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Delivered by

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TITLE
PEOPLE VS BACTERIA:
BACTERIA INNOCENT UNTIL PROVEN GUILTY

THE PEOPLE vs. BACTERIA

BACTERIA INNOCENT UNTIL PROVEN GUILTY

PREAMBLE

The title of this lecture may seem a bit unusual and confusing. First, it gives the impression that we are in a courtroom and bacteria are on trial. Secondly, you may wonder why a Professor of Microbiology appears to be flirting with Law. While I may leave you to wonder about the second, I would like to say, regarding the first, that I am not the one who has put bacteria on trial, but the world. Bacteria are blamed for more things than they are responsible for and are generally regarded as nothing but agents of disease.

Today, I have become the advocate of this highly maligned and misunderstood group of living things. My plan is to present a fuller story, giving you the good, the bad and the ugly, after which you, the members of the jury, will decide for yourselves whether the bacteria are friend, foe or maybe both.

We are innocent!Let us out!

1.0 INTRODUCTION

Some years ago, the Microbiology students, while trying to produce a new issue of their magazine, Micromedia, wrote to me requesting an article. I thought of what I could write that would be educative for them, without being too academic. I wrote an article, which I titled "Man in the world of microbes". I was striving for something catchy, but little did I know that I would, in the future, find myself making repeated reference to that statement.

In the article, I tried to highlight the place of microorganisms, particularly the bacteria, in the general scheme of things. I tried to show, using data provided by fossil records, that the bacteria were inhabitants of earth long before the first humans appeared; bacterial existence on earth dating back as far as 3.5 billion years, while the humans date back only 0.5 billion years (Avila, 1995; Di Giulio, 2003; Battistuzi *et al*., 2004). Thus, I tried to raise a controversial question: Between the bacteria and the humans, who actually has more claim to the earth? I am quite sure that if I try, I could, even today, raise a serious debate on that particular topic. But, regardless of which side anyone might care to take, one thing is certain: that Bacteria impact on the life of humans in many important ways.

Before examining the various ways in which bacteria impact on human life, it may be necessary to describe what bacteria really are.

1.1 What are Bacteria?

The bacteria (singular - bacterium) are a large group of unicellular, prokaryotic microorganisms. The term **prokaryotic** refers to cells that do not contain a nucleus and lack membrane-bound organelles. Although the term *bacteria* traditionally included all prokaryotes, the scientific classification changed after the discovery in the 1980s that prokaryotes consist of two very different groups of organisms that evolved independently from an ancient common ancestor. These evolutionary domains are called Bacteria and Archaea (Woese *et al.*, 1990).

Typically a few micrometres (0.5 - 5.0 micrometres) in length, most bacterial species are either spherical, called **cocci** (*singular* coccus, from Greek *kókkos* meaning grain or seed) or rod-shaped, called **bacilli** (*singular* bacillus, from Latin *baculus* meaning stick) (Dusenbury, 2009). Elongation is associated with swimming. Some rod-shaped bacteria, called *Vibrio*, are slightly curved or comma-shaped; others, can be spiral-shaped, called **spirilla**, or tightly coiled, called **spirochaetes**. A small number of species even have tetrahedral or cuboidal shapes (Fritz *et al.*, 2004). Some commonly known bacteria possessing some of these shapes are shown below. More recently, bacteria that grow as long rods, with a star-shaped cross-section, were discovered deep under the Earth's crust (Wanger *et al*., 2008).

This wide variety of shapes is determined by the bacterial cell wall and cytoskeleton, and is important because it can influence the ability of bacteria to acquire

nutrients, attach to surfaces, swim through liquids and escape predators.

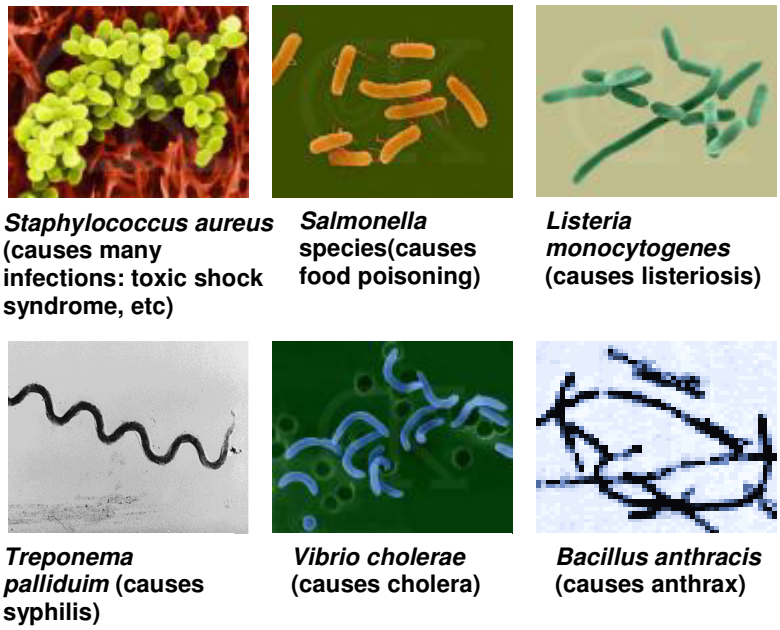

Plate 1. Composite of some commonly known bacteria, exhibiting the different shapes of bacterial cells.

Bacteria can be divided into two main groups, Gram-positive or Gram-negative, based on the structure of their cell wall and their reaction to the Gram stain (a differential staining method described by Hans Christian Gram in 1884).

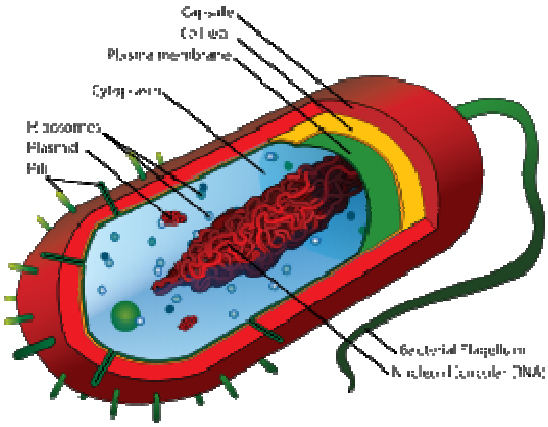

Fig. 1. Structure and contents of a typical Gram-positive bacterial cell

Bacteria are ubiquitous in every habitat on Earth, growing in soil, acidic hot springs, radioactive waste, water, and deep in the Earth's crust, as well as in organic matter and the live bodies of plants and animals. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a millilitre of fresh water. In all, there are approximately five nonillion (5×10^{30}) bacteria on Earth, forming much of the world's biomass (Whitman *et al.,* 1998).

Bacteria have a practical significance for humans. Some cause disease in humans and domestic animals, thereby affecting health and the economy. Some bacteria are useful in industry, particularly in the food, petroleum, and textile industries, some improve soil fertility, while others exist in various non-harmful relationships with human.

1.2 Ways in which Bacteria Impact on Human Life

In Industry: The use of microorganisms, particularly bacteria, in the industry cannot be overemphasized. Industrial microbiology, the major foundation of biotechnology, arose out of empirical developments in the production of wine, vinegar, beer, and *saké*, and with the traditional fermentation processes used in Asia and Africa for the production of food (Cruegar and Cruegar, 1990). For instance, traditional Nigerian foods, such as Garri, Akpu (fermented cassava foo-foo), Akamu or Ogi and condiments such as ogiri (fermented castor-oil seed or melon seed) are all results of natural microbial activities.

An experimental approach to the production of microbial metabolites began at the beginning of the 20th century, during the time of World War II, when there was need for large-scale production of the first antibiotic, penicillin. Penicillin is a natural product of a fungus *Penicillium.* In order to produce this antibiotic economically, important engineering developments had to be made, including the techniques for large-scale sterilization, aeration, and growth of the producer-

organism. In addition, genetic methods for microbial strain improvement were perfected.

From World War II up until about 1960, the major products of Industrial Microbiology were antibiotics. From 1960 through 1975, new microbial processes for the production of amino acids and flavour enhancers were developed. From 1975, biotechnology entered some important new phases and today, biotechnology, through the integrated use of microbiology, biochemistry and engineering has made possible the large-scale production of many important products such as hormones, growth factors and various enzymes.

In Food Production: Besides the traditional fermented foods already mentioned above, bacteria play other important roles in the food industry. We should note first that they are responsible for various forms of spoilage resulting in wastage of vast amounts of money all over the world each year. In contrast to their spoilage roles, however, they also play important roles in various food manufacturing processes. For example, dairy products such as cheese, yoghurt and buttermilk, which are products of major economic value, are manufactured at least in part, through the activities of various bacteria. Sauerkraut, pickles and some sausages are also produced using bacteria. There are many other foods which are produced, at least partially, through the fermentative activities of bacteria.

In Agriculture: Bacteria play very essential roles in agriculture. For example, a group of food crops called the legumes live in close association with special bacteria, which form structures called nodules on their

roots. In these root nodules, the bacteria convert atmospheric nitrogen (otherwise inaccessible to the plants) into fixed nitrogen compounds such as nitrates and nitrites that the plants can use.

Also of major agricultural importance are the microorganisms that are essential for the digestive process in ruminant animals like cattle and sheep. These important farm animals have a digestive organ called the rumen, in which microorganisms, particularly bacteria, carry out the digestive process. Enormous amounts of money from meat and milk production are linked to those rumen microorganisms and without them, cattle and sheep production would be virtually impossible.

Bacterial activities are also responsible for the cycling of important nutrients in plant nutrition, particularly carbon, nitrogen and sulphur. Bacterial activities in the soil convert these elements into forms that are readily accessible to plants.

Other roles of bacteria: In addition to the casual roles already described above, bacteria can also exist in more direct or intimate association with humans; **as agents of human disease** and **as commensals.** Medical bacteriology focuses on these two important roles of the bacteria.

In medical microbiology, as we struggle to find ways of controlling bacteria and preventing disease, we often come face to face with the fact that members of the normal bacterial flora of humans have a crucial role to play in maintenance of health. Research efforts in my laboratory over the past few years have centred in these

two areas of Microbiology. This lecture will therefore focus on these two important aspects of the association between bacteria and humans.

2.0 BACTERIA AS AGENTS OF DISEASE

Even before the discovery of the first microorganisms, their role in disease process was already suspected. For instance, at the time of Moses, the Egyptians and Hebrews had come to believe that leprosy could be transmitted by contact with lepers. In Europe, around 430 B.C. people had concluded that some plagues were contagious and by the Middle Ages, many fled cities to escape various diseases (Nester *et al*., 2004).

Despite these beliefs, however, it was not until the discovery of microorganisms by Antoin Van Leeuwenhoek in the late seventeenth century and the subsequent proof of the "germ theory" of disease by Robert Koch in 1876, that the role of microorganisms in disease was confirmed. Incidentally, the microorganism involved in Robert Koch's experiments was a bacterium known as *Bacillus anthracis*, the cause of the disease known as anthrax, a fatal disease of humans, sheep and other animals.

Because the discovery of microorgaisms in general and bacteria in particular was immediately followed by studies of their role in causing disease, their disease-causing capability is the role for which they are most commonly known. Consequently, for most lay people, the name "bacteria" is synonymous with disease and

danger. It is important therefore, to point out that every day humans are in intimate contact with an enormous number of bacteria. Every breath we take introduces some to our upper respiratory system, some are ingested with food and drinks and still more are adhered to our skin when we touch an object or surface.

The majority of these bacteria generate no ill effects whatsoever. Some may colonize various body sites, taking up residence with the variety of other organisms that live there without causing harm (some provide essential functions), while others are sloughed off with dead skin cells. Most of those swallowed are either killed in the stomach by the digestive juices or survive and are eventually eliminated with faeces (Nester *et al*., 2004).

Contrary to what most non-microbiologists think, relatively few bacteria are able to cause harm to their host. These bacteria are said to be pathogenic or called *pathogens* and have distinct patterns of interaction with their hosts enabling them to gain the upper hand in the host-parasite relationship.

In order to successfully produce disease in a host, a potential pathogen must satisfy several conditions:

- The pathogen must successfully colonize the host. This involves adherence, entry or penetration and initial multiplication of the organism.
- The pathogen must be able to spread within the host.
- The pathogen must be able to overcome the various lines of defence presented by the host,

including the anatomical barriers like intact skin and epithelia, the antimicrobial chemical substances secreted by different tissues and phagocytic and antibody immune mechanisms of the host.

- Finally the pathogen must be able to produce toxic substances with which it causes damage to host tissues.

The extent to which any bacterium is able to satisfy these conditions determines its pathogenic potential or its ability to cause disease. The ability of an organism to cause disease is known as *pathogenicity* and the degree or extent to which the pathogen can cause disease, that is the degree of pathogenicity, is known as *virulence.* The first three properties of the pathogen; that is, ability to colonize, ability to spread and ability to escape host defence, are together known as *invasiveness*, while the fourth property, the ability to produce toxic substances is known as *toxigenicity*. Thus, the virulence of any bacterium is dependent on the two properties, invasiveness and toxigenicity.

These two determinants of virulence are quantitative, ranging from very low to very high. They are also independent in the sense that a bacterium that is weakly invasive could still be very virulent if it is highly toxigenic and one which is weakly toxigenic but highly invasive could still be very virulent.

In the past, disease-causing bacteria were usually boxed into groups labelled either *true pathogens* or *opportunistic pathogens*; true pathogens referring to those bacteria that are always associated with disease

and opportunistic pathogens referring to those bacteria that cause disease only under certain circumstances, usually in circumstances of impaired or depressed immunity. However, with the increased understanding of the pathogenic mechanisms of bacteria, supplied mainly by advances in genetic studies, it is becoming increasingly clear that bacteria cannot be so strictly classified. Rather, the outcome of any interaction between a bacterium and its host depends on a variety of both host and bacterial factors operating at a particular point in time. That is to say that any particular bacterium could be a successful pathogen in one individual at a particular time but not at another or could be a pathogen in one individual and not in another. This situation can be summarized by saying that the relationship between a pathogen and its host is dynamic and is determined by both the host and bacterial factors.

Bacteria have evolved various mechanisms by which to gain the upper hand in this dynamic relationship between them and their hosts. The next section will briefly describe some of the pathogenic mechanisms of bacteria.

2.1 Mechanisms of Bacterial Pathogenicity

In the preceding section, several conditions were outlined which a potential pathogen must meet in order to successfully produce disease, starting from the successful adherence to host tissues and ending with destruction of the host tissues. This implies that the journey, from first contact between the bacterial cell and its host to the production of disease in the host, is a very

long one, along a road strewn with many obstacles and hurdles. The bacterium (potential pathogen) must essentially surmount all these obstacles and jump every hurdle before it can successfully produce disease in that host. Therefore, in this struggle between a potential pathogen and its host, the host plays a very significant role. **If one wants to be radical about it, one might even go as far as to say that a bacterial organism cannot produce disease in a host unless it is permitted by the host.** But that is only a very radical way of looking at it. What is true, however, is that production of diseases by bacterial parasites involves an inter-play of a variety of factors of both the parasite and the host. **It is a battle between two formidable opponents and only the stronger comes out the victor. If the host proves stronger, the outcome is an aborted infection. If the parasite proves stronger, the outcome is a sick individual.**

In this section, we shall discuss some of the mechanisms by which bacteria go about overcoming their hosts.

2.1.1 *Mechanisms of colonization*

Colonization is the first step towards the production of disease by an organism. As previously mentioned, it is the composite of three separate events: adherence, penetration and initial multiplication. Before damage can be done to host tissues, the pathogen must gain access to the host tissues and multiply. In most cases, this requires that the pathogen penetrate mucous membranes or epithelial surfaces at the site of entry. **Sites of entry in**

human hosts include the skin, the urogenital tract, the digestive tract, and the respiratory tract among others. These are surfaces which normally act as microbial barriers and are provided with various types of defence mechanisms. The pathogen must therefore overcome these defence mechanisms in order to enter through these sites.

The first event in colonization is **adherence**. Bacterial adherence requires the participation of two factors: a receptor on the host cell surface and an adhesin on the bacterial cell surface. The receptor is usually a specific carbohydrate or peptide residue on the surface of the host cell, with which the bacterial adhesin (typically a macromolecular component of the bacterial cell surface) can interact (Todar, 2002). The interactions between adhesins and their receptors are usually complementary and specific. Some adhesins of various pathogenic bacteria and their receptors are shown in Table 1 below.

TABLE 1. ADHESINS OF VARIOUS PATHOGENIC BACTERIA AND THEIR RECEPTORS

Bacterium	**Adhesin**	**Receptor**	**Attachment site**	**Disease**
Streptococcus pyogenes	Protein F	Amino terminus of fibronectin	Pharyngeal epithelium	Sore throat
Streptococcus mutans	Glycosyl transferase	Salivary glycoprotein	Pellicle of tooth	Dental caries
Streptococcus pneumoniae	Cell-bound protein	N-acetylhexosamine-galactose disaccharide	Mucosal epithelium	Pneumonia
Staphylococcus aureus	Cell-bound protein	Amino terminus of fibronectin	Mucosal epithelium	Various
Neisseria gonorrhoeae	N-methylphenyl-alanine pili	Glucosamine-galactose carbohydrate	Urethral/cervical epithelium	Gonorrhea
Enterotoxigenic E. coli	Type-1 fimbriae	Species-specific carbohydrate(s)	Intestinal epithelium	Diarrhea
Uropathogenic *E. coli*	Type 1 fimbriae	Complex carbohydrate	Urethral epithelium	Urethritis
Uropathogenic *E. coli*	P-pili (pap)	Globobiose linked to ceramide lipid	Upper urinary tract	Pyelonephritis
Bordetella pertussis	Fimbriae ("filamentous hemagglutinin")	Galactose on sulfated glycolipids	Respiratory epithelium	Whooping cough
Vibrio cholerae	N-methylphenylalanine pili	Fucose and mannose carbohydrate	Intestinal epithelium	Cholera
Treponema pallidum	Peptide in outer membrane	Surface protein(fibronectin)	Mucosal epithelium	Syphilis
Mycoplasma	Membrane protein	Sialic acid	Respiratory epithelium	Pneumonia
Chlamydia	Unknown	Sialic acid	Conjunctival or urethral epithelium	Conjunctivitis or urethritis

Source: Todar, 2002.

A single strain of a particular bacterial species may possess several distinct types of adhesins, encoded by distinct regions of its chromosome or plasmids. This genetic diversity enables the organism to quickly adapt to its changing environment and exploit opportunities presented by different host surfaces (Todar, 2002).

The second event is penetration. In some cases, a pathogen is able to cause damage without penetrating or going beyond the point of attachment, but in most cases, the pathogen penetrates the epithelium and either grows in the sub-mucosa or spreads to other parts of the body where growth is initiated. Penetration may be accomplished through breaks in the mucous membranes, motility and chemotaxis and by transport via the lymphatic system.

Recent studies have revealed some novel methods by which some pathogens gain entrance to host tissues. One method is that exhibited by *Salmonella* and *Shigella* species. This is a **protein secretion pathway** that serves to inject signalling proteins from the microbe into the host cell. The injected proteins then activate host cell signalling pathways that cause the host cell to internalize the microbe. Another method is that exhibited by enteropathogenic *E. coli*. The pathogen injects its own protein receptor into the host cell membrane. Once in the membrane, the receptor then binds to the pathogen's adhesin aiding adherence of the pathogen.

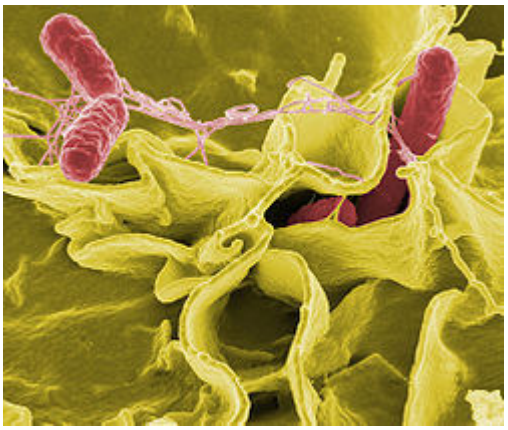

Fig. 3. Color-enhanced scanning electron micrograph showing *Salmonella typhimurium* (red) invading cultured human cells

Following adherence and entry, there often needs to be initial multiplication in order to complete colonization and establish the pathogen. The initial inoculum is rarely sufficient to cause damage. Therefore, the pathogen must grow within the host tissues in order to produce disease. This is not such an easy feat because for the organism to have the chance of growing, it must evade host defence mechanisms operating at that level and also obtain nutrients needed for growth and multiplication.

2.1.2 *Mechanisms of spr*ead

Once adhered to a host surface, some pathogens gain deeper access into the host to perpetuate the infection cycle. This pathogenic principle is termed invasion. Invasion can be divided into two types: extracellular and intracellular. Extracellular invasion occurs when a microbe breaks down the barriers of a tissue to disseminate in the host while remaining outside of host cells. This is a strategy used by many bacterial pathogens. These bacteria produce extracellular enzymes, called **invasins**, which act by breaking down primary and secondary defences of the body, thereby facilitating the spread of the organisms within the host tissues (Todar, 2002). A list of some bacterial invasins and their mechanisms of action is presented in Table 2.

Extracellular invasion allows these pathogens access to niches in tissues where they are able to proliferate, disseminate to other sites in the body, express toxins, and initiate inflammatory responses.

TABLE 2. SOME EXTRACELLULAR BACTERIAL PROTEINS THAT ARE CONSIDERED INVASINS

Invasin	**Bacteria Involved**	**Activity**
Hyaluronidase	Streptococci, staphylococci and clostridia	Degrades hyaluronic acid of connective tissue
Collagenase	*Clostridium* species	Dissolves collagen framework of muscles
Neuraminidase	*Vibrio cholerae* and *Shigella dysenteriae*	Degrades neuraminic acid of intestinal mucosa
Coagulase	*Staphylococcus aureus*	Converts fibrinogen to fibrin which causes clotting
Kinases	Staphylococci and streptococci	Converts plasminogen to plasmin which digests fibrin
Leukocidin	*Staphylococcus aureus*	Disrupts neutrophil membranes and causes discharge of lysosomal granules
Streptolysin	*Streptococcus pyogenes*	Repels phagocytes and disrupts phagocyte membrane and causes discharge of lysosomal granules
Hemolysins	Streptococci, staphylococci and clostridia	Phospholipases or lecithinases that destroy red blood cells (and other cells) by lysis
Lecithinases	*Clostridium perfringens*	Destroy lecithin in cell membranes
Phospholipases	*Clostridium perfringens*	Destroy phospholipids in cell membrane
Anthrax EF	*Bacillus anthracis*	One component (EF) is an adenylate cyclase which causes increased levels of intracellular cyclic AMP
Pertussis AC	*Bordetella pertussis*	One toxin component is an adenylate cyclase that acts locally producing an increase in intracellular cyclic AMP

Source: Todar, 2002.

Intracellular invasion occurs when a microbe actually penetrates the cells of a host tissue and survives within this environment. A number of Gram negative, Gram positive, and mycobacterial pathogens have been shown to have the ability to enter host cells, and both phagocytic and non-phagocytic cell types can serve as targets for invasion (Finlay and Falkow, 1997; Cleary and Cue, 2000; Bermudez and Sangari, 2000; Dehio *et al*., 2000). Some pathogens have an obligate intracellular lifecycle which absolutely requires a mammalian cell for growth. These include *Chlamydia* spp, *Rickettsia* spp, and *Mycobacterium leprae*. Other pathogens are facultatively intracellular, using their ability to enter and survive within host cells as a means of proliferation or spreading to other tissues.

2.1.3 *Mechanisms for evading host defence*

Some pathogenic bacteria are inherently able to resist the bactericidal components of host tissues. For example, the polysaccharide capsules of most pathogenic bacteria protect the bacteria from phagocytosis by not allowing recognition by phagocytic cells of the immune system; the outer membrane of Gram-negative bacteria is a formidable permeability barrier that is not easily penetrated by hydrophobic compounds such as bile salts which are harmful to the bacteria; pathogenic mycobacteria have a waxy cell wall that resists attack or digestion by most tissue bactericides; and intact lipopolysaccharides (LPS) of Gram-negative pathogens may protect the cells from complement-mediated lysis or the action of lysozyme.

Most successful pathogens, however, possess additional structural or biochemical features which allow them to resist the main lines of host internal defence against them, i.e., the phagocytic and immune responses of the host.

To overcome the hosts' phagocytic defence, for example, some bacterial pathogens have evolved mechanisms to avoid contact with the phagocytes, inhibit engulfment by the phagocytes, survive inside the phagocytes or even kill the phagocytes. Bacteria such as *Mycobacterium tuberculosis, S. aureus, Pseudomonas aeruginosa, Listeria monocytogens* and *Chlamydia* are very adept at escaping the host phagocytic defense (Todar, 2002).

Once the phagocytic defence of the host has been bypassed or overcome by the pathogen, the last line of defence is provided by components of the adaptive immune response; the antibody-mediated and cell-mediated immune responses. Many successful pathogens also exhibit various mechanisms for overcoming the hosts' antibody and cell mediated immune responses. They achieve this by a number of mechanisms including antigenic disguise, immunosuppression and persistence at sites inaccessible to the cells of the immune system.

2.1.4 *Production of toxins*

As mentioned previously, this is the second determinant of virulence.

Toxins have been likened to biological weapons, in that they are substances produced by bacteria to damage or destroy the host cell (Wilson *et al*., 2001). Bacteria

produce two types of toxins called **exotoxins** and **endotoxins**. **Exotoxins** are released from bacterial cells and may act at tissue sites removed from the site of bacterial growth. **Endotoxins** are cell-associated substances that are structural components of the cell walls of Gram-negative bacteria. However, endotoxins may be released from growing bacterial cells or from cells which are lysed as a result of effective host defence (e.g. lysozyme) or the activities of certain antibiotics (e.g. penicillins and cephalosporins). Hence, bacterial toxins, both soluble and cell-associated, may be transported by blood and lymph and cause cytotoxic effects at tissue sites remote from the original point of invasion or growth. Some bacterial toxins may also act at the site of colonization and play a role in invasion (Todar, 2002).

From the above discussions, it should be clear that while some bacteria may be involved in some disease processes, ranging from very mild to deadly diseases, the number of bacterial species capable of causing disease is but a minute fraction of the total number of bacterial species known to exist. The journey, for a bacterial cell, from contact with a potential host to successful invasion and initiation of disease process is a long one, along a road strewn with obstacles. Only those bacteria that are capable of surmounting these obstacles presented by the host can successfully initiate disease.

Can we then say that while bacteria are capable of causing disease, they can only do so, with our permission or our cooperation?

2.2 Non-Conventional Phenomena Associated with Bacterial Pathogenicity

With more understanding of the mechanisms by which bacteria cause disease, it becomes easier to control and combat disease-causing bacteria, even as the bacteria themselves evolve and present yet more diverse mechanisms for winning the battle. Two non-conventional phenomena enhancing bacterial virulence are discussed below.

Antibiotic resistance

One of the more troubling phenomena exhibited by bacteria today is that of antibiotic resistance. The problem of antibiotic resistance is not a new one; being discovered just a short time following the discovery of the first antibiotics. What is worrisome today, however, is the rate at which bacteria develop resistance to new antibiotics and also the phenomenon of multiple antibiotic resistance.

Many pathogenic bacteria today exhibit multiple antibiotic resistance. Multiple drug resistant bacteria are defined as bacteria that are resistant to one or more classes of antimicrobial agents. Multiple drug resistance has also been defined as a condition enabling a disease-causing organism to grow in the presence of distinct drugs or chemicals of a wide variety of structure and function targeted at eradicating them (Wikipedia). Indeed it is not unusual to encounter a bacterial organism that is resistant to all antibiotics that may be

available at any given time. How then can such organisms be controlled? One notable organism in this category is *Staphylococcus aureus*. Many strains of this species exhibit multiple antibiotic resistance. A particular strain of the organism, known as **methicillin resistant *Staphylococcus aureus* (MRSA)** is currently responsible for many cases of mortality around the world. MRSA strains are resistant to almost all known antibiotics. Consequently, infections by this strain usually result in death, particularly if the organism finds its way into the blood stream, a condition known as bacteraemia.

Besides *Staphylococcus aureus*, there are other pathogens that also exhibit multiple antibiotic resistance. These drug resistant strains are prevalent in hospital environments where they are associated with hospital acquired infections (nosocomial infections). They are also found in the community where they are involved in community acquired infections. One of the questions being asked today is whether these strains originate from the community and are then carried into the hospital environment or whether they originate from the hospital from where they are disseminated into the community. The argument is still raging today, with proponents on either side. We have even waded into the argument in my laboratory. In a recent study, we investigated the distribution and antibiotic resistance profiles of *Staphylococcus aureus* strains in community and hospital environments in Nsukka metropolis. Our results indicated that while there were a lot of cases of resistance among the bacterial isolates, the resistance

was mainly to older antibiotics such as ampicillin, floxapen, lincomycin and ampiclox while the strains remained sensitive to the relatively newer fluoroquinolone antibiotics like ciprofloxacin. Also, there was homogeneity between the community and hospital strains (Ezeonu and Ayogu, 2009). This suggests that the pattern of resistance among hospital and community strains may not be so much a question of where the strains originated from, but a question of what factors are responsible for the development of resistance or selection of the resistant phenotype.

In the Nigerian environment, where antibiotics are obtained, used and misused as easily in the community as they are in the hospital, selection of antibiotic resistant strains could well be occurring at about the same rates in the community as in the hospital environments. Indeed, other investigations and correlation studies have pointed to similar factors being responsible for spread of drug resistance in different environments in Nigeria (Eze *et al.*, 2011). **Is this not a case of bacteria becoming a problem with our co-operation and encouragement?**

Virulence genes located on movable DNA

Besides the issue of drug resistance, another major issue plaguing the world of medical bacteriology is the issue of occurrence and transfer of virulence genes in bacteria. Recent evidence has shown that virulence genes in bacteria may be found on both chromosomal and non-chromosomal DNA. The non-chromosomal DNA includes transposons and plasmids. The

implication of this is that genes encoding virulence or enabling organisms to be pathogenic, if present on these movable pieces of DNA, can easily be transferred among bacteria. Thus, the line between virulence and non-virulence of bacterial strains is virtually obliterated, because a so-called non-virulent strain may become virulent if it acquires these movable DNA segments carrying the virulence genes and vice versa. An example comes to mind here.

Six species in the genus *Bacillus* namely: *Bacillus anthracis, Bacillus cereus, Bacillus thuringiensis, Bacillus weihenstephanensis, Bacillus mycoides* and *Bacillus pseudomycoides* share over 99% homology chromosomally, but differ in their plasmid-encoded features (Rasko *et al*., 2005). Three of the species are well-known pathogens: *B. anthracis* is the cause of anthrax disease; *Bacillus thuringiensis* (BT) is the well known insecticide and *Bacillus cereus* is the causative agent of two forms of food poisoning. The genes responsible for the different virulence profiles of these pathogenic species are located on plasmids which may be acquired or lost. For example, *Bacillus anthracis* contains two plasmids, pXO1 and pXO2, encoding toxin production and encapsulation, respectively, that define this species' pathogenic potential, the presence of a Bt toxin-encoding plasmid defines *Bacillus thuringiensis* isolates, whereas different plasmids define the emetic and diarrhoeal *Bacillus cereus* isolates. The implication is that if any member of the group should lose its identifying plasmid and acquire another, its identity

would also change. In other words, *B. anthracis* can become *B. thuringiensis* and vice versa.

These plasmid-based species definitions have resulted in the classification of members of the *B. cereus* group that are not valid even when molecular typing is applied. Recent studies have suggested that there may be more diversity within this group of pathogens than previously thought (Apetroaie *et al.,* 2005). This was confirmed by a recent study conducted in our laboratory in which we characterized over 300 *B. cereus* isolates from different foods, in an attempt to identify features for separating the more dangerous emetic strains from the diarrhoeal strains. The results showed that even atypical *B. cereus* strains (strains having significantly different morphological features from reference strains) shared the same plasmid profile with typical strains (Ezeonu and Ugwu, 2009).

The summary is that, given these recently discovered phenomena, it is more difficult to really say which organism is a pathogen and which is not. How then do humans stay safe? **Once again, we come down to the issue of our co-operation with the bacteria.**

Studies have shown that most bacterial diseases can be prevented by proper behavioural and hygienic practices. For instance, food-borne bacterial diseases can be prevented by (1) preventing the initial contamination of the raw food by bacteria (2) where initial contamination cannot be prevented, the food should be cooked properly to kill contaminants (3) already cooked and processed foods should be consumed within a short time after processing (4) if food must be stored, it should

be stored under conditions that inhibit growth of bacteria and production of their toxins (e.g. in the refrigerator). Similarly, sexually transmitted diseases can be prevented by avoiding sex with infected individuals and avoiding sex with multiple partners.

3.0 BACTERIA AS COMMENSALS

From the time of discovery of bacteria, their roles in disease production have been widely studied. In fact, although there are more bacteria involved in beneficial relationships with humans than in disease production, the study of bacteria in health and medicine has been dominated by the study of their disease-causing mechanisms. However, the focus is gradually shifting as scientists try to understand the complex relationships between humans and bacteria.

In a healthy animal (including humans), the internal tissues, e.g. blood, brain, muscle, etc., are normally free of microorganisms. However, the surface tissues, i.e., skin and mucous membranes are constantly in contact with environmental organisms and become readily colonized by various microbial species. The mixture of organisms regularly found at any anatomical site is referred to as the **normal flora**, except by researchers in the field who prefer the term "**indigenous microbiota**". The normal flora of humans consists of a few eucaryotic fungi and protists, but bacteria are the most numerous and obvious microbial components of the normal flora (Todar, 2011). Some of the predominant bacterial flora of humans are shown in Table 3 below.

Table 3. Some bacteria commonly found on the surfaces of the human body.

BACTERIUM	Skin	Con-junc-tiva	Nose	Pharynx	Mouth	Lower GI	Ant. ure-thra	Vagina
Staphylococcus epidermidis	++	+	++	++	++	+	++	++
Staphylococcus aureus*	+	+/-	+	+	+	++	+/-	+
Streptococcus mitis				+	++	+/-	+	+
Streptococcus salivarius				++	++			
Streptococcus mutans*				+	++			
Enterococcus faecalis*				+/-	+	++	+	+
Streptococcus pneumoniae*		+/-	+/-	+	+			+/-
Streptococcus pyogenes*	+/-	+/-		+	+	+/-		+/-
Neisseria sp.		+	+	++	+		+	+
Neisseria meningitidis*			+	++	+			+
Enterobacteriaceae*(Escherichia coli)		+/-	+/-	+/-	+	++	+	+
Proteus sp.		+/-	+	+	+	+	+	+
Pseudomonas aeruginosa*				+/-	+/-	+	+/-	
Haemophilus influenzae*		+/-	+	+	+			
Bacteroides sp.*						++	+	+/-
Bifidobacterium bifidum						++		
Lactobacillus sp.				+	++	++		++
Clostridium sp.*					+/-	++		
Corynebacteria	++	+	++	+	+	+	+	+
Actinomycetes				+	+			

++ = nearly 100 percent + = common (about 25 percent) +/- = rare (less than 5%) * = potential pathogen

According to Cummings (1997), **the human body lives in a heavily contaminated bacterial environment and symbiosis with these microbes seems a condition for survival in most occasions.** There are approximately ten times as many bacterial cells in the human flora as there are human cells in the body, with large numbers of bacteria on the skin and as gut flora (Sears, 2005). These bacteria inhabiting the human body carry out useful processes leading to the overall health of the human individual.

Two examples are given below:

(1) In the intestinal tract, members of the bacterial flora produce vitamins which are then absorbed by the host through the intestine, inhibit pathogens, release additional energy from undigested food ingredients and maintain the lining of the tract.

(2) In the urinary and genital tracts, the commensal bacteria help to maintain an acid pH, thereby inhibiting colonization by pathogenic bacteria.

In this lecture, we will examine the roles of the intestinal (gut) bacteria as a case study of the beneficial roles played by bacteria.

3.1 The Roles of the Gut Flora

By far, the most heavily colonized organ of the human body is the gastrointestinal tract (GIT). It has an estimated surface area of 200 m^2 and as such represents a major surface for microbial colonization. Additionally, the GIT is rich in molecules that can be used as nutrients

by microbes, making it a preferred site for colonization. The colon alone is estimated to contain over 70% of all the microbes in the human body (Whitman *et al*., 1998; Ley *et al*., 2006).

The gastrointestinal tract of humans represents one of the most complex microbial ecosystems. It is widely accepted that the GIT contains more than 1,000 bacterial species, but some studies suggest that there may be as many as 35,000 species (Salminen *et al.,* 2005; Frank *et al*., 2007). The intestinal microbiota is not homogeneous. The number of bacterial cells present in the mammalian gut shows a continuum that goes from 10^1 to 10^3 bacteria/g of contents in the stomach and duodenum, progressing to 10^4 to 10^7 bacteria/g in the jejunum and ileum and culminating in 10^{11} to 10^{12} cells/g in the colon (O'Hara and Shanahan, 2006).

Colonization of the human gut with microbes begins immediately at birth. Upon passage through the birth canal, infants are exposed to a complex microbial population. After the initial establishment of the intestinal microbiota and during the first year of life, the microbial composition of the mammalian intestine is relatively simple and varies widely between different individuals and also with time. However, after 1 yr of age, the intestinal microbiota of children starts to resemble that of a young adult and stabilizes (Mandar and Mikelsaar, 1996; Mackie *et al*., 1999).

Besides the mother's microbiota composition, many other factors contribute to the microbial makeup of the mammalian GIT, including diet and host genetic factors working through the host metabolism. Despite these

factors, however, the composition of the human microbiota is fairly stable at the phylum level. The major groups that dominate the human intestine are conserved between all individuals, although the proportions of these groups can vary (Eckburg *et al*., 2005; Gill *et al*., 2006). Bacteria from two phyla, Bacteroidetes and the Firmicutes dominate the GIT, whereas Proteobacteria, Verrucomicrobia, Actinobacteria, Fusobacteria, and Cyanobacteria are present in minor proportions (Eckburg *et al*., 2005).

It has been suggested that the relationship between humans and their intestinal flora is not merely commensal (a non-harmful coexistence) but a mutually beneficial symbiotic relationship. The normal flora derive from their host a steady supply of nutrients, a stable environment, and protection and transport, while the host obtains from the normal flora certain nutritional and digestive benefits, stimulation of the development and activity of immune system, and protection against colonization and infection by pathogenic microbes (Sears, 2005; Todar, 2011). Different gut microbiota components affect different aspects of normal host development (Sears, 2005). According to some authors, the intestinal flora - the sum of all indigenous bacteria that reside inside the intestinal tract (the host) – may be considered an organ in itself, just like the liver or bone marrow, because the bacteria perform a range of essential, health-critical functions that cannot be reliably duplicated by any other means (Monastyrsky, 2011).

Some of the beneficial roles of the gut flora are as follows:

3.1.1 Synthesis of vitamins – The normal flora synthesize and excrete vitamins in excess of their own needs, which can be absorbed as nutrients by their host. For example, in humans, gut bacteria secrete Vitamin K and Vitamin B12, and lactic acid bacteria produce certain B-vitamins (Todar, 2011).

3.1.2 ***Digestion of complex 'undigestible' carbohydrates*** – Perhaps the major metabolic function of the gut flora is the fermentation of non-digestible carbohydrates, which are key sources of energy in the colon. Such non-digestible carbohydrates include large polysaccharides (that is resistant starches, pectins, cellulose), some oligosaccharides that escape digestion, as well as unabsorbed sugars and alcohols. The primary metabolic endpoint of such fermentation is the generation of short-chain fatty acids (Cummings and McFarlane, 1991; Canny and McCormick, 2008).

The short-chain fatty acids (SCFA) are absorbed by the perfused human colon in a concentration-dependent manner and are the major respiratory fuels for colonocytes, supplying up to 60 to 70% of their energy needs. SCFA also stimulate the growth of colorectal mucosal cells, retard mucosal atrophy, and decrease the risk of malignant transformation in the colon. Butyrate, in particular, has been shown to be particularly effective in decreasing the risk of malignant transformation of the colon (Rossi *et al*., 2005).

3.1.3 Inhibition of pathogens – In addition to the impact on the host, the intestinal flora also affect one another (Todar, 2011). The normal bacterial intestinal flora represents an extremely important defence mechanism, which effectively interferes with the establishment of many important enteric pathogens. In the absence of the full complement of normal flora, opportunistic microorganisms such as antibiotic-resistant *Staphylococcus, Proteus, Clostridium difficile* or the yeast *Candida albicans* can become established, and retention of these opportunistic pathogens can lead to a harmful alteration of digestive function and eventually disease (Madigan, 2008).

The ability of the microflora to inhibit non-indigenous species or pathogens from colonizing the surface is called antagonism. There are three main ways that the normal flora protects the host from the invasion of the pathogens (Todar, 2008). These are:

(1) Competition - Bacteria of the normal flora are highly adapted to the tissues of their hosts. This adaptation makes it easier for them to colonize the environment and outgrow the non-indigenous or pathogenic species and thus stop the pathogens from invading the host.

(2) Specific antagonism - In this type of antagonism, the bacteria of the normal flora produce very specific proteins called bacteriocins**,** which kill or inhibit other closely related bacterial species. For instance, there is production of colicins by some strains of *E. coli*, marcescins by *Serratia,* pyocins by *Pseudomonas* and bacitracins by *Bacillus.* Some

strains of *Lactobacillus* also produce bacteriocins. The bacteriocins as stated earlier are lethal to other bacterial species including pathogenic strains (Todar, 2008).

(3) Non-specific antagonism – bacteria of the normal flora protect the host by producing a variety of metabolites and end products that inhibit other microorganisms. These products include the short chain fatty acids, peroxides and antibiotics (Todar, 2008). The short chain fatty acids exert their inhibitory effects by lowering the pH of the colon, making the environment unconducive for non-indigenous species (Lievin *et al*., 2000; Rastall *et al*., 2005).

3.1.4 Modulation of gut immunity – The gut bacteria can stimulate innate and adaptive immunity (e.g increased IgA production), proliferation of intra-epithelial lymphocytes, regulation of the balance of T-helper 1 (Th1) and T-helper 2 (Th2) and cell mediated immune responses (by increasing the phagocytic activities of the white blood cells) in the host, against pathogens (MacFarlane and Cummings, 1999; Cogan *et al*., 2007).

Gut bacteria produce substances that help in proliferation of the intestinal epithelial cells, blood leucocytes, B and T lymphocytes and other accessory cells of the immune system (Schiffrin *et al*., 1997). For instance, *Eubacterium rectale* has been found to produce butyrate in large amounts. The butyrate is absorbed in the colon and utilized for the proliferation of the

leukocytes and enterocytes (Rastall *et al*., 2005). Also, as previously stated, butyrate is effective in decreasing the risk of malignant transformation of the colon (Rossi *et al*., 2005). Gut bacteria such as the bifidobacteria and enterococci produce folate in large amounts (Crittenden *et al*., 2003). Folate is involved in many metabolic pathways such as methyl group biogenesis, nucleotide synthesis, vitamins and some amino acids synthesis and folate availability increases the efficiency of DNA replication, repair and methylation.

3.1.5 Formation of normal stool

Stool is composed primarily of water, live intestinal bacteria, dead bacteria and cells shed by the body, mineral salts, colouring pigments and traces of fat. Intestinal bacteria are by far the largest component of stools, making up 30 to 50% of the total dry matter of faeces (O'Hara and Shanahan, 2006; O'Keefe, 2008; Todar, 2011). Single cell organisms, such as bacteria, contain mostly water, encircled by impenetrable membranes. In large quantities, they provide normal stools with its amorphous qualities (Todar, 2011).

Since bacteria represent the most dominant component of normal stool, their absence may therefore cause persistent hard stools; that's why dry stools reliably point to disbacteriosis (a change of composition and/or volume of normal intestinal flora).

The important role of the intestinal bacteria in formation of normal stool can further be illustrated by a closer look at caecotroph animals like rabbits. For these animals, the other important factor in the digestive

system, besides fibre, is the consumption of the caecotrophs (the first set of faeces). These smelly, sticky faeces contain bacteria and vitamins and provide vital nutrients for a healthy gut. These bacteria consumed in the caecotrophs then carry out full digestion of the rabbit feed to produce the hard pellets that make up the normal rabbit stool. If a rabbit is unable to eat this nutritious first faeces either because it becomes too overweight to reach around or for any other reason, the ultimate result is disease (Clive, 2011).

Some of these important roles of the intestinal bacteria were first discovered by comparative studies on germ-free animals (*gnotobiotic* animals) and conventional animals (Rolfe, 1984; Madigan *et al*., 2009; Todar, 2011).

3.2 Problems Associated with Removal of Essential Bacterial Flora

The human bowel flora is considered to be healthy if the host is not suffering, clinically, any disorders that might be related to it and generally has painless, soft daily bowel movements without any bleeding, and a normal amount of flatus. Furthermore, on examination, the normal components of the flora should be in their usual, accepted qualitative/quantitative numbers and there should be no detectable pathogens present (Todar, 2011). The following are some problems associated with unhealthy bowel flora.

Antibiotic-associated diarrhoea

Diarrhoea is defined as the frequent passing of loose or watery stools (usually three or more liquid bowel movements in a day). It is a symptom of a variety of disease conditions of the GIT, including infection, food poisoning and gastrointestinal tumor. However, some occurrences of diarrhoea are more specifically associated with particular infections or diseases.

One of the best known complications arising following antibiotic therapy is antibiotic-associated diarrhoea, which can be due to the pathological overgrowth of *Clostridium difficile* in the antibiotic-treated GIT (Borody, 2003; McFarland, 2008). It is not the antibiotic that causes the infection. Rather, the antibiotic use weakens the defence of the bowel bacteria thus permitting *C. difficile* to implant in the bowel (Kyne and Kelly, 2000).

Clostridium difficile is a spore-forming bacterium which is probably most commonly 'caught' through the mouth often in hospitals, especially after the use of antibiotics. It can result in syndromes of varying severity including transient diarrhoea, a carrier status, a mild colitis-like illness, pseudomembranous colitis (*C. difficile* colitis, characterized by offensive-smelling diarrhoea, fever and abdominal pain), and even toxic megacolon with possible mortality.

Most affected patients begin to get better once the responsible antibiotic is discontinued and **specific antibiotics** such as vancomycin, rifampicin or bacitracin, which affect *C. difficile,* are used. However, in up to 25% or more of patients, symptoms may recur.

This is because the normal flora may not at this time be capable of eradicating the persisting clostridial spores. In many patients the *C. difficile* spores remain and a chronic, relapsing disease can continue (Kyne and Kelly, 2000). Treatment of this recurrent *C. difficile* diarrhoea can be particularly difficult but some approaches to therapy have included resins such as cholestyramine (Questran) and colestid (Colestipol) granules, specific probiotics such as *Lactobacillus* GG, *Saccharomyces boulardii* and intravenous immunoglobulin (Leung *et al*., 1991). In cases where these medical therapies fail, re-colonisation of the colon using human faecal origin probiotics has also been used and reported to be successful in eradicating spores (Persky and Brandt, 2000).

Diarrhoea-predominant irritable bowel syndrome (IBS)

Irritable bowel syndrome (IBS) is a common disorder of the intestine, where patients suffer with symptoms such as cramping abdominal pain, bloating, flatulence and change in bowel habit – all suggestive of a chronic bowel flora infection.

Diarrhoea-predominant IBS is a common disorder of the gastrointestinal tract that can manifest with cramping pain, diarrhoea, bloating, explosive stools, urgency, incontinence and rectal bleeding if the stools are very frequent. Although the cause of IBS is unknown it has been termed "a functional disorder" because there is no sign of any disease when the bowels are examined (normal structure) and it is presumed that only the function has changed. Recently, it has been suggested

that the condition could be caused by inflammation or a chronic infection that has not been detected by standard stool tests, particularly infections due to some undetectable bacteria or uncommon parasites such as *Dientamoeba fragilis, Blastocystis hominis,* and *Entamoeba histolytica* (Horwitz and Fisher, 2001; Hasler, 2001). Observations of improvement in the condition, following purging of the bowel and infusion of normal flora, strengthen this suggestion (Borody, 2000).

Constipation-predominant irritable bowel syndrome

Individuals with constipation predominant IBS typically suffer bloating especially after meals, abdominal pains at times, tiredness, nausea, gas, reflux and in some situations headaches. The problem is known not to be caused by lack of fibre in the diet, inactivity or lack of water intake. However, many lay-people will advise the suffering patient to increase exercise levels and to drink more water in spite of the fact that this rarely helps. In those with mild constipation, increase of fibre intake can improve some symptoms, the fibre acting as a drug, but can worsen symptoms in others, particularly aggravating bloating. Accumulating evidence suggests that the constipation is acquired as a bacterial infection of the bowel flora with the most likely culprit being a *Clostridium* (Borody *et al.,* 2003).

Colitis

Colitis is inflammation of the bowel that can be acute or chronic. Acute inflammatory changes are usually self-

resolving and are caused by specific infections by bacteria such as *Shigella, Salmonella,* and *Campylobacter jejuni* to give a few examples. Chronic inflammation, on the other hand, can be caused by chronic infections by microorganisms such as *C. difficile, Dientamoeba fragilis*, and *E. histolytica* (Shein and Gelb, 1983).

In patients with chronic colitis of unknown origin various subdivisions of the condition have been made. Patients can have a microscopic colitis, which presents as chronic diarrhoea and cramping but colonoscopically there are no areas of inflammation until one takes a biopsy and then typical inflammatory cells are visible under the microscope. Other patients have classic distal or ulcerative colitis where colonoscopically inflammatory changes begin at the rectum and can extend to a variable distance up the bowel. In some patients the entire bowel is involved in colitis and this is called pan-colitis. In general, the more of the colon that is affected, the worse the symptoms are. Crohn's and collagenous colitis make up other subdivisions.

Intestinal Candidiasis

Candida alibicans is one of many fungi that are present in most people's intestinal tracts, and other places, where it is kept in check and causes no harm. If the intestinal balance is disrupted and healthy bacteria killed, *Candida albicans* will flourish and release toxic chemicals into the bloodstream.

When there is overgrowth, *Candida,* through the production of pseudohyphae, pushes its way into the

intestinal lining, destroying cells and brush borders. This damage allows macromolecules of partially digested food to pass through the lining, a condition known as **'leaky gut syndrome'**. The macromolecules are the perfect size for antibodies to respond to. The immune system then goes on alert for these specific foods. So, the next time they are eaten, the antibodies will be waiting to attack. The result is increased sensitivity to foods and other food substances and the environment. Also the yeast by products - metabolites that are toxic, also pass through the intestinal wall and are absorbed into the body. One of the major toxins produced by the yeast is acetic aldehyde, through conversion by the enzyme aldehyde dehydrogenase. Its multiple effects can be devastating. It is converted by the liver into alcohol, depleting the body of magnesium and potassium, reducing cell energy, and causing symptoms of intoxication, disorientation, dizziness, or mental confusion.

The 'spaciness' or 'mental fog', often described by observers of people affected, is one of the most frequent symptoms of candidiasis. This and other symptoms listed above are also the usual symptoms exhibited by children who are diagnosed as having **late-onset-autism.** On closer examination, these children may also have physical signs of yeast overgrowth that include a distinct 'yeasty' smell, and cravings for foods that yeast thrives on, such as sugars and carbohydrates, as well as a whitish coating of the tongue, diaper rash, and/or itching around the anus. In fact, there is increasing evidence

associating *Candida* overgrowth and autism (Crook, 2012).

There is increasing prevalence of intestinal candidiasis in many parts of the world today, all associated with clinical overuse of antibiotics. In light of this and the fact that antibiotics are both overused and misused in Nigeria, we have begun an investigation into the incidence of intestinal candidiasis among children in Nsukka area. Preliminary results have already shown an unusually high occurrence of the condition in children visiting the District hospital in Enugu Ezike (paper yet to be published). This should sound an alert to medical practitioners and self-medicating parents to reduce the amount of antibiotics administered to infants and children.

Other problems

The problems associated with absence of essential intestinal flora, are not limited to the GIT alone, but may spread beyond the GIT to other areas of the body. The intestinal microbiota has been linked to a number of diseases that are associated with remote organ systems. A few of these diseases are discussed below:

- **Obesity**

  Considering the previously presented discussion on the importance of microbiota for nutrient acquisition and production of vitamins and other bioactive molecules, it should not be surprising that research into the role of microbiota in the development of obesity has yielded interesting results. Researchers have demonstrated that the

gastrointestinal microbiota is directly involved in the regulation of energy homeostasis in murine and human hosts and comparisons of obese and lean distal gut microbiotas from mice and humans indicate that the community structures are very similar at the division level.

- **Allergy**

Recent studies by many researchers suggest that perturbations in the gastrointestinal microbiota, as a result of reduced microbial exposure due to changes in diet and antibiotic use, result in an underdeveloped microbiota. This "immature" microbiota delays proper maturation of the immune system, disrupting the normal sequence of events that promote the development of immunological tolerance, increasing the incidence of allergic hypersensitivity.

- **Autism**

Very little is known about the underlying etiology of autism, but extensive antibiotic use is commonly associated with late-onset autism (18–24 months of age). This has led some to hypothesize that disruptions in the normal microbiota may allow colonization by autism-triggering microorganism(s), or promote the overgrowth of neurotoxin-producing bacteria like *Clostridium tetani*. The link between the intestinal microbiota and autism is supported by the following observations: *1*) disease onset often follows antimicrobial therapy, *2*) gastrointestinal

abnormalities are often present at the onset of autism and frequently persist, and *3*) autistic symptoms have sometimes been reduced by oral vancomycin treatment, which is effective against clostridia, while relapse occurs following cessation of treatment.

Other problems not associated with the intestinal tract include blood-clotting problems, which may be linked to vitamin K deficiency (vitamin K is a metabolic by-product of intestinal bacteria such as *Escherichia coli*); some neurological disorders and anaemia (associated with deficiency in vitamin B12, which is also produced in the body by intestinal bacteria); and various immunological disorders (intestinal bacteria are also involved in modulation of the immune system and therefore their absence could affect immunity).

3.3 Causes of Disbacteriosis and Remedies

A change of composition and/or volume of normal intestinal flora is known as **disbacteriosis** or **dysbiosis**. Disbacteriosis is gradually coming to be recognized as a medical disorder. Disbacteriosis has many causes including age, disease, use of immunosuppressants such as steroids, alcohol, stress, poor nutrition and poor hygiene among others, but the most common cause is antibiotic use.

Well, anything that kills *bad* bacteria also kills *good* bacteria. Therefore, when antibiotics are taken, they kill both the pathogens and the commensal bacteria. Regardless of the cause of the disbacteriosis, the summary is that when the bacteria are suffering,

everything else suffers too: the immune system doesn't protect as well as it used to, the blood doesn't coagulate, the stools lack moisture, and the colon gets irritable and inflamed from a multitude of factors. Opportunistic pathogens also fluorish.

So, how can one prevent all these problems associated with disbacteriosis? The answer is – **restore the balance**.

There are several ways of restoring the intestinal microflora. These include the use of probiotics, prebiotics, synbiotics and lately, faecal bacteriotherapy.

Probiotics, Prebiotics and Synbiotics:

Probiotics are preparations of live microorganisms, which when administered in adequate amounts, have beneficial effects on the health of the person or animal (Hamilton-Miller *et al*., 2003). Most microorganisms used in probiotics are strains of Gram-positive bacteria of the genera *Bacillus* (*B. cereus, var. Toyoi, B. licheniformis, B subtilis*), *Enterococcus* (*E. faecium*), *Lactobacillus* (*L. acidophilus, L. casei, L. plantarum, L. farciminis, L. rhamnosus*), *Pediococcus* (*P. acidilactici*), and *Streptococcus* (*S. infantarius*). Some yeasts, particularly strains of *Sacccharomyces cerevisiae,* are also used (Falcao-e-Cunha *et al.,* 2007). Important dietary sources of probiotics include kefir (cultured milk), yoghurt, sour cream, cheese, pickled vegetables (olive, ginger) and fermented soy products and tea (Chakraborti, 2011). These foods are products of the fermentative activities of various bacteria, particularly

lactic acid bacteria (LAB) which are consumed with the food product.

Most of these probiotic species are among the naturally occurring microflora of the GIT and so their mechanisms of action are as already described above. Several studies have shown positive effects of probiotics, especially in younger animals (Falcao-e-Cunha *et al.,* 2007; Chakraborti, 2011). One local study, by Ezema (2010), has also shown this. In their study, the inclusion of probiotic *Saccharomyces* in poultry feed, caused increased growth, increased egg laying and other improvements in the poultry.

Despite these positive reports, however, there are a few limitations to the use of probiotics: the live bacteria and/or yeasts of the probiotic must be able to withstand the manufacture and storage of the feeds where they are included; the probiotics must resist the animal digestive secretions and present no risk of toxicity; the probiotic organisms must attain the concentrations, in the intestine, required to have any observable effect. In addition to all these limitations, there is also the problem of consumer confidence; not many individuals are comfortable with the thought of 'eating' bacteria and hence our interest in prebiotics.

Prebiotics have been defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth, and/or activity, of one or a limited number of beneficial bacteria in the colon and thus improve host health (Gibson and Roberfroid, 1995). While probiotics are meant to bring beneficial microbes

to the gut, prebiotics are supposed to selectively stimulate the beneficial microbes that already live there. They have two clear advantages over probiotics: first, there are no critical problems with the thermal processing of the feed and the acid conditions of the stomach and second, they do not introduce foreign microbial species into the gut (Falcao-e-Cunha *et al.,* 2007).

Prebiotics are low molecular weight, short-chained carbohydrates with 2-10 degrees of polymerization. They reach the colon largely unhydrolysed and can act as a substrate for the colonic microflora, specifically increasing the numbers of bifidobacteria, lactobacilli and a few other beneficial bacteria at the expense of other microflora components such as *Bacteroides,* clostridia and *E. coli* (Liong, 2008; Burns and Rowland, 2000). Hence, they are foods or supplements which help the beneficial bacteria in the body to perform better (roles of beneficial bacteria have been described above). In addition to the well established positive influence on intestinal microflora, however, prebiotics have certain indirect effects that include cancer prevention, positive effects on lipid metabolism, stimulation of mineral adsorption and immuno-modulatory properties (Falcao-e-Cunha *et al.,* 2007; Chakraborti, 2011).

The most widely studied prebiotics are inulin, resistant starch and non-digestible oligosaccharides, particularly fructo-oligosaccharides. However other candidate prebiotics are polyols like xylitol, sorbitol, mannitol; disaccharides like lactulose, lactilol; oligosaccharides like raffinose, soybean, and other non-

digestible oligosaccharides (Chakraborti, 2011). Some of these prebiotics have been found to be naturally present in a variety of plant foods such as bananas, barley, chicory, garlic, leeks, onions and wheat (Van Loo *et al*., 1995), but they may also be present in other foods not yet identified.

We have recently delved into this area of research and are investigating a variety of local foods for prebiotic properties. So far, we have recorded positive results with *Vernonia amygdalina* (commonly known as bitter leaf). Results from our studies showed that oral administration of aqueous extracts of *V. amygdalina* leaves to human volunteers had a positive influence on bowel function and increased the numbers of beneficial bacteria in the intestine (Ukwah and Ezeonu, 2008; Ezeonu and Ukwah, 2009). Similarly, in rabbit models, administration of the extracts selectively stimulated the growth of beneficial bacteria, while reducing the numbers of some non-beneficial organisms. In addition, administration of the extracts protected the animals against infection and colonization by *E. coli* and *Staphylococcus aureus* (Ezeonu *et al*., 2012). Studies are ongoing to identify the specific prebiotic(s) contained in these leaves and also identify other local foods with prebiotic properties. Studies are also being conducted to evaluate some synbiotics.

Synbiotics are foods or food supplements which contain live cells of beneficial bacteria (probiotic) and selective substrates (prebiotics). A synbiotic therefore contains and provides the combined beneficial effects of both probiotics and prebiotics. In a still on-going

collaborative study with researchers from the Department of Animal Production, we have evaluated the effect of a local synbiotic blend (*V. amygdalina* leaves and *Saccharomyces*) on broiler chickens. Preliminary results from our investigations showed that there was significant increase in the weight of the birds, improved blood profile and reduced counts of pathogenic bacteria in the faecal wastes of the birds (paper yet to be published).

Faecal bacteriotherapy or Human probiotic infusion (HPI)

Human probiotic infusion is a kind of therapy involving the replacement of an unhealthy bowel flora with a healthy one. HPIs can be carried out in several ways. Most commonly a short course of antibiotics is given to reduce the bacterial load in the bowel and the mucosal surfaces of the bowel. Then, the flora is removed by a lavage solution consumed by the patient similar to that carried out prior to colonoscopy. The bowel flora is then replaced by a series of enemas, thus implanting new bacteria to eradicate the opportunistic pathogens by the incoming flora.

Generally, doctors collect “fresh” faeces from pre-screened donors, mix them with isotonic solution (0.9% sodium chloride in distilled water), and inject the resulting suspension directly into the recipient’s large intestine by means of a flexible tube. The tube is inserted as far as possible into the colon, so the bacteria can reach the caecum (blind gut, the first section of the large intestine). To assure the bacteria’s survival, the

procedure is repeated several times (Borody *et al*., 2003).

This treatment has major advantages over laxative therapies because it can be curative and also alleviate other motor, fermentative and peristalsis abnormalities such as slow gastric emptying, reflux, bloating, nausea, dysphagia and in some patients, chronic tiredness (Persky and Brandt, 2000; Borody, 2000). It has been found to be successful for the treatment of many gastrointestinal illnesses due to disbacteriosis, especially *C. difficile* infection where a cure rate of 90 – 95% has been recorded. However, the treatment is less successful in colitis than it is in *C. difficile* diarrhoea and pseudomembranous colitis.

HPI offers a therapy of last resort to patients where other therapies have been ineffective or where symptoms continue in spite of treatment.

4.0 SUMMARY AND CONCLUSION

Before concluding my case, I would like to review the facts as presented to this distinguished audience and these facts are as follows:

1. Bacteria are best known for their role as agents of disease.
2. Of the nonillions of bacteria that exist on earth, only a very minute fraction is actually involved in causing disease.
3. For any bacterium to cause disease, it must surmount the many obstacles presented by the human immune system.

4. Some bacteria have evolved various mechanisms to overcome the host immune system and cause infection and disease.
5. Most bacterial infections can be prevented by maintaining healthy living habits, safe practices and healthy immune systems.
6. The majority of bacteria that interact with humans are friendly bacteria, involved in beneficial relationships.
7. Beneficial bacteria promote health through their various activities including production of vitamins, maintenance of healthy bowel function, maintenance of epithelial surfaces, modulation of the immune system and inhibition of harmful bacteria.
8. Activities and life styles, which reduce or eliminate our friendly bacteria, expose us to diseases.
9. Diseases associated with absence of the friendly bacteria include constipation, diarrhoea, opportunistic infections, autism etc.
10. The beneficial activities of our friendly bacteria can be maintained and enhanced by consciously cultivating these friendly organisms.

Mr Vice Chancellor, ladies and gentlemen, having presented my case in its entirety, it is now up to you, the jury, to decide whether bacteria are the enemies they are made out to be or not, but I submit to you that many of the bacteria that eventually cause harm to us, do so with our permission and sometimes, our co-operation and our fear and bad attitude towards the Bacteria are totally unwarranted. We should rather concentrate on adjusting our habits and lifestyles in order not to leave room for

attack by the few harmful bacteria. We should also cultivate the many friendly bacteria in and around us, to help in maintaining our health. It is only in this way that judgement will be given in favour of the people. And it is on this note that I rest my case!

Free at last!

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