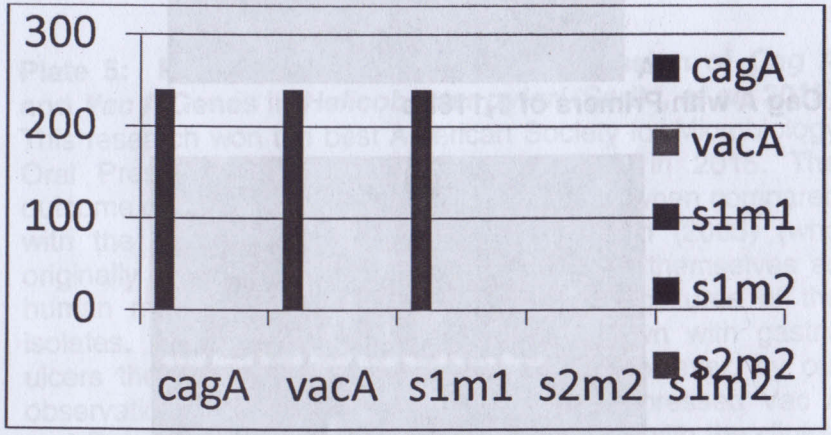
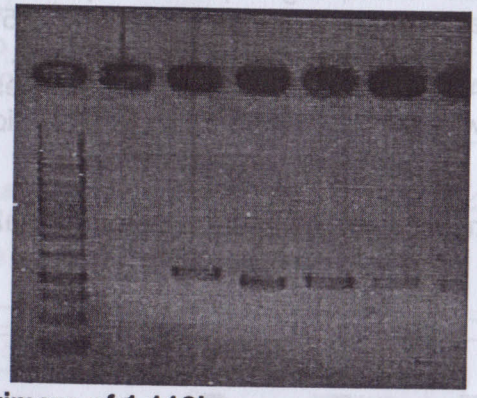


Fig. 3: Virulent Genes of *Helicobacter pylori* (Seriki, *et al.*, 2015)

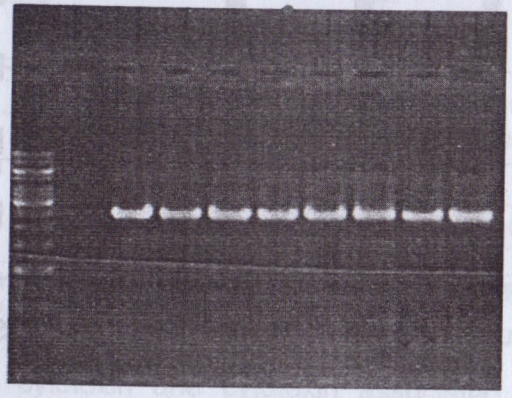
Table 11: Protein Expression of *Cag A* and *Vac A* Genes by Western Blotting (Seriki, *et al.*, 2015)

	Cag A Protein Antrum	Cag A Protein Corpus	Vac A Protein Antrum	Vac A Protein Corpus
Positive	120	119	117	117
Negative	0	1	3	3
Total	120	120	120	120



A

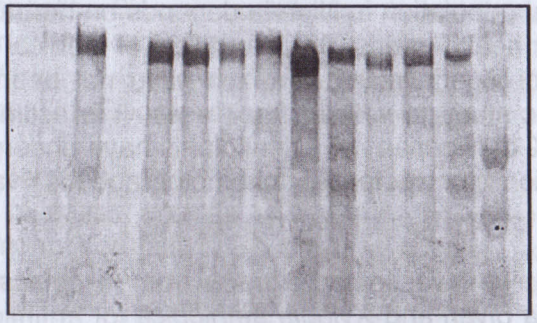
Cag A with Primers of 1,118bp



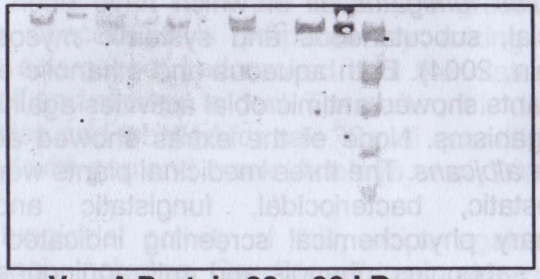
B

Vac A with Primers of 2533bp

C



Cag A Protein (120–145 kDa)



Vac A Protein 88 – 90 kDa

Plate 5: PCR Results and Protein Expression of *Cag* A and *Vac* A Genes in *Helicobacter pylori* (Seriki, *et al.*, 2015)
This research won the best American Society for Microbiology Oral Presentation at the NSM Conference in 2015. The outcome of this experiment is quite interesting when compared with the experience of Marshall and Warren (2005) (who originally discovered the organism) and used themselves as human models by physically drinking broth cultures of the isolates. Both researchers in time came down with gastric ulcers thereby providing proof of concept. However, by our observation using molecular methods, the expressed Vac A and Cag A genes had no statistical correlation with the clinical outcomes. Perhaps, we need to emphasise that old procedures for investigating virulence factors should not be completely jettisoned for the so called modern techniques.

My Contributions in the Area of Medicinal Plants

In spite of the influence of orthodox medicine, about 80% of the rural population in Nigeria depends on herbal medical care for their health needs. A wide variety of medical plants can be found in Nigeria many of which have not been subjected to scientific study to validate their uses. It is estimated that less than 2% of Nigerian medicinal plants have undergone scientific evaluation for potential chemotherapeutic value (Inyang, 2004).

In 2002, we investigated extracts from *Anogeissus leoicarpus, Daniellia diveri* and *Xylopia aethiopica* for antimicrobial activity against *Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, Trichophyton mentagrophytes* and *Aspergillus fumigatus* all of which have been implicated in superficial, subcutaneous and systemic mycoses (**Adeleye** and Amin, 2004). Both aqueous and ethanolic extracts of the three plants showed antimicrobial activities against at least two microorganisms. None of the extras showed activity against *Candida albicans.* The three medicinal plants were found to be bacteriostatic, bacteriocidal, fungistatic and fungicidal. Preliminary phytochemical screening indicated presence of tannins, saponnins, phenols and anthraquinones (**Adeleye** *et al.*, 2003).

Using standard procedures, we also screened extracts of leaves of *Alchornea cordifolia, Boerhavia diffusa* and *Bridellia micranthal* for antibacterial activity against *Helicobacter pylori, Salmonella typhi, Salmonella enteritidis, Shigella flexneri* and Enteroheamorrhagic *Escherichia coli* (EHEC). Results showed that the ethanolic extracts of the three plants and aqueous extracts inhibited the growth of all the organsims tested. However, the minimal inhibitory concentration (MIC) ranged between 15.6 and 31.25 mg/ml while the extracts were bacteriocidal at concentration ranging between 31.25 and 250 mg/ml. This indicates that leaf extracts of the three plants are of great potential in treating gastric ulcer and diarrhoea caused by the aforementioned bacteria (**Adeleye** *et al.*, 2008e).

Studies on "Epa-Ijebu"

Mr. Vice Chancellor Sir, my contribution in medicinal plants and crude drug research include evaluation of theraupeutic potentials of "original" Epa-Ijebu; my publications on Epa-Ijebu is very prominent in "Google" search on this topic.

"Epa-Ijebu" is regarded as a "wonder cure" concoction used in curing many diseases and as an antidote to scorpion and snake bite among Yorubas in South West Nigeria. According to information received from local herbalists, the recipe include juice from *Citrus aurantofolia, C. aurantium*, fruit of *Aframonium meligueta* as well as animal parts including a type of rat *Rattus norvegivm*, snake heads (various types) and scorpion. All the animal parts are dried and grounded into powder. The concoction is prepared by mixing all the ingredients in a large pot and cooked until the materials are reduced by half and allowed to cool. Thereafter, it is dispensed into small bottles and labelled for sale. Small quantities of the paste, is mixed with pap and drank (**Adeleye** *et al.*, 2008f).

We studied fungicidal activity of this recipe against some pathogenic fungi, including *Candida albicans, Microsporium* spp., *Trichophyton metagrophyte* and *Aspergillus fumigatus.* Also investigated was its bactericidal activity against clinical isolates of *Helicobacter pylori,* Salmonella Typhi, Salmonella Enteritidis, *Shigella flexneri* and Enteroheamorrhagic *Escherichia coli.* Toxicological studies was also carried out. The Epa-Ijebu concoction at 25.0-50.0 mg inhibited the growth of all fungi tested and was fungicidal at 50.0-100 mg/ml. These figures compared favourably with known antifungals (Table 12).

Table 12: Comparison of the Fungicidal Activity of the Epa-ljebu and of Griseofulvin, Nystatin and Itraconazole (Zone of inhibition measured in mm) (Adeleye *et al.* 2008f)

Organisms	Epa-ljebu (100mg/ml)	Griseofulvin (10mg/ml)		Nystatin (10mg/ml)		Itraconazole (10mg/ml)	
	MIC MFC	MIC	MFC	MIC MFC		MIC	MFC
Candida albicans	25.0 50.0	25.0	25.0	25.0	25.0	25.0	25.0
T. metagrophte	25.0 50.0	25.0	25.0	25.0	25.0	25.0	25.0
Aspergillus fumigatus	50.0 100.0	25.0	25.0	25.0	50.0	25.0	50.0
Microsporum spp.	50.0 50.0	25.0	50.0	25.0	50.0	25.0	50.0

The concoction also inhibited the growth of all the bacteria screened with MIC ranging between 15.6-125 mg/ml and MBC of between 31.5-250 mg/ml (Table 13).

Table 13: Minimal Inhibitory Concentration (MIC) and Minimal Bacteriocidal Concentration (MFC) in mg/ml of Epa-ljebu Concoction on Bacterial Isolates (Adeleye *et al.* 2008f)

Organisms	MIC (mg/ml)	MBC (mg/ml)
H. pylori	125	250
S. Typhi	15.625	31.25
S. Enteritidis	31.25	62.50
S. flexneri	15.625	31.25
Enterohaemorrhagic *E. coli* (EHEC)	31.25	125.0

However, toxicological assays showed that the concoction was toxic to the animal (mice) at concentration of 0.2-0.8 g/ml leading to their deaths within 24 hours of being fed. Pathological examination of the stomach, liver and kidneys showed profound erosion of tissue with marked area of karyolysis and karyorrhesis (Table 14, Plates A,B,C,D,E,F.). This study confirmed the antifungal and antibacterial properties

of the "wonder cure" concoction in-vitro but its use as an antidote to many ailments need to be moderated because of its toxicity to mice.

Table 14: Histological Examination of Dead and Sacrificed Animal Tissues (Adeleye *et al.*, 2008f)

ORGANS		LIVER			KIDNEY			STOMACH				
Group	Avg. body Weight	Level of degradation	Erosion	Area affected	Level of degeneration	Erosion	Areas affected	Level of degeneration	Erosion	Areas affected	Significant/Non Significant	Comment
Control	17.03	-	-	Total	-	-	Total	-	-	Total	NS	Normal
Group I(0.02g/ml)	19.73	-	-	Total	-	-	Total	-	-	Total	NS	Normal
Group II (0.02g/ml)	18.56	-	-	Total	-	-	Total	-	-	Total	NS	Normal
Group III (0.10g/ml)	20.89	-	-	Total	-	-	Total	-	-	Total	NS	Normal
Group IV (0.20g/ml)	20.36	+	-	Total	-	-	Total	-	-	Total	S	Abnormal
Group V (0.40g/ml)	17.74	++	+	Total	++	++	Total	++	++	Total	S	Abnormal
Group VI (0.80g/ml)	19.34	++	++	Total	++	++	Total	++	+++	Total	S	Abnormal

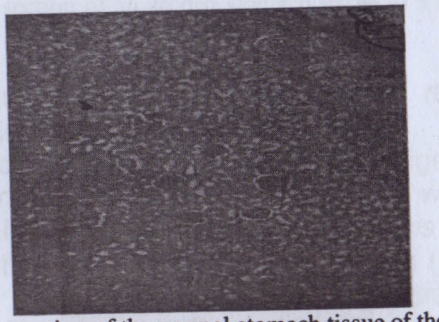


Plate 6a: Histologic section of the normal stomach tissue of the laboratory mouse fed with 0.025 g/ml of the freeze dried concoction

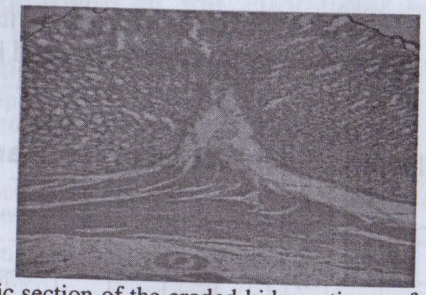


Plate 6b: Histologic section of the eroded kidney tissue of a laboratory mouse fed with 0.8 g/ml of the freeze dried concoction

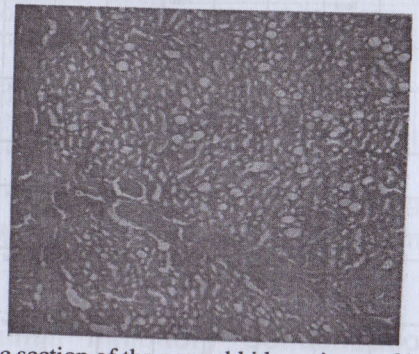


Plate 6c: Histologic section of the normal kidney tissue ofthe laboratory mouse fed with 0.025 g/ml of the freeze dried concoction.

Plate 6d: histology section of an eroded liver tissue of the laboratory mouse fed with 0.8 g/ml of the freeze dried concoction.

Plate 6e: histology section of the normal liver tissue of thelaboratory mouse fed with 0.025 g/ml of the freeze dried concoction

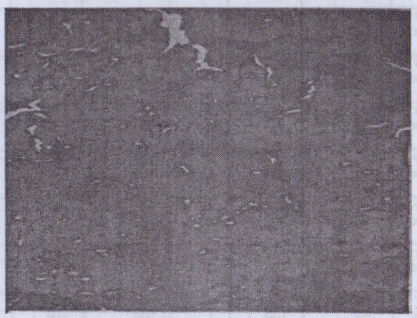


Plate 6f: histology section of an eroded stomach tissue of a laboratory mouse fed with 0.8 g/ml of the freeze dried concoction

In a related study and in collaboration with researchers at Nigerian Institute for Medical Research (NIMR), the antimicrobial activity of extracts of twelve Nigeria medicinal plant species and the wonder cure [Epa-Ijebu], were evaluated for activity against *Mycobacterium tuberculosis* isolates from tuberculosis patients sputum and control strain (H37RV). Both ethanolic and aqueous solution of the extract of *Allium ascalonicum, Terminalia glaucerceas, Allium sepa* and *Securidaca longepedunculata* (Ethanol) at 0.05g/ml as well as aqueous solution "wonder cure" concoction inhibited the growth of *M. tuberculosis* (Table 15). However at lower concentration 0.2mg/ml (critical propotion level of the control drug) Isoniazide, *M. tuberculosis* was resistant to both aqueous and ethanolic extracts of the plants and the wonder cure. Our results offered a scientific basis for the traditional

use of aqueous and ethanolic extracts of the said plants and Epa-ljebu concoction at high concentration in treating tuberculosis (**Adeleye** *et al.*, 2008g).

Table 15: Critical Proportion Calculations Using Isoniazide as the Standard at Higher Concentration of Herb Extracts (0.05g/ml) (Adeleye *et al.* 2008g)

Name of Extract/Concoction	solvent	(1)Control 10^{-3}	(2)Herb Extract 10^{-3}	B (1+2)	(3)control 10^{-3}	4)Herb Extract 10^{-3}	A (3+4)	Critical Proportion B+A%	Sensitivity
Crinum jagus	Water Ethanol	100 100	100 100	1 1	100 100	100 100	1 1	≥1 ≥1	Resistant Resistant
Allium cepa	Water Ethanol	100 100	0 0	0 0	100 100	0 0	0 0	≤1 ≤1	Sensitive Sensitive
Xylopia aethiopica	Water Ethanol	100 100	100 100	1 1	100 100	100 100	1 1	≥1 ≥1	Resistant Resistant
Aprus precatorius	Water Ethanol	100 100	100 100	1 1	100 100	100 100	1 1	≥1 ≥1	Resistant Resistant
Allium Ascalonicum	Water Ethanol	100 100	0 0	0 0	100 100	0 0	1 1	≤1 ≤1	Sensitive Sensitive
Allium sativum	Water Ethanol	100 100	100 100	1 1	100 100	100 100	1 1	≥1 ≥1	Resistant Resistant
Aframomum melegueta	Water Ethanol	100 100	50 80	2 1.25	100 100	40 50	2.5 2	≥5 ≥2.5	Resistant Resistant
Terminalia glaucescens	Water Ethanol	100 100	0 0	0 0	100 100	0 0	0 0	≤1 ≤1	Sensitive Sensitive
Tetrapleura tetraptera	Water Ethanol	100 100	100 100	1 1	100 100	100 100	1 1	≥1 ≥1	Resistant Resistant
Grarcing kola	Water Ethanol	100 100	100 100	1 1	100 100	100 100	1 1	≥1 ≥1	Resistant Resistant
Nicotina tabacum	Water Ethanol	100 100	100 100	1 1	100 100	100 100	1 1	≥1 ≥1	Resistant Resistant
Securidaca longepedunculata	Water Ethanol	100 100	50 0	1 1	100 100	40 0	2.5 0	≥5 ≤1	Resistant Sensitive
Salt	Water Ethanol	100 100	100 100	2 0	100 100	100 100	1 1	≥1 ≥1	Resistant Resistant
Epa-ljebu	Water Ethanol	100 100	0 0	1 1	100 100	0 0	0 0	≤1 ≤1	Sensitive Sensitive
Isoniazide	Water	100	0	0	100	0	0	≤1	Sensitive

We also evaluated the twelve plants and Epa-ljebu against the following bacterial pathogens: *Staphylococcus aureus, Escherichia coli*, Salmonella Typhi, *Proteus vulgaris, Shigella sonnei* using "punch-hole" diffusion method. Seven of the plant species namely *Allium sativum, Terminalia glaucescens, Allium cepa, Xylopia aethiopiea, Allum ascalonicum, Aframomom melegueta, Securidaca longipeduncalata,* and Epa-ljebu were inhibitory against the bacterial pathogen, however, most activity was recorded with Epa-ljebu (**Adeleye** *et al.*, 2008h).

Table 16: Actimicrobial Activity of Plant Extracts and 'Epa-ljebu' Concoction Using Agar Diffusion Method (Adeleye *et al* ., 2008h)

Name of extract/Concoction	solvent	*S. aureus* (mm)	*E. coli* (mm)	*S. sonnei* (mm)	S. Typhi (mm)	*Proteus Vulgaris* (mm)
Crinum jagus	Water Ethanol	0 0	0 0	0 0	0 0	0 0
Allium ascalonicum	Water Ethanol	10 10	0 0	0 0	11 11	0 0
Allium cepa	Water Ethanol	8 6	0 0	0 0	10 10	0 0
Xylopia aethiopica	Water Ethanol	10 10	12 10	10 10	11 11	13 13
Abrus precatorius	Water Ethanol	0 0	0 0	0 0	0 0	0 0
Allium sativum	Water Ethanol	10 10	12 11	11 11	10 10	12 12
Aframomum melegueta	Water Ethanol	8 8	10 10	12 12	10 11	10 10
Terminalia glaucescens	Water Ethanol	10 9	14 12	10 10	15 10	12 10
Tetrapleura tetraptera	Water Ethanol	0 0	0 0	0 0	0 0	0 0
Grarcing kola	Water Ethanol	0 0	0 0	0 0	0 0	0 0
Nicotina tabacum	Water Ethanol	0 0	0 0	0 0	0 0	0 0
Securidaca longepedunculata	Water Ethanol	0 0	0 0	0 0	0 0	0 0
Epa-ljebu	Water Ethanol	11 10	15 14	10 10	15 15	13 12

in an attempt to determine the major constituents of Epa-ljebu responsible for the acclaimed currative ability, we extrasted essential oils and volatile compounds by hydrodistillation and analysed both using gas chromatography/mass spectrometry (GC/MS). Twelve major organic compounds were identified of which Fatty acids were most prevalent (35.52%). This was followed by normal alkanes such as nonadecene, hexadecane, heptadecane, octadecane and heneicosane (constituting 26.5%), 2-p-nitrophenyl-oxadiazol-1,3,4-one-5 (18.18%), Quinoline (5.96%), Benzothiazole (4.87%), Alcohol (2.48%) and other compounds in traces. The prevalence of **fatty acid** extracts as well as quinoline, benzothiazole and alchohol

may be responsible for the anti-bacterial and antifungal as well as other curative ability of this local concoction. (**Adeleye** *et al.*, 2011b). We could not investigate the legendry anti-snake venom potency of this "concoction" because of lack of adequate experimental model. However, we postulate that the "Epa-Ijebu" could easily pass for a crude vaccine given its composition. Louise Pasteur made a crude vaccine against rabies in 1885 by using dried brain tissues of dead rabid dogs dissolved in formalin. Epa-Ijebu contains dried snake head grounded into powered among other items. This could serve as killed toxoids (which is heat-killed protein vaccine).

In another study, we investigated the essential oil obtained by hydrodistillation method as well as aqueous and ethanolic extracts of *Gongronema latifolium* leaves (called Utazi and Arokeke in South East and Western Nigeria respectively) for antimicrobial activity against bacteria isolated from blood streams of HIV patients in Lagos. The oil and extracts showed moderate inhibitory activity against *Staphylococcus* spp., *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Onchrobactrum authropi* and *Candida albicans*. The MIC for essential oil ranged between 5-40 µg/ml while the MBC ranged between 10-50 µg/ml. The MIC and MBC for ethanol extract ranged between 3.12-12.5 mg/ml and 3.125-25.0 mg/ml respectively (Table 17). Analysis of the essential oil using Gas Chromatography/Mass Spectrophotometry (GC-MS) (Table 18) showed that it was dominated by linear aliphatic compounds (27.06%), unsaturated fatty acids, characterised by high percentage of phthalic acid (18.61%), oleic acid (5.2%), arachidic acid (2.34%), fumanic acid (2.22%) and monoterpenes including camphor, β-cymene and phytol (**Adeleye** *et al.*, 2011c).

Table 17: MIC and MBC of Aqueous, Ethanolic and Essential Oil Extract of *Geongronema latifolium* (Adeleye *et al.*, 2011c)

Test organisms	Aqueous Extract			Ethanolic Extract			Essential Oil		
	MIC ($mg\ mL^{-1}$)	MBC ($mg\ mL^{-1}$)	Zone of inhibition (mm)	MIC ($mg\ mL^{-1}$)	MBC ($mg\ mL^{-1}$)	Zone of inhibition (mm)	MIC ($pg\ mL^{-1}$)	MBC ($pg\ mL^{-1}$)	Zone of inhibition (mm)
Staphylococcus. Urealyticus	6.25	25	10.0	6.25	12.50	10.0	5.0	5.0	10.00
Staphylococcus C. aureus	6.25	6.25	9.0	6.25	12.50	9.3	10.0	10.0	10.50
Staphylococcus chromogene	6.25	6.25	9.3	6.25	12.50	9.0	10.0	5.0	10.00
Staphylococcus cohni cohnii	12.50	25.00	8.8	12.50	25.00	9.8	5.0	10.0	10.00
Staphylococcus sciuri	12.50	12.50	9.5	12.50	25.00	10.0	5.0	5.0	9.70
Staphylococcus warneri	6.25	12.50	9.5	12.50	25.00	9.5	5.0	5.0	10.75
Staphylococcus epidermidis	6.25	6.25	9.5	12.50	25.00	9.8	5.0	5.0	10.00
Pseudomonas florescence	6.25	6.25	9.3	3.125	3.125	9.5	5.0	10.0	9.75
Pseudomonas aeruginosa	6.25	6.25	9.3	3.125	3.125	7.8	10.0	10.0	8.50
Shigella flexneri	6.25	12.50	9.7	6.25	12.50	10.3	10.0	10.0	11.25
Shigella dysentriae	12.50	12.50	10.0	6.25	12.50	10.3	5.0	20.0	11.00
Salmonella typhi	12.50	25.00	8.2	3.125	6.25	9.5	20.0	20.0	10.00
Salmonella ryhimurium	12.50	25.00	7.5	3.125	6.25	8.3	10.0	20.0	7.50
Klebsirlla Pneumonia	25.00	25.00	7.0	3.125	6.25	7.3	10.0	40.0	6.30
Onchrobactrum anthropi	12.50	12.50	6.2	6.25	12.50	6.5	40.0	10.0	6.30
Escherichia coli	6.25	6.25	6.5	6.25	12.50	7.0	10.0	10.0	7.00
Candida albicons	-	-		-	-		5.0	10.0	11.20
Escherichia coli NCTC 10148	6.25	6.25	7.0	6.25	12.50	7.8	10.0	10.0	7.00
Staphylococcus aureus ATCC 25923	6.25	12.50	8.6	12.50	12.50	9.0	10.0	10.0	8.40

Table 18: Compounds Identified from Essential Oil of Gongronema latifolium (Adeleye *et al.*, 2011c)

IUPAC name	Common name	Retention time	Composition(%
Dichloroxylenol	B-cymen	11.391	0.41
Hexadecane	Haxadecane	14.107	2.72
Dodecane	Dodecane	14.190	1.36
Octadecane	ctadecane	14.360	0.99
Heptadecane	Magaric acid	14.446	0.67
Eicosane	Arachidic acid	14.469	0.57
n-Octadecane	Octadecane	15.024	1.9
I-Bromodocosane	Camphor	15.144	1.20
Octadecane	Octadecane	15.144	1.20
2-bromododecane	Bromodocane	15.815	10.49
Hexadecane	Hexadecane	15.871	4.88
Triacontane	Tricontane	16.565	5.36
3.Chloro-IH-Pyridoquinoline	Quinoline	16.648	1.58
7,12-Dihydrobenzo(k) Fluoranrhene	Fluoranthene	16.678	0.48
Ergoline-8-methanol	n-Methanol	16.824	2.22
2,4-dimethylpent-3-y-lunfrcyl ester	FumRIC cid	16,824	2.22
6H-indolo(2,3-b) quinoxaline	Quinoline	16,824	1.22
Octadecanoic acid	Oleic acid	16,878	1.36
Hesatriacotane	Hexatriancontane	17.163	5.46
1-nonadecene	Nonadecene	17.279	2.20
2-Pentadecanol	n-alcohol	17.711	0.44
4-trifluoroacetoxytradecane	Trifluoroacetate	17.944	1.87
1.2 Benzenedicarboxylic acid	Phthalic acid	18.083	6.06
Nonacosance	Nonamcosane	18.302	1.18
Eicosylacetate	achinoic acid	18.442	1.27
Octacosyl acetate	Lainanyl acetate	18.545	0.511
1-H exacosanol	oleic acid	19.026	1.50
n-prophy 11-cotadecenoate		19.133	0.23
1,2-Benzenedicaboxylioc acid	Phthalic acid	19.219	2.40
Trans-13-Octadecenoic acid	Oleic acid	19.302	2.26
3-Beta-acetoxy-6 nitroadrost	Phytol	20.970	1.66
1-hexacosanol	n-alchol	21.282	1.66
Phthalic acid cycloheptyl isohesy easter	Phthalic acid	25.437	10.15

In Nigeria, there has been a wide but unsubstantiated claim by local snuff takers as to the efficacy of tobacco snuff in ameliorating lung diseases. In order to verify this claim we screened tobacco leave extracts against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae* as well as *Candida albicans* and *Aspergillus fumigatus*. The result showed that aqueous methanol and ethanol extracts of

the plant did not exhibit any significant antimicrobial or antifungal activity against the tested organism. On the contrary, aqueous extract of the leave was found to promote growth of *S. pneumoniae* at concentrations of 40 and 80 mg/ml. We, therefore concluded that snuff taking was not a beneficial therapy for respiratory tract infection (**Adeleye** and Uwagboe, 2003).

Coughing is one of the many symptoms of respiratory tract infections whose aetiology include bacteria, viruses and fungi. In a related study, we screened for six common ingredients used in local cough mixture against a battery of bacteria causing upper respiratory tract infections. The result showed that lime, garlic onion, onion and honey were active against *S. auerus, Streptococcus* spp., *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella* spp. Palm kernel oil and bitter kola did not show any antibacterial activity (**Adeleye** and Opiah, 2003).

In one of our recent study, the anti-plasmodial properties of the aqueous leaf extract of *Morinda lucida*, Benth (Oruwo) a traditional curative herb found in South West Nigeria was determined. A four-day suppressive test was used to determine the anti-plasmodial properties of the leaf extract. Three weeks old albino mice were infected with standard concentration of *Plasmodium berghei* and after four days different concentration ranging between 200mg and 800mg of water extracts of the leaves of *Morinda lucida* was administered intraperitoneally. *Plasmodium berghei* density was then determined to monitor the level of parasitemia. The leaf extract was highly effective in suppressing *Plasmodium berghei*. Activity was dose dependent achieving >70% suppression at 800mg/ml. The result of phytochemical analysis of extract was positive for flavonoids, saponins and steroids. This study confirms *Morinda lucida* as a potential antiplasmodial therapy (Babatunde *et al.*, 2016a). During the course of my research into beneficial effect of herbal preparation, it was also observed that most of the preparations are highly contaminated with microbes (**Adeleye** *et al.*, 2005a).

It is therefore recommended that herbal remedies be heated for decontamination before use.

Endophytic Fungi as Sources of Novel Drugs

Mr. Vice Chancellor Sir, the success of several medicinal drugs from microbial origin has shifted the focus of drug discovery from plants to microorganisms (Balagururathau and Radhakrishman, 2007). The probability of obtaining a novel compound is higher from a novel source. As such, endophytic fungi are considered as potential sources of new antimicrobial compounds. Endophytes are microbes that colonise the internal plant tissues beneath the epidermal cell layers without causing any harm or symptomatic infection to the host. Endophytic fungi are known to contribute to their host plant by producing excessive secondary metabolites that provide protection and survival values to the plants (Walton, 1995) (Babatunde *et al.* 2016b).

Of the 300,000 plants species that exist on earth, each individual plant is host to one or more endophytes, thus providing a rich reservoir of microorganism (Strobel and Daisy, 2003). The functional metabolites produced by endophytes include alkaloids, terpenoids, quinones, steroids, isocumarin derivatives, flavonoids, phenols and phenolic acids and peptides. Among the new bioactive compounds discovered are: Novel broad spectrum antibiotics, Kakadumycins, extracted from endophytic Streptomycete associated with *Grevillea pteridfolia*, ambuic acid, and antifungal agent from several isolates of *Pestalotiopsis microspore*. Recently, (Abass *et al.* 2015) (Abass et al. 2016) we investigated the endophytic fungi diversity and antimicrobial potential of endophytic fungi of three medicinal plants namely, *Alstonia boonei* (Ahun), *Enantra chlorantha* (Awopa) and *Kigelia Africana* (pandoro) that have ethnobotanical history in Nigeria. A total of ten endophytic fungi were isolated from the stem bark of the plants using standard procedure. Seven of these fungi were identified as *Aspergillus niger, Microphomina* spp., *Trichoderma* spp. and four different *Penicillum* spp. The cell free fermentation broth of the fungal isolates was inhibitory to six bacterial isolates tested with varying zones of inhibition comparable to

standard antibiotics (Table 19). Extracts from this broth cultures when subjected to GC/MS yielded a host of bioactive compounds which included Coumarin, Cinnamic acids, Penicillin, etc., as seen in Table 20. This was the first report of extraction of bioactive compounds from endophytic fungi in Nigeria. No doubt these compounds may be contributing to the curative properties of these plants. For example, Cinnamic acid has been found to be active against bacteria, fungi and viruses (Sova, 2012). Penicillin and Griseofulvin are known antibacterial agents widely used for therapeutic purposes world over (Park *et al.*, 2005).

Table 19: Antimicrobial Activity of Endophytic Fungi (zone of inhibition in mm)(Abass *et al.*, 2015)

Fungi Isolate	Endophytic Fungi	*E. coli* (mm)	*P. aeruginosa* (mm)	*E. faecalis* (mm)	*S. aureus* (mm)	S. Typhi (mm)	*C. albicans* (mm)
AA	*Microphomina* spp.	10	12	N*	N*	N*	N*
AG	*Aspergillus niger*	14	16	6	N*	N*	N*
BB	*Penicillium* sp 1	N*	25	N*	20	N*	N*
BA	*Penicillium citrinum*	N*	28	N*	N*	N*	N*
CC	Unidentified	N*	N*	N*	20	N*	38
CD	Unidentified	12	N*	N*	N*	N*	30
CBP	*Penicillium sp. 2*	N*	N*	6	N*	N*	40
CBG	*Penicillium nigricans*	N*	N*	N*	N*	N*	35
AD	*Trichoderma* sp.	N*	N*	N*	N*	N*	26

*No inhibition.

Table 20: Bioactive Compounds Characterised from Extracts of Fermentation Broths from Endophytic Fungi (Abass *et al.*, 2016)

S/N	Pure compounds	Endophytic Fungi Producing Bioactive Metabolites				
		Macrophomina	*A.niger*	*P.citrinum*	Penicillium sp1	Penicillium sp2
1	Cinnamic acids	+ve	-ve	-ve	+ve	-ve
2	alpha-Amorphen	-ve	-ve	-ve	-ve	+ve
3	5,8-dimethylquinoline	+ve	+ve	+ve	+ve	+ve
4	4a-methyl-trans-2-decali	-ve	-ve	-ve	-ve	-ve
5	Penicillin	-ve	-ve	-ve	-ve	+ve
6	alpha-Cadinol	+ve	+ve	+ve	-ve	+ve
7	Griseofulvin	-ve	-ve	-ve	-ve	+ve
8	n-Tridecanoic acid	+ve	-ve	-ve	+ve	+ve
9	Palmitaldehyde	+ve	+ve	+ve	-ve	+ve
10	n-Tetradecanoic acid	-ve	+ve	+ve	-ve	+ve
11	Homosyringic acid	+ve	+ve	+ve	+ve	+ve
12	8,11-Octadecadienoic acid	+ve	+ve	+ve	+ve	+ve
13	Olealdehyde	-ve	+ve	-ve	-ve	+ve
14	2-Pentadeconone	+ve	-ve	+ve	+ve	+ve
15	Cis-9-hexadecenal	-ve	-ve	-ve	-ve	+ve
16	Cis-2-methoxy cinnamic	-ve	-ve	-ve	-ve	+ve
17	Coumarin	-ve	+ve	+ve	-ve	+ve
18	Cinnamaldehyde	-ve	+ve	+ve	-ve	-ve
19	2-Furaldehyde-5-hydroxym	+ve	-ve	-ve	+ve	-ve
20	Cubenol	+ve	-ve	-ve	-ve	-ve
21	Erythritol	-ve	-ve	+ve	-ve	+ve

"+ve" bioactive compounds found

"-ve" bioactive compounds not found

The Marine as a Source of New Therapeutic Compounds

Mr.Vice-Chancellor Sir, *"A woman's mind is an ocean of too many secrets"* (Rose Dawson, TITANIC, 1996). The bowel of the sea is the reservoir of many novel bioactive molecules. My contributions to research into marine drug and natural products have produced a couple of doctoral and masters students. Available treatments for many infectious diseases caused by bacteria, fungi and viruses are limited due to increasing drug resistance. This has led to increased search for new sources of therapeutic compounds (Levy, 2002). David (1998) reported the role of oceans in the development of new drugs. Not only evolutionary biologists were interested in marine life forms but the pharmaceutical companies were increasingly searching the seas for new drugs.

This is as a result of the need for new drugs as commonly used ones are being faced with antibiotic resistance. Researchers have focused on terrestrial plants and microorganisms for many years, mainly because specimens

are easy to obtain (Gravalos, 2009). But a growing proportion of today's promising, pharmaceutic research focuses on the sea, where marine organisms have evolved biologically unique molecules. Innumerable organisms, displaying rich biodiversity, populate the ocean depth. They are extremely diverse species of invertebrates-fixed or sessile-many in plant form and others capable of slow, primitive movement. These invertebrates possess no physical defense such as protective shells or spines; instead, they have developed biologically active molecules - secondary metabolic substance - which they use to attack prey or defend their habitat. The fascinating variety of marine organisms hints at a myriads of new possibilities of drug discovery.

Marine drug discovery began in the 1970s by early investigators demonstrating unequivocally, that marine plants and animals were genetically and biochemically unique. Over 18,000 structurally unique and often highly bioactive metabolites have now been isolated from marine plants and animals.

Marine drugs are compounds obtained from marine plants, animals and microorganisms. About 70% of earth surface is covered with water and it harbours 500,000 live species divided into 30 different phyla (Das et al., 2006). The marine environment is an exceptional reservoir of bioactive natural products. The ocean is packed with unique organisms, which have compounds possessing different pharmacological activities. These compounds possess unique mechanism of action, structurally novel and possess potent biological activities different from terrestrial organisms (Newman *et al.*, 2011).

Research into the pharmacological properties of marine natural products has led to discovery of many potent active agents considered worthy of clinical application. The marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural/chemical features not found in terrestrial natural products (Carte, 1996). Because of the physical and chemical conditions in the marine

environment, almost every class of marine organism exhibits a variety of molecules with unique structural features (Anake and Pichan, 2004). Marine ecosystem comprise of a continuous resource of immeasurable biological activities and vast chemical entities. Given such a background, the chemistry of marine natural products has been progressing at an unprecedented rate, resulting in a multitude of discoveries of carbon skeleton and molecules hitherto unseen on land. The diversity has provided a unique source of chemical compounds with potential bioactivities that could lead to potential new drugs candidates (Cabrita *et al.*, 2010).

Reports have shown an emerging source of new bio-actives may result from many recent studies of microbial diversity in the marine environment (Rheineimer, 1992; Rohwer *et al.*, 2002; Bourne and Munn 2005; Enticknap *et al.*, 2006; Fenical and Jensen, 2006; Webster and Bourne, 2007; Martinez-Garcia *et al.*, 2007; Longford *et al.*, 2007; Perez-Matos *et al.*, 2007). Researchers have isolated approximately 7,000 marine natural products, 25% of which are from algae, 33% from sponges, 18% from coelenterates (sea whips, sea fans, and soft corals), and 24% from representatives of other invertebrate phyla such as ascidians (also called turnicates), opisthobranch molluscs (nudibranchs, sea hares, etc.), echinoderms (star, sea cucumbers) and bryozoans (moss animals).

Research Contributions in Bioactive Compounds from Marine Algae

In a study initiated by me, a total of seventeen (17) different species of macro-algae were obtained from West and South African coasts and examined for bioactive compounds (Daniels *et al.*, 2014). They are green algae *Cauplepa taxifolia, Chaetamorpha antennina, Chaetamorpha linum, Chladophora vagabunda, Codium* spp. *Enteromorpha flexuosa (Plate 7), Ulva fasciata, Ulva lactuca* and brown algae: *Padina antilarum, Sargassum vulgare, Sargassum heterophyllum (Plate 8), Padina durvillae,* and red algae: *Centroceras clavalatum, Gelidium corneum, Hydropuntia dentate, Hypnea musciformis* and *Plocamium rigidiun.*

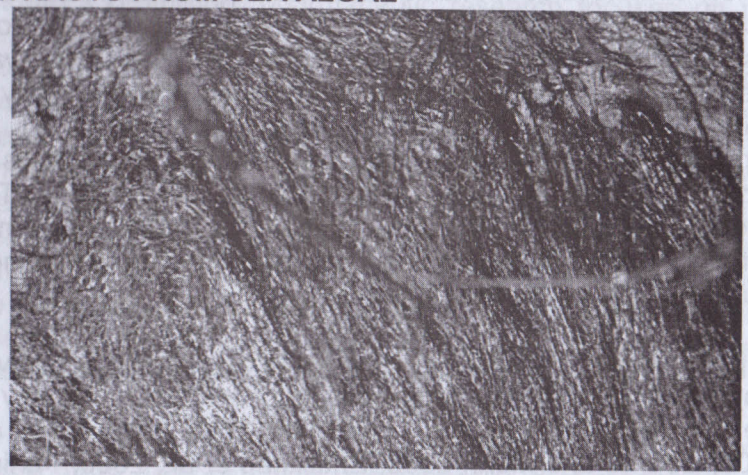


Plate 7: *Enteromorpha flexuosa*

Plate 8: Sargassum heterophyllum

Crude extracts were obtained using the following solvents Dichloromethane/Methanol (DCM/MeOH), chloroform/ methanol (CHL/MeOH) 90% (v/v), Ethanol and Diethyl ether. The radical scavenging abilities of selected algae were evaluated by qualitative antioxidant method using 2,2-

Diphenyl-1-picryl hydrazyl (DPPH) solution. Antibiofilm analysis was carried out on *Sargassum heterophyllum*, *Sagassum vulgare* and *Fasciata.* Antibacterial activity of crude extract was carried out on 7 Gram positive bacterial strains (*Staphylococcus aureus* clinical strain, *Staphylococcus aureus* laboratory strain ATTCC 25922, *Bacillus substilis, Streptococcus pneumoniae, Sreptococcus faecalis* and *Mycobacterium aurum*) and 11 Gram negative bacterial strain (*Escherichia coli* clinical strain, *E. coli* laboratory strain NCTC 10418, E. coli ATCC 25923, *Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Pseudomonas putida,* Salmonella Typhi clinical, S. Typhi NCTC 8385 and *Klebsiella pneumoniae*) by disc and agar diffusion methods and the minimum inhibitory concentration (MIC) was evaluated by broth microtitre and agar dilutions. The acute oral toxicity assay was carried out using laboratory rats with doses 300mg/kg, 2,000mg/kg and 5,000mg/kg body weight. Bactericidal kinetics of *S. heterophyllum* crude extracts was determined against antibiotic resistant *S. aureus* at 80µg (the Minimum Bactericidal concentration-MBC) and its multiple-2MBC and 3MBC. Bioactivity guided purification of crude extracts was done by fractionation of crude extracts through Step Gradient Column Separation. Purification of fractions was achieved by column fractionation and High Performance Liquid Chromatography. Metabolites were purified and characterised by full NMR Spectrometry including HNMR, CNMR, COSY and HSQC. The percentage yield of the algae ranged between 2.2 and 9.0%. Thirteen out of the seventeen (76%) algal species screened showed antibacterial activity to at least one of the test organism (Table 20a,b).

Table 21a: Antibacterial Activity of Crude Extracts of *E. flexuosa* on Gram Negative Bacteria (Daniel *et al.*, 2014) (zone of inhibition in mm)

Extract	Ec I	Ec II	Ec III	Ec IV	Pv	Pm	Pp	Pa	Kp	Stl	Stll
DEEF	ND	ND	ND	ND	ND	ND	ND	ND	0	0	0
DCEF	13.33	9.00	12.33	11.67	ND	9.00	7.17	11.67	NA	NA	NA
ETEF	0	0	0	0	0	0	0	0	0	0	0
CHEF	11.33	9.17	11	11.17	ND	8.67	6.00	ND	ND	ND	ND
Ciprofloxacin (10 mg)	20	19	19	20	14	14.67	18	14.17	19	17.17	18

DEEF: diethylether *Enteromorpha flexuosa*, DCEF: dichloromethane/methanol *Enteromorpha flexuosa*, ETEF ethanol *Enteromorpha flexuosa*, CHEF chloroform/methanol *Enteromorpha flexuosa*.

KEY: EcI- *E coli clinical I, E. coli clinical II, Ec II- E. coli NCTC 10418, E. coli IV- E. coli ATCC 25923, Pv –Proteus vulgaris, Pm- Proteus mirabilis, Pa- Pseudomonas aeruginosa, Pp- Pseudomonas putida, St I- Salmonella typhi clinical, StII- Salmonella typhi, NA: Not active, ND: Not Determined*

Table 21b: Antibacterial Activity of Crude Extracts of *E. flexuosa* on Gram Positive Bacteria (zone of inhibition in mm)

Extract	Sa I	*Sa* II	*Sa* III	*Bs*	*Ma*	*Sf*	*Sp*
DEEF	ND	ND	ND	ND	ND	ND	ND
DCEF	13	10.67	11	10.33	NA	ND	ND
ETEF	0	0	0	0	0	0	0
CHEF	11.33	10.67	11.33	NA	NA	ND	ND
Gentamycin (30 mg)	18	19	18.17	16.133	17	19	12.37

DEEF diethylether *Enteromorpha flexuosa*, DCEF dichloromethane/methanol *Enteromorpha flexuosa*, ETEF ethanol *Enteromorpha flexuosa*, CHEF chloroform/methanol *Enteromorpha flexuosa*.

Key: Sa I *S. aureus* clinical strain, Sa II- *S. aureus* laboratory strain,
Sa III- *S. aureus* ATCC 25922,
BS- *Bacillus subtilis*, Sp- *Streptococcus pneumonia*,

Sf- *Streptococcus faecalis*, *Mycobacterium aurum*, *NA- not active*,
ND- not determined

MIC values for the pure compounds screened ranged from 20µg-160µg/ml while MIC for crude extracts ranged from 80 µg-640 µg/ml. The bactericidal kinetics revealed that a total kill was achieved in *S. heterophyllum* extract against *S. aureus* strain. Antioxidant activity was expressed in 9 out of 11 (81.8%) of the tested crude extract. Good antibiofilm activity was also recorded for the three tested algae. Fucosterol, a phytosterol was isolated from *Enteromorpha flexuosa* (Fig 4a) while Fucoxanthin (Fig 4b) was extracted from the two *Sargassum* spp. tested in this study.

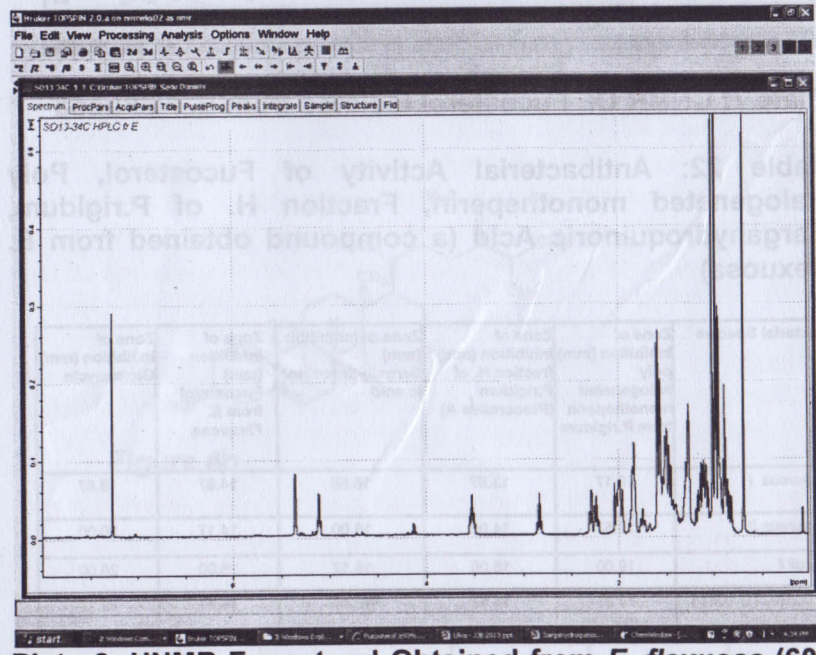


Plate 6: HNMR Fucosterol Obtained from *E. flexuosa* (600 MHZ : $CDCL_3$)

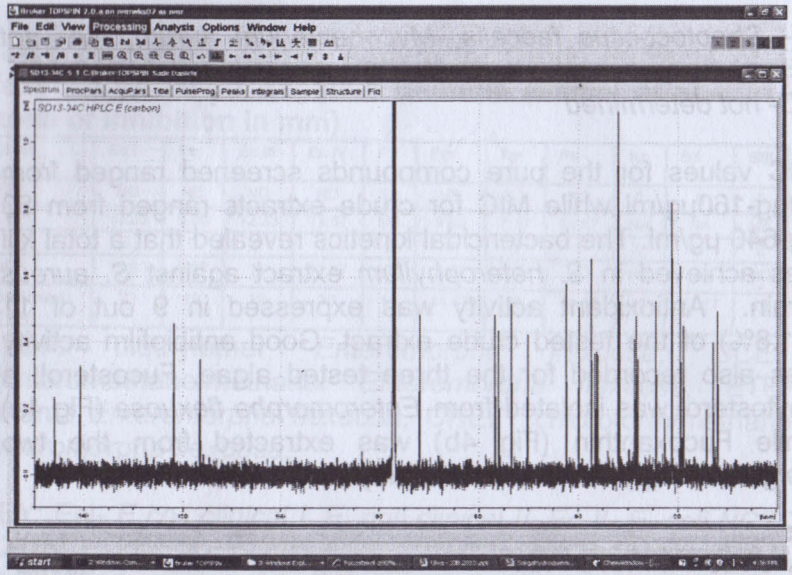


Plate 7: CNMR OF Fucosterol Isolated from E. Flexuosa

Table 22: Antibacterial Activity of Fucosterol, Poly Halogeneted monotheperin, Fraction H. of P.rigidum, Sargahydroquinoric Acid (a compound obtained from E. flexuosa)

Bacterial Species	Zone of Inhibition (mm) poly halogeneted monotheperin from P.rigidum	Zone of Inhibition (mm) fraction H. of P.rigidum (Plocoralide A)	Zone of Inhibition (mm) Sargahydroquinoric acid	Zone of Inhibition (mm) Fucosterol from *E. Flexuosa*	Zone of Inhibition (mm) Gentamycin
S. aureus I	16.17	13.67	16.68	14.67	18.67
S. aureus II	18.57	14.00	16.00	14.17	20.00
E. coli I	19.00	15.00	16.17	18.00	20.00
E. coli NCTC 10418	19.27	14.17	15.67	16.17	19.17

A poly-halogenated monoterpene **(Plocoralide A)** was isolated from fraction H of *P. rigidum* (Fig 4c) while two diterpenes (Sargahydroquinoic acid and Sargaquionoic) acid were isolated from *S. heterophyllum* (Fig. 4d). All pure compounds displayed antibacterial activities comparable to standard

antibiotics (Table 22). Acute oral toxicity assay revealed the lethal dosage (LD_{50}) for *Sargassum heterophyllum* to be 2500mg/kg while *E. flexuosa* had an LD_{50} of 5000mg/kg. The activities of crude extract were observed to be dose-dependent. The results of this study revealed that micro-algae from West and South African coast have antibacterial potentials which could lead to the development of novel therapeutic compounds and this may provide answers to the problem of antimicrobial resistance.

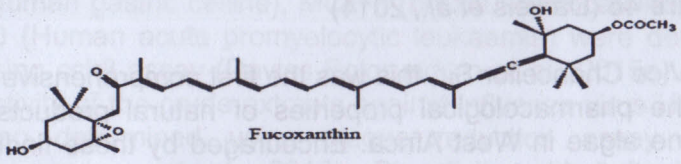


Figure 4a: Fucosterol

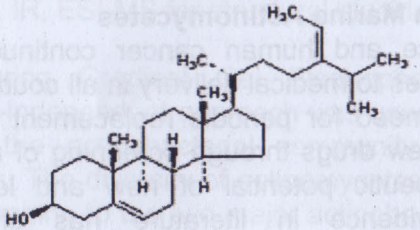
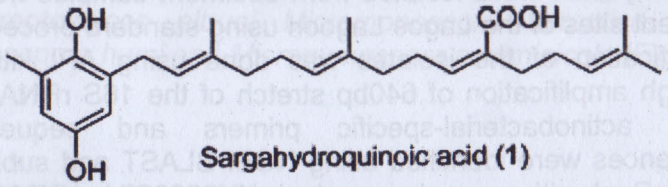
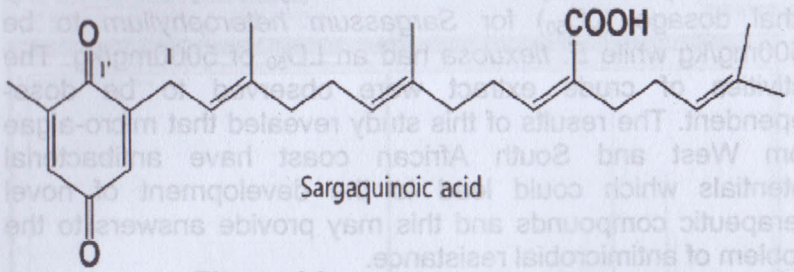


Figure 4b



Sargahydroquinoic acid (1)

Figure 4c



Sargaquinoic acid

Figure 4d

Figure 4e (Daniels *et al.*, 2014)

Mr. Vice Chancellor Sir, this was the first comprehensive study on the pharmacological properties of natural products from marine algae in West Africa. Encouraged by these findings, I proceeded to investigating antitumour and antimicrobial potentials of another group of marine microorganisms - The Actinomycete Bacteria.

My Research Contributions in Novel Antitumour and Antimicrobial from Marine Actinomycetes

Antibiotic resistance and human cancer continue to pose formidable challenges to medical delivery in all countries of the world. There is a need for periodic replacement of existing therapeutics with new drugs through screening of unexplored habitats for therapeutic potential of new and less known actinomycetes. Evidence in literature has shown that indigenous actinomycetes of the marine environment in West Africa have not been explored for their antibiotic and other therapeutic potentials. In a study initiated by me which won the best Nigerian Society of Microbiogy (NSM) oral and poster presentation at the last NSM Conference in 2015, actinomycetes were isolated from sediment samples from 12 different sites of the Lagos Lagoon using standard procedures. Identification of the isolates was done using API kits and through amplification of 640bp stretch of the 16S rRNA gene using actinobacterial-specific primers and sequencing. Sequences were identified using NCBI BLAST and submitted to GenBank with accession numbers KX352057 to KX352088.

Phylogenetic tree was constructed to determine the evolutionary relationship among the strains compared with already known strains. The actinomycete isolates were screened to determine their antimicrobial potentials against test strains *E.coli, P. aeruginosa, S. flexneri, S. epidermidis, S. typhi* and *E. faecalis* using cross streak method. For antifungal activities, Crude extracts were also screened against *C. albicans, Trichophyton mentagrophytes* A1, *Trichophyton mentangrophytes* A2 and *Trichophyton rubrum*. Anticancer activity of their crude extracts on cell-lines K562 (Human acute myelocytic leukemia), HeLa (cervical carcinoma), AGS (Human gastric celline), MCF-7 (breast adenocarcinoma), HL-60 (Human acute promyelocytic leukaemia) were determined using cck8 assay (Davies-Bolorunduro *et al.*, 2015a). Antiviral activity of the crude extracts against influenza virus H1N2 was also determined using plaque reduction assay (Davies-Bolorunduro *et al.*, 2016). Bioactivity-guided fractionation, using flash column chromatography was done to obtain purified antimicrobial and anticancer compounds using column packed with silica gel. Spectroscopic analyses was carried out on the purified compounds using ^{1}H-NMR, ^{13}C-NMR, COSY, HMBC, IR, ESI-MS for structural elucidation of the compounds.

Denaturing Gradient Gel Electrophoresis (DGGE), as a culture-independent approach was used to provide information about the actinobacterial community profile in the Lagos Lagoon. The diversity of actinomycetes showed their identities as belonging to the two major actinobacteria genera which are *Streptomyces* and *Micromonospora*. Twenty four of the isolates were species belonging to the genera *Streptomyces* while 7 of the isolates were species belonging to *Micromonospora* and 1 belong to *Agromyces* (Davies-Bolorunduro *et al.*, 2015b). The isolated species include *Streptomyces fulvissimus, Streptomyces bingchenggensis, Streptomyces albus. Micromonospora aurantica, Micromonospora humi* and *Micromonospora sediminicola* (Fig. 3).

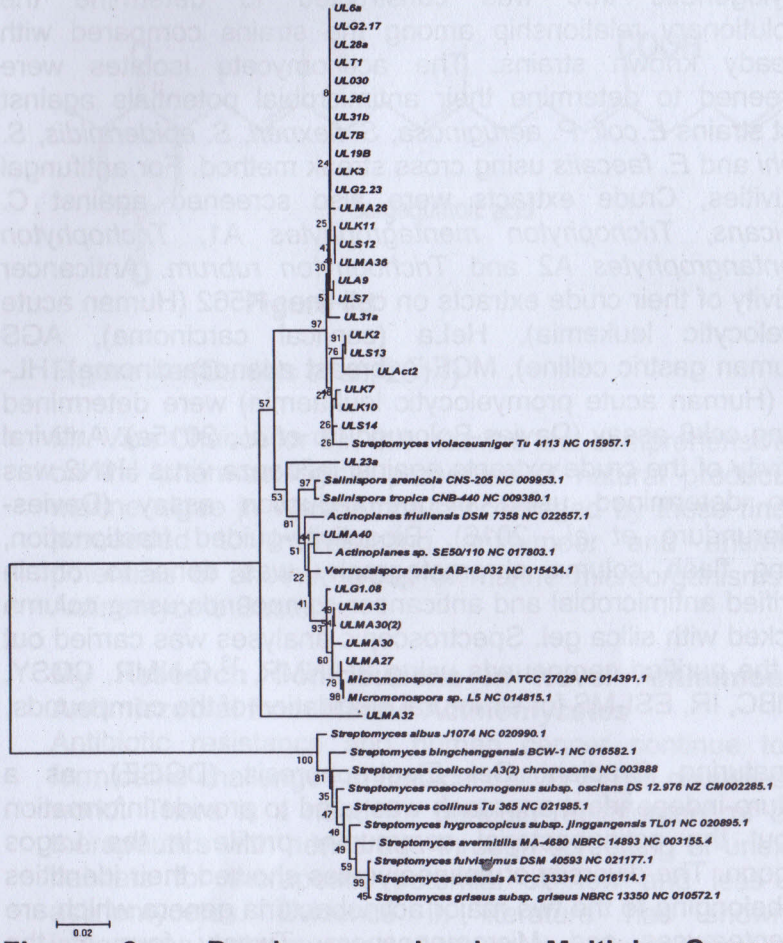


Figure 3: Dendogram showing Multiple Sequence Alignment of 16S rRNA Gene Sequences of Actinomycetes Isolated in Lagos Lagoon and other Marine Environments Worldwide and some other known Strains. Pairwise phylogenetic distances were calculated based on 16S rRNA gene (Davies –Bolorunduro *et al.*, 2015b)

The crude extracts of 14 of the 32 isolates showed significant activity against the test bacterial and fungal isolates (Table 23 & 24). MIC ranged from 0.078 mg/mL to 10 mg/mL (bacteria) and 0.156 mg/mL to 5 mg/mL(fungi). (Table 25 and 26). MBC ranged from 0.313 mg/mL to 10

mg/mL while MFC ranged from 0.156 mg/mL to 10 mg/mL. All the isolates showed antitumour activity against at least one of the cell-lines screened with IC50 ranging from 0.030mg/ml to 4.4mg/ml. (Table 27, Fig. 5 & 6). Isolates, UL7B, ULS13, ULS14 and ULK2, inhibited all the cancer lines screened at low concentrations.

Table 23: Antibacterial Activity of Bioactive Actinomycetes Strains (cross-streaking assay) (Davies *et al.*, 2015a)

Fractions	*E.coli*	*Pseudomonas aeruginosa*	*Shigella Flexneri*	*Staphylococcus epidermidis*	*Salmonella typhi*	*Enterococcus faecalis*
UL7B	-	-	-	5mm	-	7mm
ULS7	-	-	-	5mm	-	5mm
ULK2	-	-	-	-	6mm	5mm
ULS12	-	-	-	6mm	-	-
ULS13	4mm	-	4mm	7mm	-	-
ULK3	-	-	10mm	-	-	-
ULK10	-	-	5mm	-	-	7mm
ULK11	-	-	5mm	7mm	-	-
ULMA40	-	-	10mm	7mm	-	5mm
ULG2.17	-	-	-	4mm	-	-
UL28f	-	-	-	-	5mm	-
UL28a	-	-	-	7mm	-	-
UL23a	-	-	-	3mm	-	-
ULMa27	5mm	-	-	-	-	4mm
ULS14	15mm	2mm	15mm	27mm	25mm	10mm
ULT1	-	-	-	7mm	-	-
ULMa36	-	-	8mm	-	-	6mm
UL28d	-	-	-	5mm	-	-
ULG1.08	-	-	5mm	-	-	-
UL31c	-	-	-	5mm	-	-
UL19b	-		-	5mm	-	-

Table 24: Antifungal Activity of Bioactive Crude Extracts of Actinomycetes Strains (Kirby-Bauer assay) (Davies – Bolorunduro *et al.*, 2016)

Test Organisms	*Trichophyton mentagrophytes* A1	*Trichophyton mentagrophytes* A2	*Trichophyton rubrum*	*Candida albicans*
UL7B	33mm	33mm	29mm	16mm
ULS7	32mm	34mm	24mm	20mm
ULK2	28mm	30mm	26mm	20mm
ULS12	25mm	26mm	23mm	18mm
ULK7	32mm	27mm	21mm	-
ULK3	24mm	29mm	22mm	23mm
ULK10	-	-	-	15mm
ULK11	26mm	29mm	26mm	12mm
ULMA40	28mm	29mm	21mm	4mm
ULMa27	29mm	32mm	27mm	-
ULS14	27mm	28mm	24mm	20mm
ULS13	-	-	-	5mm

Table 25: Minimum Inhibitory Concentration (MIC) (mg/ml) of Bioactive Crude Extracts against Bacterial Pathogens (Davies – Bolorunduro et al 2015b)

Test Organisms	Gram Reaction	UL7B	ULS7	ULS12	ULS13	ULK3	ULK10	ULK11	ULMA40	ULMA27	ULS14	ULK2
E. coli	-	-	-	-	-	-	-	-	-	10	5	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-
Shigella flexneri	-	-	-	-	-	5	-	-	2.5	-	2.5	-
Staphylococcus epidermidis	+	10	10	10	10	-	-	10	10	-	0.078	-
Salmonella typhi	-	-	-	-	-	-	-	-	-	-	0.156	10
Enterococcus faecalis	+	10	10	-	-	-	10	-	-	10	5	10

Table 26: Minimum Inhibitory Concentration (MIC) (mg/ml) of Bioactive Crude Extracts against Fungal Pathogens (Davies – Bolorunduro *et al.*, 2015b)

Test Organisms	UL7B	ULS7	ULK7	ULS12	ULS13	ULK3	ULK10	ULK11	ULMA40	ULMA27	ULS14	ULK2
Candida albicans	5	1.25	0.625	1.25	0.156	0.156	2.5	2.5	-	-	0.625	1.25
Trichophyton mentagrophytes A1	0.156	0.156	0.156	0.156	-	0.156	-	0.156	0.156	0.156	0.156	0.156
Trichophyton mentagrophytes A2	0.156	0.156	0.156	0.156	-	0.313	-	0.625	0.156	0.156	0.156	0.313
Trichophyton rubrum	0.156	0.156	-	0.156	-	0.313	-	0.156	0.156	0.156	0.156	0.313

Table 27: Antitumour Effect of Actinomycete Crude Extracts on Cellines (mg/mL) (Davies – Bolorunduro *et al.*, 2015a)

Cell lines	UL7B	ULS7	ULK7	ULS12	ULS13	ULK3	ULK10	ULK11	ULMA40	ULMA36	ULMA27	ULS14	ULK2	ULG1.08
HL-60	0.083	-	-	-	0.096	-	-	-	-	-	-	0.640	0.083	-
AGS	0.095	2.320	1.231	1.981	0.030	-	2.14	-	-	-	-	0.075	1.543	-
K562	0.045	-	1.250	2.371	0.101	-	3.124	-	-	-	-	0.203	0.078	-
MCF-7	1.315	1.181	2.251	2.082	0.125	-	2.163	-	-	-	-	0.139	2.176	-
HeLa	2.277	0.312	1.251	2.031	0.070	-	0.240	-	-	-	4.401	0.040	0.034	-

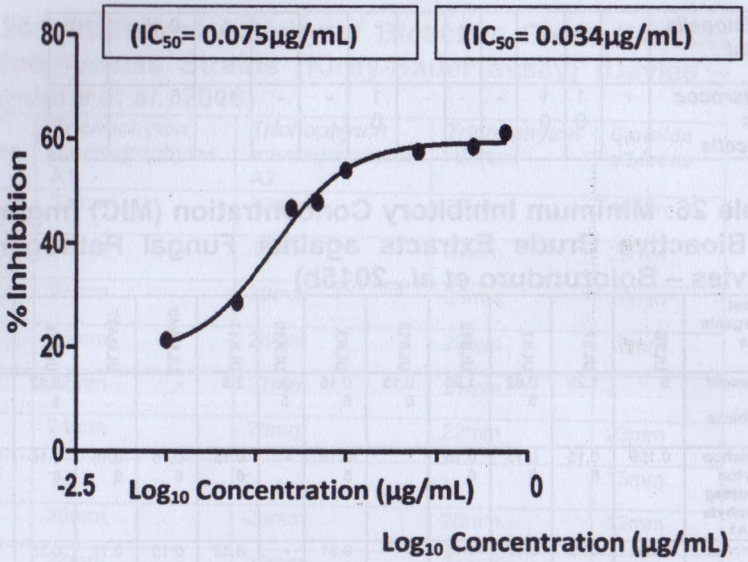


Figure 5:- Inhibition Concentration (IC_{50}) of Compound ULDF4 against HeLa celline

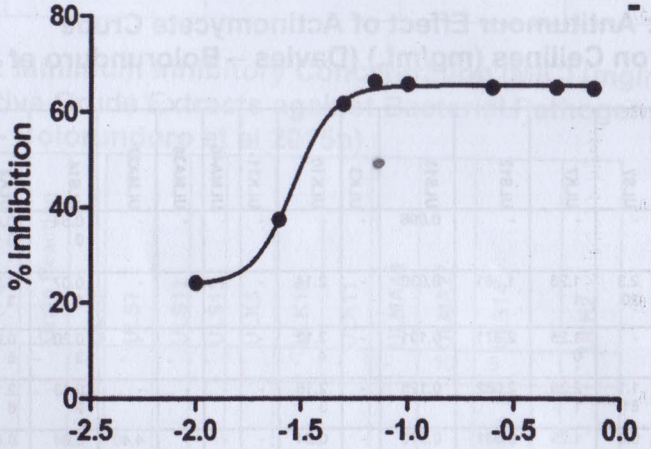
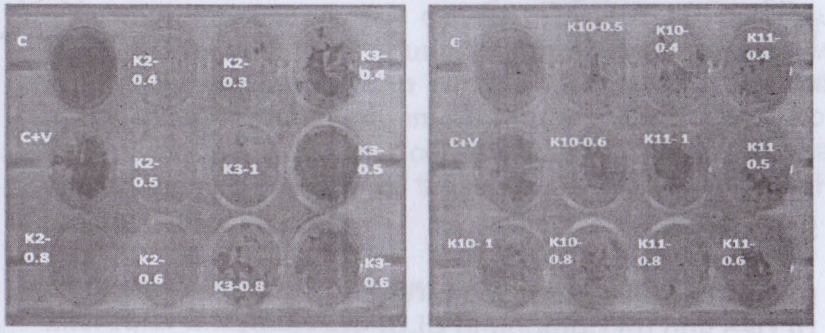


Figure 6:- Inhibition Concentration (IC_{50}) of Compound ULDF5 against HeLa celline

However, extract from the isolate *Streptomyces bingchenggensis* ULS14 was the most active. *Streptomyces avermitilis* ULK3 showed significant antiviral activity when screened against influenza virs HIN2, having activity at concentration as low as 0.5mg/ml (Plate 8a &b).



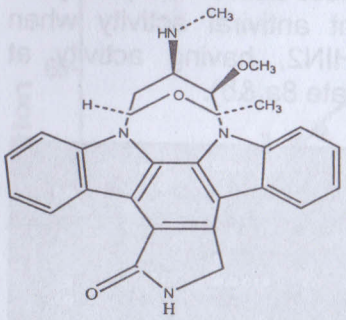
A B

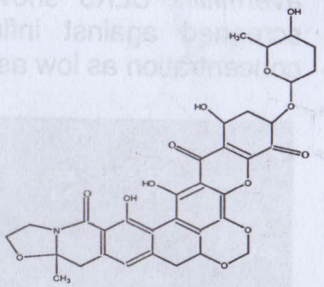
Plate 8: Antiviral Activity of Crude Extracts of Actinomyetes against H3N2 Influenza Virus (Davies-Bolorunduro *et al.*, 2016).

Legend: C= control (cells) without virus, C+V= control (cells) infected with virus, K2 (0.8-0.3) = concentrations of crude extracts of ULK2, K3 (1-0.3) = concentrations of crude extracts of ULK3 in mg/mL, K10 (1-0.4) = concentrations of crude extracts of ULK10 in mg/mL, K11 (1-0.4) = concentrations of crude extracts of ULK11 in mg/mL

The diversity of the actinobacterial community determined using DGGE identified more species such as *Arthrobacter phenanthrenivorans, Arthrobacter arilaitensis* and *Micrococcus luteus* (Davies – Bolorunduro *et al.*, 2015b). Two bioactive compounds with antimicrobial and antitumour properties were isolated, purified, and structural elucidation based on ESI-MS, IR and NMR data obtained revealed the compounds were structurally related to staurosporine and kigamicin.

(a) (b)





Staurosporine Kigamycin

Figure 7a & 7b: Proposed Structures of (a) Compound ULDF5 and (b) Compound ULDF4

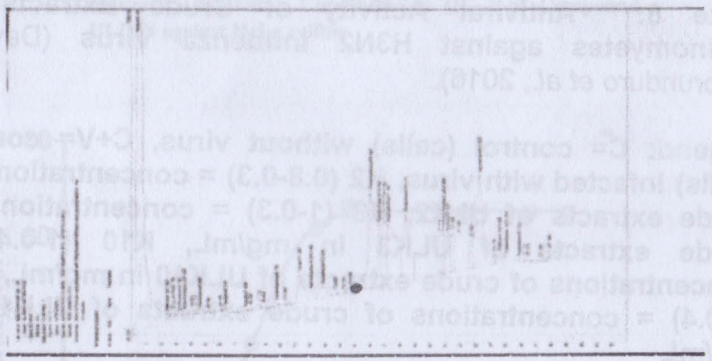


Figure 8: GCMS Results of Antibiotics Profile of Isolates S12

The GC/MS results showed more than twenty antibiotic (Fig. 8), peaks from extracts of each isolate and this milieu of antibiotics include Rifamycin, Erythromycin, Tetracenomycin D, Kanamycin, Nystatin, Oxytetracycline, Marinomycin, Asukamycin, Chloramphenicol, Streptomycin, Himalomycin A, (Davies *et al.*, 2015c), Hyoscymine (Davies *et al.*, 2015d) Antimethicillin resistance agents (Davies *et al.*, 2015e) etc.

Other unidentifiable peaks were also seen which may indicate novel and not-yet-identified antimicrobials.

Mr. Vice-Chancellor Sir, this study revealed for the first time, actinomycete diversity of Lagos Lagoon and their potential as sources of novel antibacterial, antifungal, antitumour and antiviral compounds which can be exploited for therapeutic purpose. **Indeed, the four Actinomycete species with antitumour properties can serve as lead drug candidates in the quest for new antitumour therapy worldwide.** I would also like to reveal that our collaborators in South Africa have offered to screen some of these Actinomycetes for anti-HIV properties.

Contributions in other Areas of Microbiology

Microbial Enzymology

My first exposure to research during my Masters degree at Ife was in the area of enzymology and my first two publications in this field on alpha amylase still remain evergreen:

ADELEYE, I. A. (1990a).

ADELEYE, I. A. (1990).

Other contributions in this field include the following:

ADELEYE, I. A. and OMOTOSHO, G. D. (2003)

ADELEYE, I. A. and LASHEBIKAN, O. M. (2003)

ADELEYE, I. A. and LASHEBIKAN, O. M. (2004)

Environmental Microbiology: I also made a forays into this area of field microbiology and contributed the following papers:

ADELEYE, I. A. and ADEBIYI, A. A. (2003). ADELEYE, I. A. and ERUBA, S. (2004). ADELEYE, I. A., OKORODUDU, E. and LAWAL, A. K. (2004). ADELEYE, I. A. and OSHIDIPE, O. O. (2004).

ADELEYE, I. A. and ESAN, A. A. (2001).

Food Microbiology

My Sole contribution in this important area of microbiology is on fermentation by wara (local cheese).

(1). ADELEYE, I. A. and ONASANYA, O. A. (2000).

Recommendations

1. Funding of marine Drug Research: following from my research finding, the Lagos, Lagoon just behind us is a repository of many microbes that can be exploited for therapeutic purposes. In this direction, Mr. Vice Chancellor sir, I wish to suggest the need for funding of research activities focused on the discovery and development of new and effective antimicrobial and antitumour agents from marine environments.
2. Improved Sectoral Allocation to Education & Research: This has been a recurring problem with successive government since our independence. It is even worse under this present dispensation, UNESCO recommendation of 26% sectoral allocation should be a benchmark in order to improve our educational standard. It is an established fact that the leel of academic attainment is directly related to the amount of fund available for research. We can learn from South African model whereby every published article attracts financial reward. Not only this, researchers receive grants for attracting post graduate students from other countries to work in their laboratories and in the event of that student being visited by his or her home based supervisor, special grant is made available to sponsor such visits. All these go a long way to foster international collaboration.
3. Formation of Research Groups: We need to streamline our research efforts by forging collaborations among individuals researches in identical area. I am happy the University of Lagos is looking toward this direction through the office of Research and Innovation centre. The advantage is very obvious because no individual can be an expert in all the various components but rather a strong team work can deliver a robust research output. Lecturers should be made to key into established research groups rather than floating new ones. In this way, faculties can have clearly defined research forcus for the purpose of attracting grants locally and internationally. Duplication of research proposals should be discouraged.

4. Solving the Accommodation Problems FOR Staff: Mr. Vice Chancellor Sir, one of the greatest problems in Lagos is accommodation and there is so uch competition for the few ones on campus much so that young and vibrant lecturers are denied on account of length of service and rank. I want to suggest, Sir, that existing boys-quarters be allocated to this group of lecturers to ameliorate their suffering since many cannot afford to rent flats outside the campus. Moreover, there should be a time line for occupying university quarters by individuals. A stipulated maximum period of say 20 years should be put in place after which the occupant is expected to have acquired a personal house and give room to others on the queue.

The University quarters should not become "old people's homes" or hospices for geriatrics. If we adopt these measures staff will be encouraged to build and occupy their houses rather than live in the University quarters for eternity.

Conclusion

Mr. Vice – Chancellor Sir, distinguished members of this audience, after listening to this lecture, you will agree with me that HIV and its deadly co-travellers and "partners-in-crime" can still be put in check. Although, there is no vaccine against the virus, mankind can take solace in the fact that the virus itself does not kill but the co-travellers are the killers. Medicinal plants do come handy in tackling the drug resistant "co-travellers" and research into marine environment is unearthing new and more potent therapeutic agents that will give succour to mankind.

Acknowledgement

Mr. Vice-Chancellor Sir, I am grateful to the Almighty God for His faithfulness, goodness and mercy over me and for making today a reality. I remain grateful to Late Professor Adetokunbo Sofoluwe, in whose tenure I became a professor. I immensely thank and appreciate the Vice-Chancellor, Professor Rahamon Bello; the former and current DVC Academic and Research, Professors Babajide Alo, Oluwatoyin Ogundipe,as well as the current DVC Management Prof. Duro Oni. I also thank the entire University management team for the support given to the Department of Microbiology while hosting of the 38th Edition of the Nigerian Society for Microbiology Annual General Meeting and Conference (Unilag 2015) during my tenure as the Head of Department. The hosting has been adjudged the best so far in the history of that association.

I appreciate my parents, Late Pa Michael Dada Adeleye and Mrs. Abigail Mopelola Adeleye, who although not lettered, made sure I received decent education. I want to thank my uncles, the Olojos; Uncle Femi, Chief Tunde, Late Wale, Jide, Boladale, my "twin" brother "Kuge", for their encouragement and assistance all through the years. My nieces and nephews Funmilola Faleye, the Dosunmus, the Awodeles, the Adesolas, my cousins; Durojaiyes, Babatundes, Dr. and Dr. (Mrs.) Adedayo, Segun Olalemi are all appreciated. Thanks are also due to my in-laws especially my father-in-law; Late Pa Gabriel Akinwunmi Olaleye, my mother-in-law; Mrs. Sabaina Taiwo Olaleye and all the Olaleye family at Ilesa, the Adeloyes especially the Modakeke extended family.

I would also like to appreciate my spiritual father; Rt. Rev. Samson Osundina, the Bishop of Ikorodu Diocese Methodist Church, Nigeria. So also the lay President of MCN, Ikorodu Diocese, Sir Ayodele Eleso J.P. and the executive of the Men's Fellowship both at the Lagos Arch-Diocese and Ikorodu Diocese.

I appreciate my direct siblings, Mr. Adedokun Adeleye, Mrs. Arike Dosumu, Mrs. Mowunmi Faleye and all the neighbours at Olusojo Street, Bariga where I grew up. Let me apologise that if your name is not listed or mentioned in this book, it is not because

I forgot you but it is simply because you exist in the sublime softcopy which is in the innermost recess of my heart and too precious to let out.

I am eternally grateful to all my classmates at the Ilesa Grammar School Class of 1969. I was the smallest and almost the youngest and hopefully I have not disappointed you all. I cannot but mention a few names: The President, Mr. Diran Akinjogbin, Mr. Segun Ayeni, Prof. Bisi Balogun (Former VC FUTA), Mr. Olu "Eja" Adebiyi, Prof. Wole Agbede and our own Prof. Ajike Osanyin, Barristers Awobiyi, Muyiwa Aduroja (SAN), Engr. Kola Adedeji, Arch. Femi Ikotun, Moji David and a host of others too numerous to mention.

During my A-Level days, my path crossed many individuals but two made a lasting impression and I will mention them. First is Omo-oba Yemisi Shyllon, an Engineer, a Lawyer, an Industrialist and foremost Art Historian collector and a Patron of many clubs including Kegites Club Ilya du Lagoon, so also Chief Titi Odimayo, an Industrialist who still remains a close friend.

At this juncture, I would like to appreciate my academic fathers, first is Prof. Patrick O. Olutiola, one of the foremost microbial physiologist, who initiated me into the rigours of academic research. Second is Prof. Igbekele Adeyemi ADETOSOYE who said to me that Ph.D is a 3 letter word, I can give you a Ph.D in record time but I want to train you well so that you will be able to train others. Sir, I appreciate your training and mentorship which opened new vistas in my research life.

I am indebted to all my research collaborators especially those who admitted my Doctoral students into the various laboratories in various continents. To mention a few, they are: Professor Pascal Bessong, University of Venda, South Africa; Prof. Denzil Beukkes of Rhodes University, South Africa, Prof. George Wang and Prof. Ming Luo of Georgia State University, Atlanta, Georgia, USA and Prof. Rainer Haas, LMU School of Hygiene and Medical Microbiology, Munich, Germany.

At local level, Dr. Stella Smith of Nigerian Institute for Medical Research, Prof. Sulaimon Akanmu Department of Haematology and Drug Transmission; Lagos University Teaching Hospital Idi – araba, and Mr. Solomon Bamiro; Department of Obstetrics and Gynaecology, College of Medicine University of Lagos.

I appreciate my students over the years at both postgraduate and undergraduate levels especially Mr. Chinedu Obosi, the first M.Sc. student to work on bacterial blood stream infection of HIV patients in Lagos and who accidentally inoculated himself in the process of recapping the syringe used for drawing blood from an HIV patient. Luckily, he was placed on antiretroviral medication immediately and tested negative a month later. Others are Dr. Shade Daniels, Bisi Davies-Bolorunduro, Morrison Opara, Biodun Seriki, Chioma Kunle-ope, Bose Elesinnla, Abraham Ajayi, Felix Alao and many others.

Worthy of special mention are my colleagues in the Faculty of Science most especially staff of the Department of Microbiology (academic, non-academic and administrative staff); thank you all. Prof. Dele Olowokudejo, Prof. O. Omidiji, Prof. Dupe Akinola, my boss at BCOS, Prof. Wale Okunnuga, Prof. Wesley Okei, Prof. Dotun Adekunle (Dean), Prof. Caroline Umebese, Prof. Clem Edokpayi, Prof. Dike Nwankwo, Prof. M.A.C Chendu, Prof. Obinna Chukwu, Prof. Charles Nwadiae, Prof. Bayo Otitoloju, Dr. Ropo Akinsoji, Dr. Folarin Oguntoyinbo, My friends from other faculties, Prof. Ike Mowete, Prof. Iyiola Oni, Dr. Alabi Soneye, Dr. Kehinde Oladeji and my ASUU comrades; Karo Igbinaka, Dr. Laja Odukoya, and Dr. Ashiru are appreciated.

I cannot forget my old friends at Yaba Tech, Mrs. Jumoke Sobande, Dr. Peter Egbenni, Mr. "Kayo" Durojaiye, Mr. "Balo" Balogun, etc.

On the social front, thanks are due to members of Ijesa Socialite Club; Hon. Tunde Ajilore, Mr. Niran Obembe (SPIFCO MD). Mr. Sola Ogedengbe, Mr. Bode Ekunseitan and Mr. Bosun Esan. Also, President of Ijesa Sports Club, Engr. Garus and V.P Tunji Ademosoye and all club members are appreciated. Members of

the Elder's table Unilag Staff Club and the president, Bayo are appreciated.

On the home front, I would like to appreciate my two daughters who are the gift of love from God; Miss. Sandra Ayomide ADELEYE (my baby lawyer) and Miss. Elfrida Tolulope ADELEYE. Finally, from the bottom of my heart, I appreciate my better half, my love, my sister and my companion, Josephine Mojisola ADELEYE for all the support, encouragement and endurance.

Distinguished Ladies and Gentlemen, I thank you all for sparing the time and listening to this inaugural lecture. I wish you all God's blessing.

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