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MAN AGAINST MICROBES:

NO VICTOR, NO VANQUISHED

By

Deboye Kolawole

Professor of Medical Microbiology

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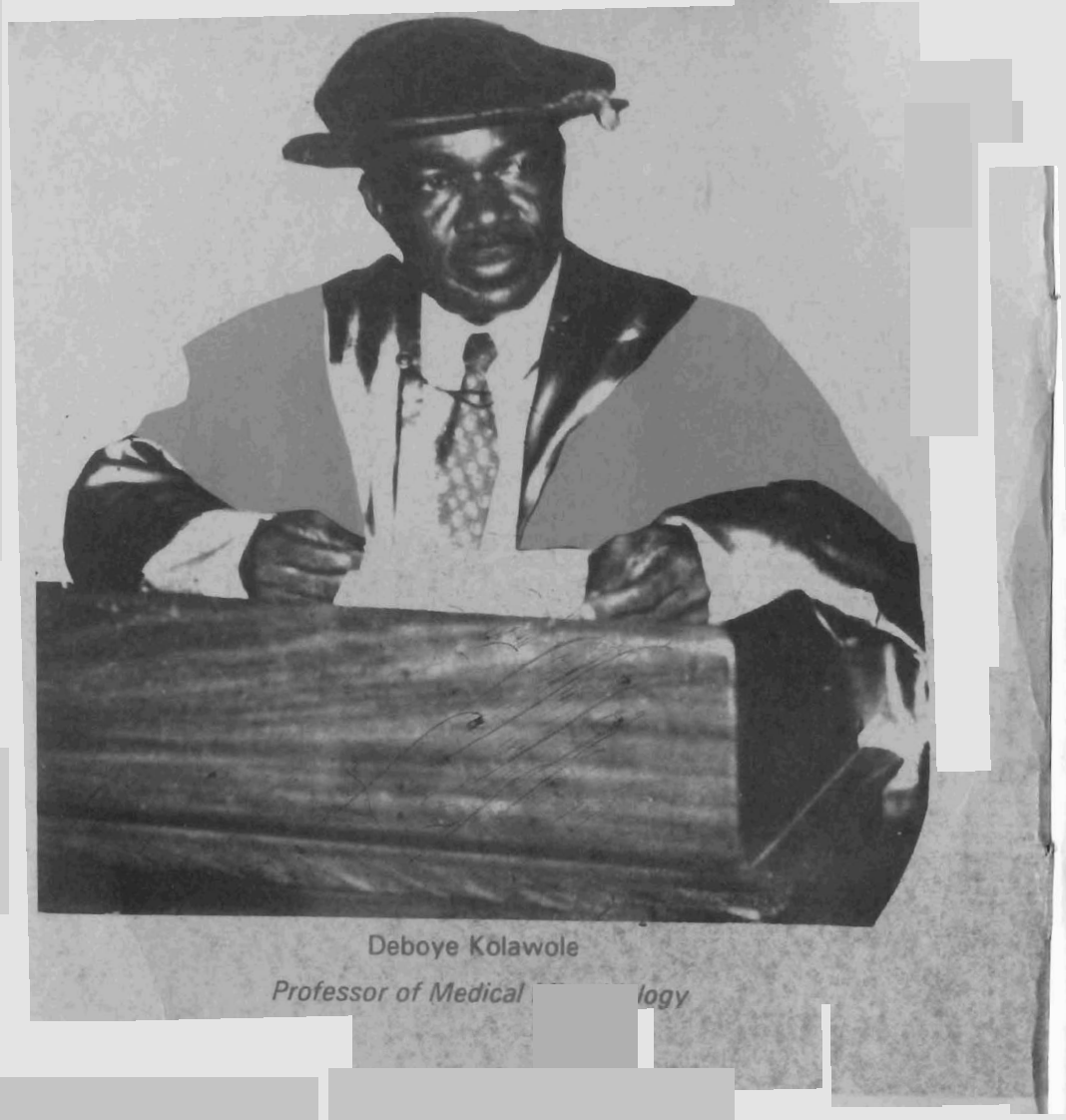

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INTRODUTION

Preamble and Background to the Topic

I give all glory to God Almighty and thank the authorities of the Obafemi Awolowo University for the privilege to give this inaugural lecture today. I was appointed by this University as an Assistant Lecturer in Microbiology a little over twenty-six years ago. I stand before you today as a Professor, a post to which I was promoted eight years ago, to share with you, my experience up-to-date.

My interest in Medical Microbiology was triggered off as a young boy in secondary school by the story of how Anton van Leeuwenhoek (1632 - 1723) a draper from Holland, made single-lensed microscopes with which he "saw" organisms in materials scraped from his own mouth. He made such accurate drawings of what he saw that they can still be recognized and identified with similar organisms seen under modern-day light microscopes. That there were things in the mouth other "than met the naked eye" intrigued me. This interest was intensified when, in my final year B.Sc. at the University, I had the opportunity of offering Microbiology as a special topic. At the end of that year, I won a Postgraduate scholarship for being the best overall student in my class, which I utilized for Masters degree studies in Medical Microbiology in the Faculty of Medicine, Ahmadu Bello University, Zaria. This marked the beginning of my interest in disease causation and the difficulties encountered in the treatment and/or eradication of the causal agents.

The topic of my lecture today - **"MAN AGAINST MICROBES: NO VICTOR, NO VANQUISHED"** - is suggestive of a battle between man and microbes, which cannot be said to have been won or lost on either side. Man is the highest of God's creation, to whom He gave dominion over all other creatures, while microbes are organisms that are too small to be seen with the naked eye, but are present in large numbers everywhere. Both man and microbes have therefore existed side by side through the ages, with man not being able to see the microbes until in the nineteenth century, but all the while seeing and experiencing the effects of their activities, especially the harmful effects, which helped to draw attention to them. The link between microbes and these harmful effects was not easily established, as will be seen in the following account.

Linking the Unseen (Microbes) With the Seen (Harmful Effects)

Early records (Brock, 1961) referred to plagues and pestilences which were attributed (solely) to real and hypothetical factors in man's environment, including cosmic influences, witchcraft, seasonal

variations and 'bad' air. However, the spread of disease from a sick person to others in the community was recognized early and, in the Biblical Book of Leviticus, isolation of sick persons to curtail the spread of certain skin and venereal diseases was already being practised. In 1546, Hieronymus Fracastorius stated in his publication *De Contagione* - that diseases could be spread by direct contact between individuals, via inanimate objects such as clothing and personal possessions (fomites) or through the air. He even called the causal agents "invisible seeds", "germs" or "small infective particles", which could multiply and propagate their likes but he could not demonstrate their existence. Using his series of simple microscopes, Anton Van Leeuwenhoek also reported the presence of minute living creatures in water from different sources and in decaying animal and vegetable matter which had been left to stand for a week or two at room temperature.

Subsequent workers in the field who tried to explain the origins of these microbes and disease were divided into two main schools of thought. While some believed in spontaneous generation, i.e., that microbes simply just came into being, others said that they developed from seeds or germs that had come from the air, i.e., the Germ theory. Since there was still no factual support to link the microbes with diseases, the theory of spontaneous generation remained very popular and the idea of infective agents arising from their likes was not resolved for another two hundred years.

In the 18th century, the central figures in the controversy were Needham, an Irish Catholic Priest and the Italian, Lazzaro Spallanzani. Needham held the view that microbes re-appeared in infusions which had previously been heated to kill all living creatures while Spallanzani insisted that this did not occur if the heating was vigorous enough and air was subsequently excluded from the container. In 1765 and 1776, Spallanzani used Needham's own experiments to prove his point, thus dealing the first major blow to the theory of spontaneous generation and opening the way for more scientific reasoning.

In 1765, Gordon of Aberdeen in Scotland demonstrated that only women visited by doctors or nurses who had previously attended to patients affected with puerperal fever developed the disease in an epidemic. He therefore recommended the washing and changing of clothes to prevent carriage of infective particles from an infected woman to others. Towards the middle of the nineteenth century, Semmelweiss observed that the spread of puerperal infection by students and medical practitioners who habitually went from the post mortem room to the maternity wards could be reduced by the observance of regulations governing general cleanliness, e.g., washing of hands in a solution of chloride of lime. In 1835, Agustino Bassi, an

Italian Civil Servant, showed that the calcino disease of silkworms was caused by a fungus which invaded their tissues and could be transmitted by the inoculation of material from such tissues into those of a healthy one. These observations, together with the availability of improved microscopes, initiated the systematic description of microorganisms and, by 1838, Ehrenberg had introduced terms such as *bacterium, vibrio, spirillum* and *spirochaete*. In 1850, Rayer and Davaine reported the presence of rod-shaped organisms in the blood of animals that had died of anthrax and Davaine later (1886) demonstrated the transmission of this disease by inoculating blood containing such rods into healthy sheep which then developed anthrax.

The foundations of modern microbiological technique were, however, laid by a French Chemist, Louis Pasteur (1822 - 1895), in his studies of fermentation between 1855 and 1860. He showed that lactic and butyric acid fermentations were carried out by bacteria while alcoholic fermentations (in the production of beer and wine) were by yeasts. He also showed that there was a direct relationship between particular microorganisms and the type of fermentation produced and that living microorganisms were always derived from exactly similar living organisms. This confirmed the works of Spallanzani (1765, 1776) and Bassi (1835-6) and swept away, once and for all, the theory of spontaneous generation. His other works resulted in the rescue of the silkworm industry from an infectious disease, eradication of anthrax by immunization of farm animals, immunization of fowls against chicken cholera and of man against rabies. These outstanding experimental results were applied by Lister, a Professor of Surgery in Glasgow, in introducing the use of antiseptics in the management of wounds in 1867.

In 1840, Henle had pointed out that a microbe causing a disease should be present in every case and should be able to produce a similar disease in animals into which it was later inoculated. These ideas were later expanded in 1870 by Robert Koch (1843-1910), a German General Practitioner. Following from the work of Davaine on anthrax, he produced pure cultures of the rod-shaped organisms seen in the blood of animals suffering from anthrax and was able to reproduce the disease by infecting his cultures into other animals. These criteria for establishing the causal organism of a disease became what are collectively called "Koch's postulates" today. Koch and his students subsequently identified the causal organisms of tuberculosis (1882), cholera (1883), typhoid, diphtheria and many other major diseases of man and his animals. They devised the use of aniline dyes for staining microbes, of oil immersion microscope objectives for examining them and of media solidified with agar for growing them. These giant

scientific and technical advances have contributed immensely in establishing the germ theory of disease as opposed to spontaneous generation, and in making medical microbiology what it is today. They made it possible for Alexander Ogston, a Scottish Surgeon (1880-1882) to show that cocci organisms produced inflammation and suppuration and were the main causes of acute abscesses. He discovered and named staphylococci and distinguished them from streptococci. With the establishment of the link between microbes and many infectious diseases, the battle line between man and the microbes was at last clearly drawn, and the ways and means of fighting them clearer. The nature of this battle and its present status, is the theme of my lecture today.

MICROBES AND DISEASE

The range of organisms collectively called microbes overlaps across the provinces of botany and zoology with the common characteristic that they are single-celled or unicellular. They include algae, protozoa, fungi, viruses and bacteria, and can be found in widely diverse habitats ranging from hot springs to the human body, the soil and the depths of the ocean. It has been estimated that the total mass of microbes in the world is about five to twenty-five times the total mass of all animal life, both aquatic and terrestrial (Postgate, 1992).

Only a small proportion of the microbes, however, are disease producing or pathogenic, as most are free-living in soil, water and similar habitats and are unable to invade the living body (Duguid *et al.*, 1985). Disease-causing microbes are therefore in a minority. I have worked mainly in the field of bacteriology and therefore, for most of the rest of this lecture, I shall be reporting observations made mainly on bacteria, especially their involvement in disease and man's efforts to curb them.

Bacteria as Pathogens

Any microbe which, given suitable circumstances, can cause disease, is called a pathogen. Bacteria were the first disease-causing microbes to be so identified, as shown in the account above, before pathogens were recognized among the fungi, viruses and protozoa. Compared with the large numbers of bacteria that are free-living, only a few bacteria cause disease, but this small number causes most (about 85%) of the infectious diseases known, thus exerting an enormous impact upon human welfare (Mims *et al.*, 1992).

Two major categories of pathogens are recognized-true and opportunistic pathogens. True pathogens are those bacteria which possess innate properties which enable them to overcome the body defences and invade the tissues of a normal healthy subject. Their growth in the tissues, or their production of poisonous substances (otherwise called toxins), damages the tissues and causes the manifestation of disease (Duguid *et al.* 1985). Opportunistic pathogens are those which do not usually behave as pathogens but may in fact be part of the body's own microflora, living on the skin and on the mucous membranes of the upper respiratory tract, intestines and vagina, and obtaining their nourishment from the secretions and food residues. Normally, they do not invade the blood or tissues, and are generally harmless, maintaining a delicate balance which can be upturned at any time, to result in disease, as we will see later. A good example is of *E. coli* organisms which are harmless commensals in the gut but which cause infection in the urinary tract.

Some diseases can be caused only by a particular pathogen or particular strains of that species, i.e., the link between disease and pathogen is highly specific (Singleton, 1992). An example is anthrax by *Bacillus anthracis*. In other cases, a disease may be due to any of several different causal agents, e.g., gas gangrene can be caused by more than one specie of *Clostridium*.

Bacterial pathogenicity factors

Route of Infection and Adhesion by the Bacteria

Although the skin is normally an effective barrier, when it is broken by wounding, insect bites, surgery, etc., pathogens may gain access to cause either systemic or localized disease. The mucous membranes of the intestinal, respiratory and genitourimary tracts are usually more vulnerable than the skin and hence many infections begin at these sites when they act as portals of entry (Singleton, 1992).

In the early phase of infection, most bacteria will need to struggle against the flushing action of body secretions, e.g., in the genitourinary tract, ciliary action and peristalsis, all of which tend to discourage the establishment of a pathogen on the mucous membrane and other surfaces. Hence there is a need for attachment mechanisms to help the microbes adhere firmly to the tissues while also competing with the host's own microflora. Examples of bacteria possessing fimbriae for attachment are the enterotoxigenic *E. coli* (ETEC) associated with travellers' diarrhoea.

Pathogenesis

This is the mechanism of disease development, and it differs from one pathogen to another. Some produce toxins which adversely affect certain physiological functions while others grow and invade particular

cells or tissues. In some diseases, the symptoms result from a hypersensitivity or "over-reaction" of the body's own defence mechanisms to the presence of the bacteria.

(a) **Toxigenicity** - The bacteria produce and may liberate the toxins into the environment in the host body, where they act on the cells to produce the symptoms typical of the disease, e.g., in cases of cholera, botulism, tetanus, etc. Toxins are protein in nature and each has a single specific mode and site of action. When released into the surrounding environment, they are called exotoxins. In other cases, the toxic materials are not released by the bacteria, as they are actually components of lipopolysaccharides found in the cell walls of Gram-negative bacteria. Here they are called endotoxins and are released only after autolysis or artificial disruption of the bacterial cells. When present in blood, either on whole cells or as fragments of lysed cells, they initiate a number of non-specific reactions, e.g., endotoxic shock.

(b) **Invasiveness** - This is the capacity of bacteria to invade and multiply in healthy tissues, and is dependent upon the production of certain substances, called aggressins. The capacity of some bacteria to produce disease appears to depend almost entirely on this, e.g., pneumococcus, while in others these work hand-in-hand with toxigenicity (Duguid *et al.*, 1985). For example, *Streptococcus pyogenes*, a characteristically invasive microbe produces erythrogenic toxin while its invasiveness (and that of *Staphylococcus aureus* and *Clostridium welchii*) is due largely to the production of hyaluronidase (spreading factor). Other aggressive factors include fibrinolysin or kinase in *Strept. pyogenes* which also acts as spreading factor, depolymerizing enzymes, e.g., collagenase, a nuclease produced by *Cl. welchii* in gas gangrene and neuraminidase, a mucinase produced by many bacteria which catalyses the hydrolysis of mucoproteins at cell surface and hence facilitates attack on the cell.

(c) **Evasive factors and strategies** - These are the factors and strategies by which a pathogen evades host defences. Some pathogens have anti-phagocytic capsules, e.g., the capsule of *Strept. pyogenes* is made of hyaluronic acid which is normally a component of animal tissues; this represents a camouflage which appears to confer protection against phagocytosis. Certain pathogenic bacteria, e.g., staphylococci and streptococci produce leucocidin, a substance which can damage or even kill phagocytes (white blood cells). For example, the Panton-Valentine leucocidin of staphylococci lyses both macrophages and polymorphonuclear leucocytes. Such organisms are

therefore able to prevent being ingested and digested by these cells. Another evasive mechanism is the change in the cell-surface chemistry and, hence the antigens, such that the microbes avoid the effects of specific antibodies already produced against previous antigens, e.g., *Borrelia* sp., the causative agent of relapsing fever.

(d) **Destruction of host cells or tissues** - Some pathogens destroy host cells or tissues at the site of infection, especially on the mucous membranes of the intestinal tract and blood. Examples are *Salmonella typhi* which causes an intense inflammation of the small intestine leading to necrosis (death) and haemorrhage, and the enteroinvasive *E. coli* which invades and destroys cells in the small intestine and colon, resulting in abdominal pain, profuse watery stools and subsequent dehydration. In the oroya fever caused by *Bartonella bacilliformis,* the growth of the bacteria results in the destruction of red blood cells and, hence anaemia.

Organ Specificity

Many pathogenic microbes have affinity for specific tissues or organs in the host body, e.g., pneumococci have a predilection for lung tissue where they cause pneumonia and associated symptoms, while meningococci are attracted to serous membranes of the brain and cause meningitis.

Previous Involvement in Disease

The sustenance of virulence in certain bacteria requires frequent transfer from host to host. The enhancement of virulence by such frequent passage, whether naturally or artificially, has been demonstrated in staphylococci by several workers [Adlam *et al.*, 1970 (a), (b); Kolawole, Omolayole and Lamikanra, 1988]. Hence a microbe taken from the site of infection into another host will exhibit a greater virulence than in the previous infection

MAN AGAINST MICROBES: HIS WEAPONS

It has been shown earlier that bacteria can be found everywhere and, even though a large majority are harmless, the few pathogenic species cause about 85% of all infectious diseases that afflict man. There is therefore a need to eliminate them or to inhibit their activities, to protect man from their harmful effects. In order to do this, man has been equippped with or has developed an array of weapons against them, ranging from constitutive and adaptive body defences, to sterilization, disinfection, antisepsis and antibiosis.

Constitutive or Innate Defences of the Body:

These are the non-specific defence mechanicms already in place in the human body which are in operation all the time. They are made up of exterior and interior defence systems. The outermost of the exterior defence system is the skin which, in an unbroken state, acts as a physical barrier to bacterial infection. It also produces sweat and sebaceous secretions which contain lactic acid and fatty acids that are inhibitory to bacteria, while also giving rise to a lower pH on the skin surface. Should there be a break in the skin and bacteria get through, the membranes lining the inner surfaces of the body secrete mucus which act as a protective barrier as well as inhibit the attachment of bacteria to the epithehal cells. All bacteria and other particles caught in this trap are removed mechanically by ciliary action, coughing and sneezing. Other such mechanical strategies used to protect the epithehal surfaces include the flushing action of tears, saliva and urine. It must be noted that many of these fluids secreted by the body contain microbicidal factors, e.g., acid in gastric juice, spermine and zinc in semen, lactoperoxidase in milk and lysozyme in tears, nasal secretions and saliva. Next in line of exterior defences are the body's resident microflora, which I call "territorial controllers" with which any incoming pathogens must compete for essential nutrients. By virtue of the advantage of previous occupancy on the epithehal surfaces, coupled with the production of inhibitory substances (bactericidins) such as acid or colicins, they frequently succeed in suppressing the growth of many potentially pathogenic bacteria and other microbes.

If, in spite of the above barriers the bacteria still penetrate, two main defensive interior strategies are immediately brought into play. One is the mechanism of phagocytosis, by which macrophages and polymorphonuclear neutrophils (PMN) engulf and destroy incoming bacteria or other particles. These cells are attracted to the "foreigners" by the processes of chemotaxis and a complex series of proteins called "complement". The action of complement results in an influx of polymorphs to the site and an increase in vascular permeability leading to a potent antimicrobial inflammatory response, a key feature in innate immunity.

In addition to the above, there are other extracellular microbicidal agents in the body fluid in concentrations sufficient to inhibit infectious agents. Examples are lactoferrin, which complexes with iron to deprive bacteria of an important growth factor, interferons, which are broad-spectrum anti-viral agents, and properdins.

Adaptive Responses

These are immune responses that are 'tailor-made' to defend or

protect the body against particular species of pathogens. They act generally by linking the incoming pathogenic microbes directly into the various killing mechanisms in the innate system described above, the ensuing reaction being specific for that particular microbe. The facilitator of this specific reaction is the antibody. An antibody is a protein secreted by certain white cells in the host body called B-lymphocytes in response to contact with an infectious microbe, which acts as a foreign antigen. Each antigen, whether whole organisms, their components or products, e.g., toxins has a characteristic surface molecule that acts as its "chemical fingerprint" and the antibody induced by its presence has a recognition site that is complementary (i.e., matching) in shape to this fingerprint. This enables the antibody to bind specifically with various degrees of strength to that antigen. This antigen-antibody complex immediately activates and binds to the complement system (immune adherence) which then induces (opsonizes) the process of phagocytosis by macrophages and polymorphs as described earlier. By this process, the incoming antigen is invariably drawn into the innate mechanism of the acute inflammatory response for destruction.

At the first contact with an antigen some B-cells record the encounter, thus becoming memory cells. Whenever the same antigen comes back, these memory cells enable the body to quickly recognize and respond with a more rapid and vigorous production of specific antibodies against it.

I have described above an adaptive response which occurs naturally in the body. The production of antibodies can, however, be induced artificially by vaccination. This is a process of deliberately introducing into the body, particular antigens of a given pathogen (typically killed cells or cell components) called vaccines, to initiate the production of the corresponding antibodies and the designation of corresponding memory cells in readiness for an attack by that pathogen. This acts as a miniature infection by which the body's mechanisms are trained to recognize and respond promptly on any subsequent exposure to the pathogen.

Physical Control of Microbes

I have described so far the mechanisms with which the body is either naturally endowed or artificially enhanced to deal with invading pathogens. Man has also devised ways by which they can be dealt with outside the body

Sterilization

This is any procedure designed to get rid of all microbial life,

including endospores from an object or substance. There are physical and chemical methods of sterilization.

Physical Methods - These include the application of heat, exposure to ionizing radiation and filtration. Heat can be applied as dry heat, such as burning in incinerators, flaming and in hot air ovens, or as moist heat, e.g., pasteurization, boiling (100°C), in steam above atmospheric pressure and in autoclaves. The effectiveness of heat application in these cases will depend on time of exposure, presence of moisture, the number and condition of the microbes present. Sterilization by ionizing radiation, used mainly in industry, employs β-rays, gamma rays and X-rays and these act mainly by supplying energy for a variety of lethal chemical reactions in the contaminating microbes. Filtration is based on the fact that it is not always necessary to kill microbes to achieve sterilization. It is a process of removing them from a fluid or gas which can be passed through the small pores and passages of a filter and is applicable to those substances which cannot be heated, irradiated or chemically treated. Different pore sizes are normally made for different sizes of microbes to ensure total filtration. Since this process removes whole bodies of microbes, it is sometimes considered superior to methods which kill but leave the substance of the organisms behind.

Chemical Methods - Chemicals offer control that range from sterilization to mere inhibition of activities. Chemical sterilants are usually highly reactive and damaging to living tissues. Hence they require careful handling, and are applied on inanimate objects mainly, including instruments, apparatus, furniture, walls and floors. Examples of chemical methods commonly used are exposure to ethylene oxide, low temperature steam plus formaldehyde or immersion in, or application of liquid agents such as glutaraldehyde, B-propiolactone. A second set of chemicals act as disinfectants, i.e., they are able to destroy, inactivate or remove potentially pathogenic microbes without necessarily affecting the other organisms present, and they usually have little or no effect on bacterial endospores. The activity of disinfectants is governed by factors such as dilution, temperature, pH, the presence of organic matter or detergent and the period of exposure. Disinfectants which kill bacteria are said to be bactericidal while those which just stop or inhibit their growth and/or activities are said to be bacteriostatic. Examples of disinfectants in common use are the phenolics, chlorine, quartenary ammonium compounds (QACs) and hypochlorites. A third set of chemicals are actually disinfectants used in antisepsis, i.e., the disinfection of living tissues. The antiseptics are

disinfectants that are either sufficiently diluted or deliberately formulated to be harmless to human tissue, and can therefore be applied to the skin. They may be used to prevent (prophylactic) or treat (therapeutic) infection. Examples of antiseptics in common use are dettol (chloroxylenol), hexachlorophene, ethanol : water mixture, 'medicated' soaps, certain quartenary ammonium compounds, iodine, savlon (0.5% chlorhexidine + 0.5% cetrimide) and hibitane (0.5% chlorhexidine + 70% alcohol).

Are these sufficient?

The foregoing represents a formidable and intricately-arranged assemblage of forces available to man in combating microbial disease. Under normal circumstances therefore, man should be able to repel or survive every microbial attack or infection, but this is not always the case. The equilibrium between the microbe's virulence factors and strategies, and man's innate body defences can be easily tipped in favour of the microbe in circumstances which pre-dispose him to infectious disease. For example, contact with the HIV virus could lead to the acquisition of the dreaded immune deficiency syndrome (AIDS), thus rendering the constitutive and adaptive body defence systems incompetent to fight infections. Man could be in a state of malnourishment or intoxication of some sort which lowers the efficiency of his resistance to infection. He could be involved in an accident or some other traumatic experience in which the intact skin barrier is broken, thus opening the way for bacterial entry into the body tissues. He may even be under the bondage of bad habits like smoking and excess consumption of alcohol, both of which are known to pre-dispose to diseases. If he, in addition to any of the above, is getting old, his immune system may have been impaired such that the equilibrium tips in favour of the microbe. Apart from the above, the absence inadequacy or failure of immunization programmes such as the Expanded Programme on Immunization (E.P.I.) in Nigeria, could leave man at the mercy of the microbes. Furthermore, poor environmental sanitation and absence of external control of the organisms, as in sterilization, disinfection and antisepsis would allow them to multiply to such great numbers that could easily overwhelm man's weapons of war. Overall, therefore, infectious diseases still occur in man. Hence the need for treatment, and therefore the search for appropriate antimicrobial drugs.

The Discovery and Development of Antimicrobial Drugs

Long before the associations between particular microbes and diseases were established, naturally occurring compounds were already being used in the treatment of infections, with the first on record being the use of cinchona back extract (quinine) for the treatment of malaria in 1619. The causal agent was not identified until 1880. It was a case of fighting an enemy you could not see, and this was the case for many other diseases for a long time. Early in the twentieth century, Paul Ehrlich (1854 - 1915) initiated the search for synthetic substances specifically designed to attack pathogenic microbes. He is noted for developing the arsenical compounds, which were effective against organisms causing syphilis and trypanosomiasis. In 1929, Fleming discovered penicillin while studying the moulds that could inhibit the growth of many bacteria. Domagk introduced the first series of sulphonamides (prontosil) against streptococcal infections in 1935 and, in 1940, Chain and Florey showed that penicillin was an effective chemotherapeutic agent, thus opening the door to subsequent major advances. Within a short period of time, drugs were available to treat virtually all bacterial, fungal, protozoan and worm infections. Particularly, there was a dramatic reduction in morbidity and mortality due to bacterial infections.

In parallel with this rapid development, however, strains of bacteria began to emerge which were resistant to these agents almost as soon as they became available and this has continued to the present day (Lyon and Skurray, 1987; Neu, 1992). Studies on the mechanisms by which bacteria acquire resistance to killing by host body defence systems and various antimicrobial drugs have formed the main thrust of my research work over the last twenty-five years and this is presented in the following paragraphs.

BACTERIAL RESISTANCE

An organism is said to be resistant when it is not inhibited or killed by an antibacterial agent at concentrations of the agent achievable in the body after normal dosage. Some bacteria are naturally resistant (i.e., inherent or innate resistance) to certain antimicrobial agents, e.g., the Gram-negative rods, which possess an additional membrane layer containing sub-layers of proteins, fats and sugars outside the cell wall peptidoglycan and a periplasmic space below it. All these make the overall covering less permeable to large molecules than Gram-positive cells, which possess only the peptidoglycan layer. This impermeability prevents the attainment of an inhibitory concentration of drugs within the cells, thus placing a limitation on the treatment of infections caused by these organisms.

On the other hand, some other bacteria are considered to be innately susceptible to antibacterial agents, e.g., *Streptococcus haemolyticus* has remained sensitive to benzylpenicillin since its discovery while *Treponema pallidum*, the causative agent for syphilis, has remained sensitive to penicillin. However, certain strains within some species do develop or acquire resistance. This happens mainly as a result of attempts, by the organisms, to respond or adapt to environmental changes. The chances of acquiring or developing resistance are greatly enhanced by the rapid rates of multiplication and large sizes of bacterial populations. The emergence of resistance may be sudden in some cases whereas in others, resistance may be slow to emerge.

Mechanisms of Resistance

Previously susceptible bacteria could become resistant, either through changes in their genetic configuration or through alterations in their known biochemical reactions.

Genetic Basis of Antibacterial Drug Resistance

The genetic changes leading to emergence of resistance may be due to any one of the following:

(a) A single chromosomal mutation which occurs spontaneously in a bacterial cell resulting in the synthesis of an altered protein which eventually gives the mutant a selective advantage over the susceptible population when growing in the presence of an antibiotic. The mutants usually survive and outgrow the susceptible population.

(b) Acquisition of antibiotic resistance by the transfer of genetic information. This may be by conjugation, transformation or transduction. Cellular conjugation requires that two cells, the donor and recipient, come together. During this contact, genetic material, in form of DNA elements known as R-plasmids, is transferred through a channel between the two mating cells. Another way by which genetic information can be transferred is via the so-called "jumping genes" or transposons. These genes are able to insert themselves into different genomic sites on chromosomes or plasmids that have no homology with them. Some transposons are simple and carry only information relating to the insertion function while others may be complex, having other genes for resistance to different groups of antibiotics at the same time (multi-resistance). In transduction, the DNA from the donor is carried to the recipient inside a phage
Transfer of genetic information by transformation is found mainly

among Gram-positive bacteria. Here, the naked DNA fragments released upon cell lysis may be absorbed by competent cells and integrated into their genomes.

Biochemical Mechanisms of Resistance

There are three main types of biochemical mechanisms found among strains of drug-resistant bacteria:

(a) Change in the Drug Target

The target enzyme may be changed to a product with a lowered affinity for the antibacterial agent but which still allows normal metabolic processes. In the alternative, additional target enzyme may be produced to overwhelm the antibacterial agent. This mechanism is in operation in resistance to Streptomycin, Methicillin, Erythromycin, Trimethoprim, sulphonamides and Quinolones and appears to be the most common mechanism of resistance to antibiotics.

(b) A Reduction in Cellular Permeability to the Antibacterial Agent

A decrease in the permeability of the cell wall, will decrease the amount of drug to below the inhibitory level at the target. The plasmid-mediated tetracycline resistance in both Gram-positive and Gram-negative species is an example of antagonism to the antibiotic transport system. This amount can also be decreased by an efflux mechanism whereby the drug is pumped out of the cell, e.g., the quinolone efflux system in *E. coli.*

(c) Inactivation of Antibacterial Agent

This involves the production of enzymes which either convert an active drug to an inactive compound or which destroy it outright. This is the most common mechanism against β-lactams, Aminoglycosides, cephalosporins and chloramphenicol. An example is the conversion, by β-lactamase enzyme, of penicillin to inert penicilloic acid by the hydrolytic splitting of the β-lactam ring.

My Experience with the Staphylococcus

Strains of bacteria from the Genus *Staphylococcus* have been my most frequently used bacterial species in my research work. *Staphylococcus aureus* (more recently classified as *Micrococcus pyogenes* var *aureus*) is the causative agent of boils, pimples, spots, various drastic skin conditions, abscesses, osteomvelitis, food poisoning, blood poisoning (septicaemia), PID, Toxic shock syndrome, etc. Of all known bacteria, these organisms are probably the most

ubiquitous and have been in the frontline of identified resistance against antibiotics, having among their strains the so-called troublesome methicillin-resistant *Staph. aureus* (MRSA) which have spread to hospitals in many parts of the world since the early 1960s. Coagulase-negative staphylococci are now recognized as an important cause of human and animal disease, and are also frequently found to be resistant to antibacterial agents.

My work with *Staph aureus* is summarized in the Table 1. It started with investigations into its resistance to host body defence mechanisms before going into its resistance to antibiotic drugs, disinfectants, antiseptics, synthesized novel compounds and extracts of local plant species used in traditional African medicine.

Mechanism of Resistance to Host Body Defence Systems

Early observations on staphylococci taken directly from infected animals indicated enhancements in both their pathogenicity and resistance (Cohn and Hirsch, 1960; Beining and Kennedy, 1963). Growth of original organisms *in vivo* (i.e., deliberate initiation of disease situation in laboratory animals) also gave bacteria that were similarly enhanced in resistance to killing by rabbit PMN and their crude lysates [Adlam, Pearce and Smith, 1970(a), (b)]. This phenomenon was later shown to apply to other staphylococcal strains, thus giving a sufficiently reasonable parameter on which to base examinations of the nature of the conversion, *in vivo*, which enabled the bacteria to survive the onslaught of host defence mechanicms in a disease situation (Pearce, Kolawole and Scragg, 1976). The questions asked were:

(i) What happens to the organisms during growth *in vivo?*
(ii) What is the mechanism of the conversion *in vivo?*
(ii) How soon after inoculation was the conversion made?

First, it was necessary to devise a more accurate experimental system as well as *in vitro* cultural conditions which would simulate the environmental situation *in vivo*. Thereafter, bacteria were grown in different body fluids, including whole plasma, serum, fibrinogen, γ-globulin and bovine serum albumin (B.S.A.). Only growth in whole plasma and, in serum to some extent, produced organisms that were

Table 1: *Staphylococcus* from Various Sources or Prepared in Different Ways and the Various Antibacterial Agents or Systems against which they were Tested

SOURCE/PREPARATION OF ORGANISM	ANTIBACTERIAL AGENTS/SYSTEMS						
	WHOLE PMN PHAGOCYTIC KILLING	SOLUBLE PMN BACTERICIDINS	ANTIBIOTICS	DISINFECTANTS	ANTISEPTICS	SYNTHESIZED NOVEL COMPOUNDS	EXTRACTS OF LOCAL HERBS
(I) Infection Site	---	---	1994; 1995; 1997	1994;	1994;	---	---
(II) Growth *in vivo*	1975; 1976; 1983(a)	1983(a)	---	---	---	---	---
(III) Growth *in vitro*	1976; 1983(a)	1984(a)	---	1985	1985	1987;	1988(c),(d);
Broths	1976; 1983(a)	1983(b) 1984(b)	1984(b)	1984(b);	1984(b)	1988(a),(b),(e)	1989
High-salt, High-CHO	1976; 1983(a)	1983(a) 1984(a)	---	---	---	---	---
Plasma		---	---	---	---	---	---
Bovine serum albumen (BSA)	]	---	---	---	---	---	---
Fibrinogen	]----(1984)(e)	---	---	---	---	---	---
Serum γ-globulin	]	---	---	---	---	---	---

fully converted and were similar to bacteria grown *in vivo* in rabbits. Organisms grown in plasma were therefore selected for further analysis on the nature of the enhancement in resistance. In looking for a convenient experimental system, the now widely-used method of chloroquine phosphate stabilization of polymorph lysosomes as a tool for accurate measurement of phagocytic killing of staphylococci was devised. A soluble bactericidin system which was more stable than the crude lysates earlier employed was also prepared. These constituted modest contributions to knowledge [Kolawole, 1983(a)]. The conversion to resistant phenotypes was later shown to be rapid, the organisms acquired a surface coating and were clumped together (Kolawole, 1984). Upon sub-culture *in vitro* in broth or after treatment with either trypsin or 2M KBr (procedures by which proteins are removed from membranes), these acquired attributes were lost, indicating a mainly protective role for the surface coating and that it was protein in nature. These observations helped to answer the second and third questions on the mechanism and rate of conversion. It was concluded that the surface coating was fibrin, formed from the interaction between staphylococcal free coagulase and fibrinogen in the plasma. This fibrin deposit probably blocked possible sites of bactericidin action on the bacterial surface (Pennial, Holdbrook and Zeya 1973; Hibbit and Benians, 1973) including covering up of NADH oxidase enzyme sites on the cytoplasmic membrane. Alternatively, it could have formed complexes with basic components of soluble bactericidins, as is the case with protamines, thus rendering them ineffective. Such a conversion and surface coating occurring rapidly in an infection (and *in vivo*), could account, at least in part, for the intracellular survival of organisms within PMN cells [Adlam *et al.*, 1970(b); Kolawole, 1983(a)] and is likely to be an important virulence mechanism. Such organisms would have more chances of survival and hence of establishing an infection in the next host, despite its natural defence mechanisms.

Along the line of these investigations, other cultural conditions were found to enhance the virulence and resistance of staphylococci. One, growth in the high-salt, high-carbohydrate modified 110 *Staphylococcus* medium produced mucoid organisms surrounded by a slime layer. This layer was made up of either a complex polysaccharide and a series of amino acids (Ekstedt and Berhard, 1973), or ribitol teichoic acid, a mannan and a serologically active polypeptide (Brock and Reiter, 1972) which conferred, on the organisms, additional

resistance to killing by rabbit PMN bactericidal factors [Kolawole, 1983(b)]. Washing in saline broth or sub-culturing in infusion broth resulted in organisms which had lost the slime layer and which had reverted to the susceptible phenotype. This indicated that the slime layer either acted as a physical barrier by blocking possible sites of action of soluble bactericidins or its components formed complexes with those of soluble bactericidins in much the same way as teichoic acid precipitates proteins from PMN to render them ineffective. Secondly, prolonged incubation of bacteria in ordinary broth also resulted in enhanced resistance, which could not be reversed by treatments rendering plasma-grown organisms susceptible.

From this body of work, several mechanisms by which *Staphylococcus* acquired resistance could be identified:

(i) specific interactions between host and staphylococcal factors which resulted in the deposition of a protective layer of fibrin around the organisms [Kolawole, 1983(a); 1984(b)];

(ii) non-specific interactions relating to adaptations of bacteria to different environmental and cultural conditions, e.g.:

acquisition of a surface coating from the *in vitro* environment which is different from that of *in vivo* but which performs a similar protective function [Kolawole, 1983(b); 1984(b); 1985];

adaptation to an alternative metabolic pathway in the environment which causes organisms to become insensitive to the usual antibacterial substances [Kolawole, 1984(a)]. This would be possible when organisms shifted from a usually aerobic respiration to an anaerobic one, in response to changing requirements in the growth media.

These findings would appear to explain why staphylococcal diseases contacted from existing cases or nosocomially may be more serious and more difficult to treat. They also explain why strains of *Staph. aureus* in environments offering physiological conditions similar to those *in vivo* or *in vitro* experimental conditions used in these works could undergo conversion to resistant forms, thus becoming difficult to eliminate [Kolawole and Odu, 1984; Kolawole, 1984(b); Kolawole, 1985; Kolawole, Omolayole and Lamikanra, 1988; Kolawole and Adedayo, 1994].

Resistance to Antibiotics

Early in the antibiotic era (1940 - 41), virtually all strains of *Staph. aureus* world-wide were susceptible to penicillin G, but by 1944, strains which could destroy penicillin by means of penicillinase (β-lactamase) enzyme had emerged. By now, over 95% of *Staph aureus* world-wide

is resistant to penicillin, ampicillin and the anti-pseudomonas penicillins (Lyon and Skurray, 1987). In response to this, the pharmaceutical industry came up with methicillin in 1960, which could not be destroyed by penicillinase. However, resistance to it soon emerged and by the 1980s, it had become a problem world-wide (Lyon and Skurray, 1987). Methicillin resistant *Staph. aureus* is now known to be resistant to all β-lactams, penicillins, cephalosporins carbapenems and penems as a result of possessing a mecA gene that produces a new penicillin-binding protein (PBP) 2a that has low affinity for β-lactam antibiotics.

Our experience with *Staph aureus* over the last 15 years has followed the general pattern in the rest of the world as reported in our publications of 1984, 1985, 1994, 1995 and 1997. The paper in 1995 is a report of *Staph. aureus* from septic wounds, each of which is resistant to 3 to 4 antibiotics (i.e., mutiply resistant) while the 1997 paper reports animal staphylococci that have crossed host boundaries and found their way into human wound infections. They also show high level resistance to commonly used antibiotics. Such organisms could become important skin contaminants in humans and opportunistic zoonotics.

Overall, therefore, man has not been able to curb bacterial resistance. The efforts to find agents with improved antimicrobial activity have therefore become a running battle between man and the microbes.

Resistance to synthesized novel Compounds and Extracts of Local Plants

As a contribution to the search for new and more effective antimicrobial drugs, I have been involved in inter-disciplinary studies with colleagues from other departments in the University. In collaboration with Dr. W. O. Erhun of the Faculty of Pharmacy, my student, Dr. J. K. Oloke and myself, identified several active components from the local plant *Aframomum melegueta* which showed remarkable antibacterial and antifungal capabilities (Oloke *et al.*, 1988; 1989). These included the volatile oil and some paradols. In separate works with Professor C. A. Obafemi of the Chemistry Department, certain organotin compounds (Obafemi *et al.*, 1987; Kolawole *et al.*, 1988) and sulfonyl derivatives (Obafemi and Kolawole, 1986; Obafemi *et al.*, 1988) were synthesized which showed antibacterial activities. These activities were comparable to those of some of the standard antibiotics in use and may need to be potentiated significantly for incorporation into drugs.

Resistance to Disinfectants and Antiseptics

The work in this area was informed by the need to assess the effectiveness of the various efforts to prevent bacteria from getting to man, since man's body defence mechanisms and the therapeutic drugs available to him have not offered 100% protection. The aim of disinfection and antisepsis is to eliminate or inactivate potentially pathogenic bacteria from man's immediate environment, particularly the hospitals. Bacteria from clinical specimens, encapsulated nasal isolates and mucoid-grown bacteria possessing a slime covering were therefore tested for their susceptibilities to commonly-used disinfectants and antiseptics (Kolawole and Odu, 1984; Kolawole, 1984(b); 1985; Kolawole *et al.*, 1985; Kolawole and Adedayo, 1994). All showed remarkable resistance to killing by the antiseptics tested at the use-dilutions recommended by the manufacturers, when compared with wild strains, which were still susceptible. Powerful disinfectants such as Hycolin and Izal killed 95-98% of bacterial populations within a ten minute exposure period, not 100% killing. The resistance of bacteria to these agents suggested that there could be serious implications on the maintenance of personal hygiene and public health in the country.

WHERE DO WE GO FROM HERE?

We have seen that staphylococci possess an array of attributes and capacities for adaptations with which they can overcome or defy man's body defence mechanisms as well as physical and chemical means of control and, given the right atmosphere, to cause disease. We may then ask the following questions and attempt to answer them right away:

(1) Are these observations common to other microbes or just peculiar to staphylococci?

The answer is that it is now a common phenomenon. There is a general crisis in antibiotic resistance. The resistance of other microbes which have become world-wide problems include that of *Streptococcus pneumoniae, Streptococcus pyogenes,* the enterococci, *Haemophilus influenzae* and certain anaerobic bacteria (Neu, 1992). Only recently, a team of Nigerian Researchers (Drs. Olukoya and Olasupo) were reported in *The Guardian* Newspapers to have published, in the *Nigerian Journal of Hospital Medicine,* high incidences of plasmid-mediated resistance to commonly prescribed antibiotics among bacteria associated with diarrhoea in infants and young children. The bacteria listed included enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), *Campylobacter, Shigella,*

Salmonella, Vibrio, Aeromonas and *Plesiomonas* species, observed over a period of nine years. Malaria is a microbial disease and has remained a major killer in the tropics, especially among children. The causative organisms have developed resistance to most of the chloroquine-based drugs used in treatment and the struggle is far from being won.

(2) Will man ever win over the microbes?

The answer is, I don't know. Man's activities have contributed immensely to increased resistance found among microbes. The use of antibiotics *per se* creates selective pressures on microbial populations which lead to the emergence of resistant strains. The indiscriminate use of antibiotics for trivial illnesses, ill-defined prophylactic purposes and in animal feedstuffs has done a lot to worsen the situation. Yet, we cannot abandon antibiotics or other antimicrobial drugs. The answer therefore will be that it depends on man's management of the situation.

(3) So, What must we do to be saved?

Before listing the few suggestions I have, it will be necessary to first point out that it is not the primary intention of microbes to cause disease, as that would amount to wanting to kill the goose that lays the golden egg. They are organisms who just want to live their normal lives, whether as commensals or as parasites, in their usual habitats. Changes in the habitats or in the usual conditions in their habitats would normally trigger their adaptive and aggressive mechanisms into action and a disease could be the result. The development of disease is therefore an abberration in the relationship and not the aim or norm. With this understanding, it should become clearer how we can exercise our dominion over them. I have the following recommendations:

(1) In view of the fact that man's activities *per se* have contributed immensely to the development of resistance among microbes, the handling of microbes in any form should be controlled. Manipulations enhancing resistance must be carried out or strictly supervised by experts. Organisms showing unusual resistance in experimental work should be destroyed immediately and carefully discarded, not to create any monsters.

(2) The search for new and more effective antimicrobial drugs should continue since resistant bacteria will continue to appear, as a result of their remarkable ability to overcome each new agent soon after it becomes available. The Nigerian Government will have to put more money into research, especially in the treatment of mostly tropical diseases. The onus lies with us to find permanent cures for essentially African problems such as malaria. The interests of drug manufacturing

companies based outside the region will largely be economic. The millions of Naira spent on importing such drugs could be spent more wisely in research and development here at home.

(3) There must be well-defined and enforceable antibiotic control programmes, perhaps as Government policy, such that they are prescribed and used only when it is clearly indicated through properly conducted antibiotic sensitivity tests. As a back up, there should be continuous surveillance of the use of antibiotics - locally, nationally and internationally - while also monitoring emerging patterns of bacterial resistance. The appropriate use of antimicrobial drugs will delay and, in many cases, prevent the emergence of resistance.

A more sincere and concerted effort should also be made to curb the importation and circulation of fake drugs in the country. They only bruise microbial causative agents of disease, thus training them for greater resistance. They do not cure.

(4) Improvements in both personal and environmental hygiene through public health education, to keep potentially pathogenic microbes at bay and hence to stop their spread, particularly in the hospital are recommended. Isolation and quarantine methods should be employed and appropriate disinfectant/antiseptics used efficiently, in the care of infected and colonized patients. Controls directed against known reservoirs of disease and transmission agents should be intensified and well-planned immunization programmes carried out as and when due.

Mr. Vice-Chancellor, this lecture will be incomplete if I do not say anything about A.I.D.S., the dreaded Acquired Immune Deficiency Syndrome, which at present does not have an affordable cure for all affected. The War Against AIDS programme was first launched in Nigeria on August 23, 1991. State control committees were quickly launched and full-time AIDS coordinators appointed. Even Local Government Areas had their AIDS Committees and full-time Managers designated. The aim was to eventually integrate the AIDS programmes into Primary Health Care. That seemed to be how far we went in formulating a National Policy on AIDS. We read recently in the Newspapers (*The Guardian*, August 17, 1997) that the draft policy was made ready by four Technical Committees made up of Nigerian experts in April 1995 but that bureaucracy has since delayed its enactment. It has taken the death, on August 2nd, 1997, of Afro-beat Musician, Mr. Olufela Anikulapo-Kuti of complications arising from AIDS to jolt our policy-makers back into action. We hear that top Ministry officials are now determined to put finishing touches to the policy for early enactment. Let us hope it will not get caught in any official bottle-necks again. An all-embracing programme of education on AIDS that

will reach all and sundry will be most welcome, for a start. Prevention, after all, is better than cure. We cannot claim any more wisdom than comes from God, who has told us the ultimate way of salvation from this affliction by microbes:

> ***".....If thou wilt diligently hearken to the voice of The Lord thy God, and wilt do that which is right in His sight, and wilt give ear to His commandments, and keep all His statutes, I will put none of these diseases upon thee, which I have brought upon the Egyptians: for I am the Lord that healeth thee."*** --- ***EXODUS 15:26***

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References

ADLAM, C., PEARCE, J. H. and SMITH, H. (1970): Virulenc mechanicms of staphylococci grown *in vivo* and *in vitro*. J. Med Microbiol *3*: 147-155.

ADLAM, C., PEARCE, J. H. and SMITH, H. (1970): The interaction o staphylococci grown *in vivo* and *in vitro* with polymorphonulea leucocytes. J. Med. Microbiol 3: 157 - 163.

BEINING, P. R. and KENNEDY, E. R. (1963): Characteristics of a strair of *staphylococcus aureus* grown *in vivo* and *in vitro*. J. Bacteriol. *85*: 732-741.

BROCK, T. D. (1961): Milestones in Microbiology. Prentice-Hall Inc Biological Science Series, N.J.

BROCK, J. H., TURVEY, A. and REITER, B. (1973): Virulence of twc mastitis strains of *Staphylococcus aureus*, in bovine skin: enhancement by growth in high carbohydrate-high salt medium or in raw milk. Infect. Immun. *7*: 865-872.

COHN, Z. A. and HIRSCH, J. G. (1960): The isolation and properties ofspecific cytoplasmic granules of rabbit polymorphonuclea leucocytes. J. Exp. Med. *112*: 983-1004.

DUGUID, J. P., MARMION, B. P. and SWAIN, R.H.A. (1985): Mackie and McCartney Medical Microbiology, 13th ed. Vol. 1. Microbia infections. Churchill Livingstone, Edinburgh, London and New York.

HIBBIT, K. G. and BENIANS, M. (1973): The use of flourescen conjugates to study the site of action of antimicrobial cationic proteins on staphylococci. Res. Vet. Sc. *15*: 61-64.

HUGO, W. B. and RUSSEL, A. D. (1992): Pharmaceutical Microbilogy. 5th ed. Blackwell Scientific Publications, Oxford.

KOLAWOLE, D. O. (1983): Evaluation and use of the drug inhibition method of measuring intracellutar killing in differentiating between staphylococci grown *in vivo* and *in vitro*. J. Hyg., Camb. *91*: 211-222.

KOLAWOLE, D. O. (1983): Effect of mucoid growth of *Staphylococcus aureus* on its susceptibility to rabbit polymorph bactericidal factors. FEMS Microbiol. Letters *16*: 31-33.

KOLAWOLE, D. O. (1984): Mechanisms of resistance of staphylococci grown in plasma to polymorph bactericidins. J. Hyg., Camb. *92*: 183-192.

KOLAWOLE, D. O. (1984): Resistance mechanisms of mucoid-grown

Staphylococcus aureus to the antibacterial action of some disinfectants and antiseptics. FEMS Microbiol Letters *25*: 205-209.

KOLAWOLE, D. O. (1985): Inhibitory effects of staphylococcal slime layer on the antibacterial activities of some disinfectants and antiseptics. Zbl. Bakt. Suppl. *14*: 647-652.

KOLAWOLE, D. O. and ODU, W. D. (1984): Incidence and bacteriological investigations of osteomyelitis infections in Ile-Ife, Nigeria. Nig. J. Microbiol. *41*: 22-28.

KOLAWOLE, D. O., OMILABU, S. A. and FANIFOSI, A. O. (1985): Antibacterial effectiveness of commercial preparations of some disinfectants and antiseptics sold in Nigeria. Microbios Letters *28*: 89 - 95.

KOLAWOLE, D. O., IYOHA, D. E. and OBAFEMI, C. A. (1988): Studies on the antibacterial activities of some organo-tin compounds Nig. J. Biotechnol. *4*: -124-130.

KOLAWOLE, D. O., OMOLAYOLE, O. A. and LAMIKANRA, A. (1988): A Study of the virulence of encapsulated *Staphylococcus aureus* isolated from healthy nasal carriers. Nig. J. Microbiol., *8*: 29 - 33.

KOLAWOLE, D. O. and ADEDAYO, O. (1994): Resistance of mouse-virulent encapsulated nasal isolates of *Staphylococcus aureus* to disinfectants and antiseptics. Biomedial Letters *50*: 151-156.

KOLAWOLE, D. O. and SHITTU, A. O. (1995): Multi-resistant *Staphylococcus aureus* from septic wounds in Nigeria. Biomedical Letters *52*: 245-252.

KOLAWOLE, D. O. and SHITTU, A. O. (1997): Unusual recovery of animal staphylococci from septic wounds of hospital patients in Ile-Ife, Nigeria. Letters in Applied Microbiol., *24*: 87-90

LYON, B. R. and SKURRAY, R. (1987): Antimicrobial resistance of *Staphylococcus aureus*: Genetic Basis. Microbiol. Rev. *5*: 88 - 134.

MIMS, C. A., PLAYFAIR, J.H.L., ROITT, I. M., WAKELIN, D., WILLIAMS, R. and ANDERSON, R. M. (1993): Medical Microbiology. Mosby-Year Book Europe Ltd.

NEU, H. C. (1992): The Crisis in antibiotic resistance. Science, *257*: 1054-1073.

OBAFEMI, C. A., EJENARI, A. B., KOLAWOLE, D. O. and OLOKE, J. K. (1987): Studies of Heterocyclic Compounds x. The synthesis and properties of some organo-tin (iv) -oxygen and -nitrogen heterocycles. Inorganica Chimica Acta. *151*: 21-27.

OBAFEMI, C. A., FAGUN, Y. O. and KOLAWOLE, D. O. (1988): Synthesis and Antibacterial activity of some sulfonyl-iminoindoline derivatives. African J. Sc. and Technol. (Nairobi) *2*: 14-25.

OLOKE, J. K. and KOLAWOLE, D. O. (1988): The Antimicrobial Spectrum of inhibitory substances produced by some fungi Nig J. Biotechriol., *6*: 127-130.

OLOKE, J. K., KOLAWOLE, D. O. and ERHUN, W. O. (1988): The antibacterial and antifungal activities of certain components of *Aframomum melegueta* Roscoe fruits. Fitoterapia LIX: 383-388.

OLOKE, J. K., KOLAWOLE, D. O. and ERHUN, W. O. (1989): Antimicrobial effectiveness of six paradols, 1. A Structure-activity relationship study. J. Ethno-Pharmacology (U.S.A.) *25*: 109-115.

PEARCE, J. H., KOLAWOLE, D. O. and SCRAGG, M. A. (1976): Mechanisms of resistance of staphylococci grown *in vivo* to polymorph bactericidins. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionkrakheitan und Hygiene, I Abt Suppl. *5*: 849-854.

PENNIAL, R., HOLBROOK, J. P. and ZEYA, H. J. (1972): The inhibition of cytochrome oxidase by lysosomal cationic proteins of rabbit poly morphonuclear leucocytes. Biochem. Biophys. Res. Comm. *47*: 1270-1276.

POSTGATE, J. (1992): *Microbes and Man*. 3rd Ed. Cambridge University Press.

REINER, R. (1984): *Antibiotics - An introduction*. Nigerian Edition. New Horn Press Ltd.

SINGLETON, P. (1992): *Introduction to Bacteria*. 2nd Ed. John Wiley & Sons, Chichester.