Microbial Pathogenesis 121 (2018) 139–146

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

# Pathogenic flora composition and overview of the trends used for bacterial pathogenicity identifications

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ARTICLE INFO

*Keywords:*
pathogenic bacteria
Colonization
Tissue injury
Diseases

ABSTRACT

Over 250 species of resident flora in the class of bacteria are known to be associated with humans. These conventional flora compositions is often determined by factors which may not be limited to genetics, age, sex, stress and nutrition of humans. Man is constantly in contact with bacteria through media such as air, water, soil and food. This paper reviews the concept of bacterial pathogenesis from the sequential point of colonization to tissue injury. The paper in addition to examination of the factors which enhance virulence in bacterial pathogens also x-rayed the concept of pathogenicity islands and the next generation approaches or rather current trends/methods used in the bacterial pathogenicity investigations. In terms of pathogenicity which of course is the capacity to cause disease in animals, requires that the attacking bacterial strain is virulent, and has ability to bypass the host immune defensive mechanisms. In order to achieve or exhibit pathogenicity, the virulence factors required by microorganisms include capsule, pigments, enzymes, iron acquisition through siderophores. Bacterial Pathogenicity Islands as a distinct concept in bacterial pathogenesis are just loci on the chromosome or extra chromosomal units which are acquired by horizontal gene transfer within pathogens in a microbial community or biofilm. In the area of laboratory investigations, bacterial pathogenesis was initially carried out using culture dependent approaches, which can only detect about 1% of human and veterinary-important pathogens. However, in the recent paradigms shift, the use of proteomics, metagenomics, phylogenetic tree analyses, spoligotyping, and finger printing etc. have made it possible that 100% of the bacterial pathogens in nature can be extensively studied.

## 1. Introduction to basic concepts in bacterial pathogenesis

### 1.1. Normal flora of humans

There is absent of microorganisms including bacteria in the tissues, brain and muscles of healthy animals. However, the protective tissues of animals like skin and other anatomical sites and mucous membranes are colonised by bacterial species as humans come in contact with the environment. The consortium of bacteria regularly encountered in tissues of human body especially surface tissues of the skin act to protect the body parts against invasion by other groups of microorganism. They are also referred to as normal microbiota or indigenous microbiota by many authorities [1]. In the course of the Development of Microbiology and Medicine as fields of study, these conventional floras were grouped into two broad categorized namely: (see Figs. 1–3).

Resident flora - absolutely present
Transient flora –their presence is just for a short while

The normal flora of humans comprises of over 250 bacteria isolates. This normal flora composition is often determined by factors which may not be limited to genetics, age of the host, sex, stress and nutrition pattern of the host. Interestingly, there are wide range of reports on the developmental changes in humans including; weaning at infant stage, the cutting of all the set of teeth, and the commencement and seizure of ovulation, which indirectly hold a lot of influence on the spectra of the

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https://doi.org/10.1016/j.micpath.2018.05.006
Received 8 April 2018; Received in revised form 28 April 2018; Accepted 4 May 2018
Available online 05 May 2018
0882-4010/ 

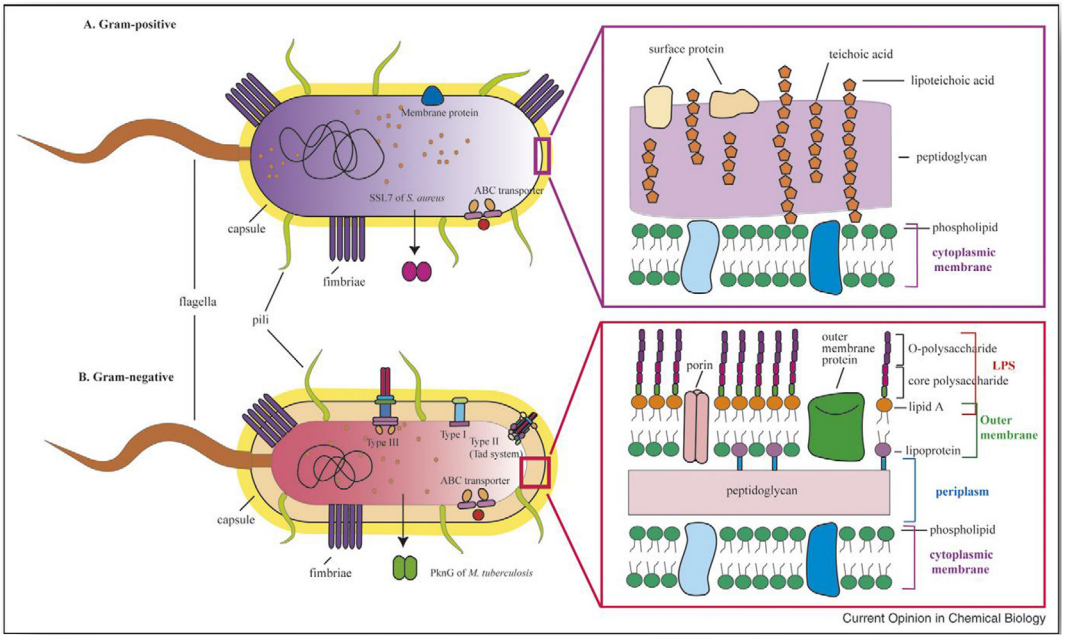


**Fig. 1.** The schematic representation of some salient virulence factors in pathogenic Gram-positive (A) and Gram-negative bacteria (B) as reported Hsing-Ju et al. [38].
**Source**: Hsing-Juet al. [38].

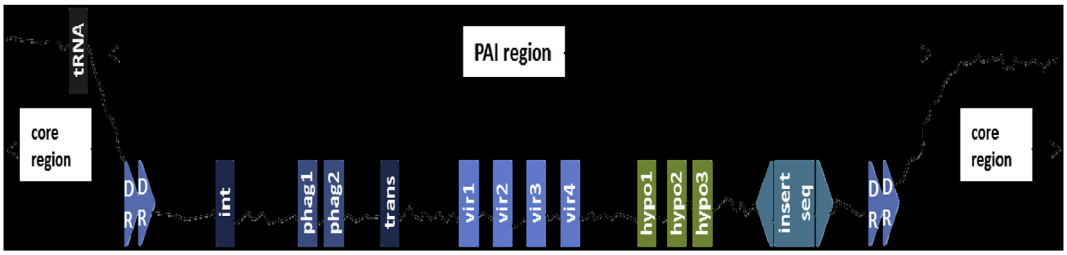


**Fig. 2.** A typical diagrammatic presentation of pathogenicity islands showing the various the associated virulence genes (vir1, vir2, vir3, and vir4).
Source: Che et al. [31].

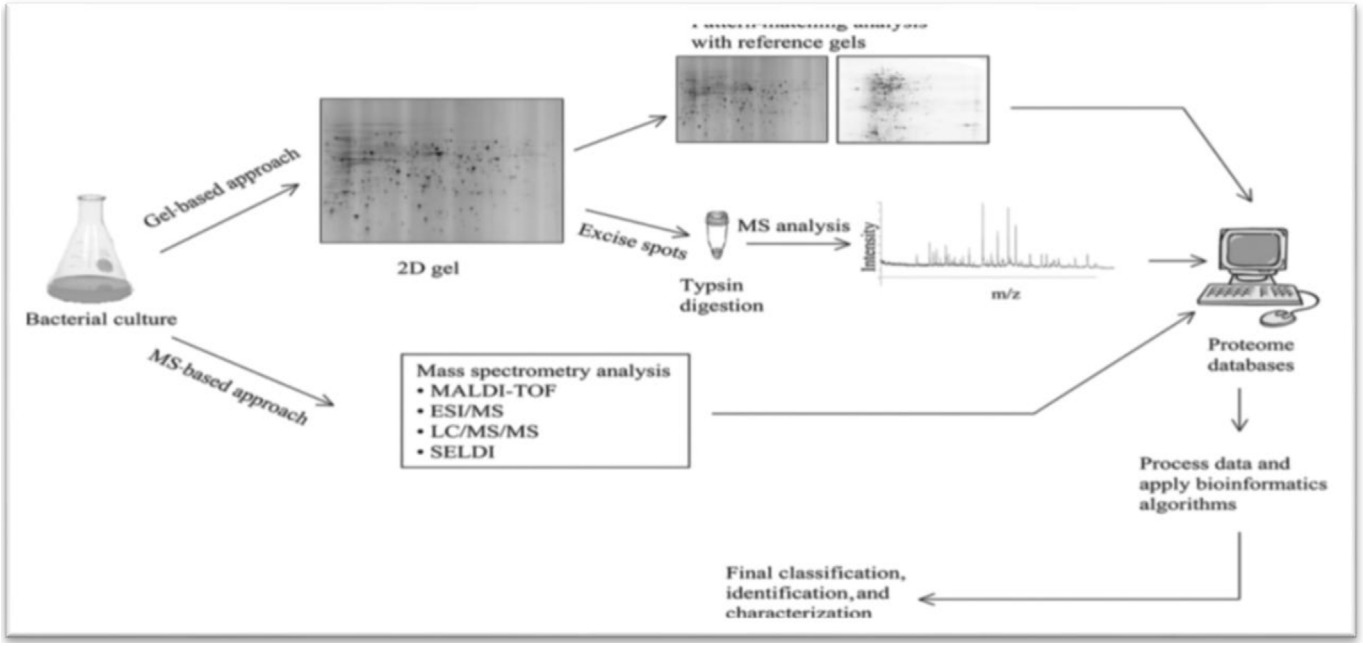


**Fig. 3.** Proteomic procedures for identification of bacteria using culture-independent approach.

host's flora found in various organs and sites of the body such as, the intestinal tract, the oral cavity, and the vagina [2].

Generally, humans have benefitted from these floras in the under listed ways:

i The normal flora prevents colonization of human tissues and organs by bacterial pathogens. In order to prevent colonization by pathogens, these floras often colonize the tissues sites in such a way that there is no available position for the bacterial pathogens to attach and struggle for nutrients such as carbon, nitrogen and phosphorus sources.

ii Another route, through which the flora of humans has benefitted man, is by knocking off bacterial pathogens through the production of secondary metabolites which inhibit bacterial pathogens through bacteriostatic process. Typically, the bacteria residing in the intestines of humans produce a variety of secondary metabolites from the polyketide pathway, or intact glucose molecules, peroxides, and related compounds, which inhibit or kill other bacteria.

iii The normal floras of humans have the potential of releasing some antibodies in an intact host. The ability of these normal floras to possess antigens and become immunologically recognised in the body of humans and lower animals, antibodies specific to them are often produced. Thereafter the initial immune sensitization with related flora, subsequent attack by bacterial pathogens will be resisted through production of specific antibodies [3].

Generally, in both human and veterinary cases, there is a continuous exposure to bacteria through media like air, water, soil and food. In the case of hosts with intact immune system, these conventional or normal floras remain harmless. However, if the host's immune system is compromised and defences are weakened, the bacteria often cause infections referred to as opportunistic infectious diseases with various forms of manifestation including septicaemia. However, if the infections are hospital acquired or nosocomial infection, the tendencies or probability that the causative pathogen possesses resistant genes to common antibiotics is high [4].

In order to understand the concept of pathogenesis, it is important to have a clear understanding of what a pathogen is. Pathogens are microorganisms including bacteria that possess the genetic and phenotypic tendencies to cause diseases to humans and lower animals, while pathogenicity is a microbial phenomenon showing the tendencies of microorganisms including bacteria to cause diseases in a host. Some microorganisms may not be able to establish any disease condition in an intact host unless the immune system of the host is compromised or weakened by some factors [4].

### 1.2. Opportunistic pathogens

In the case of bacteria which cause diseases in an immuno-compromised host which would not occur in healthy hosts represent the group of disease agents called opportunistic pathogens. It is in the case of underlying factors like defective immune system that *some* bacterial isolates behave as pathogens and are able to initiate opportunistic infections. In a case history, where a normal flora is able to establish an infectious disease, the disease is called an endogegenous bacterial disease [5]. Many opportunistic pathogens such as coagulase-negative *Staphylococcus* and *Escherichia coli* are integral aspects of the normal human flora and are usually carried on the surfaces of skin or mucosal. Tortora [6] opined that the introduction of these normal flora into sites in the human body where they are not normally found, or withdrawal of some associated bacteria by the use of wide-spectrum antibiotics such as the beta-lactams, aminoglycosides, quinolones, will never allow multiplication of these bacterial pathogens and their subsequent development into full blown bacterial disease.

### 1.3. Innate and acquired immunity: overview

Innate immunity is a form of disease defence common to both healthy populations of human and lower animals. The defensive tools of an intact host usually will enhance protection against the invasive actions of the conventional flora and the diseases caused by bacteria. Innate immunity, which is also known as natural immunity therefore, will involve the protective actions of the structures like skin, nose hairs, antigen-antibody reaction and phagocytosis by immunoglobulins [5].

Acquired immunity are host protective powers derived by an intact host on exposure to a bacterial pathogen. This is quite different from the innate immunity because acquired immunity can only be triggered if the host is appropriately exposed to a pathogen or group of pathogens in the case of poly-bacterial infection of diseases.

Invariably, it must be clearly mentioned that acquired immunity is generally quite specific and is usually directed against a pathogen invading an intact host. Thus, while innate immunity is mostly directed towards general body defence, it is also worthy to mention that the innate immunity is not adequate on its own to protect a host from bacterial of general microbial infection.

The innate immune response to invasive pathogens has remained another major drive that determines if antigen of bacteria during interaction with bacteria will establish an infectious disease. There is need for advanced research in immunochemistry using state-of the art-scientific tools in order to have a better understanding of the whole process by which innate immune take molecular cognizance of bacterial antigens and the sequence of reaction that leads to danger signals like cellular stress. An intact host with immune system that has not been compromised by any factor will equally respond to a wide spectrum of bacterial pathogens by recruiting cells of inflammation, and also secretes antimicrobial mediators.

In Immunochemistry, the Toll-like receptors (TLRs) and nuclear oligomerization domain (NOD)-like receptors (NLRs) are two major families of germ-line encoded receptors that recognize PAMPS and initiate immune signalling and thus are important players in the host response to bacterial infections. Notwithstanding these sophisticated war against pathogens, bacterial pathogens have devised sophisticated mechanisms to bypass human innate immunity [7].

### 1.4. Mutual interactions between bacterial pathogens and hosts

Virulence of pathogenic bacteria cannot be exhibited until there is a mutual interaction between the pathogens and the hosts (Humans and Lower Animals). The specificity of bacterial pathogens to various hosts cell lines and compartments of cells leads to host-pathogen interaction that generates potential virulence [8]. There are overwhelming scientific evidence which shows that the pathways of metabolism of microbial pathogens are regulated during the infection process in order to adapt to the dynamic conditions of the host environment.

Pathogenic microorganisms which include bacteria that often colonize the surface tissues of the animal are the normal flora. These bacteria have a broad range of relationship with their hosts. In terms of mutual interaction between bacteria and higher animals, there are other sub-relationships on the inherent benefit and position of each of the organisms in the relationship. Some of them have symbiotic relationship with their host, if and only if the two organisms live in an environment and closely with themselves [9].

The symbiotic relationships between microorganisms and higher animals have been further reclassified into mutualism and commensalism. Mutualism is an association where both members of the association (the bacteria and the hosts) benefit. A typical example in humans is the association between the lactic acid bacteria (LAB) and the female genital tract. The LAB physiological group of bacteria are genetically equipped in the natural environment to provide lactic acid which help in protecting the normal flora of the vagina and also knock off foreign pathogens (yeasts and bacteria) capable of establishing

urinary tract infection and related diseases. On the other hand, vagina of humans provides the required temperature for their survival [10].

In a commensalism type of relationship, no observable harm is experienced by each member in the association.

In addition, parasitism is a form of relationship between bacterial pathogens and hosts that has become a public health challenge.

Generally in pathogenic bacteriology, parasitic bacteria survive in the body of human or other lower animals, deriving nutrition and protection from the host, while there is no apparent benefit the host derives from the pathogen. In biological sciences, observations have shown that the mode of existence of a parasite can bring debilitating, annihilating or deleterious conditions to the hosts [11].

Casadevall and Pirofski [12] had previously put forward the opinion that in serious attempts to understand the differences between pathogenic and non-pathogenic microbes, early researchers/workers tried to identify characteristics that allowed pathogenic microbes to cause disease. Scientific studies in the areas of pathogenic bacteriology and microbial metabolism have taken position that microorganisms are able to produce metabolites within human tissues in order to cause debilitating diseases [12].

## 2. Bacterial pathogenesis

The ability of a microbial strain to establish disease is simply called pathogenicity. It requires the quality of communicability from a host or reservoir to a fresh host, ability to sustain life in the new host, infectivity or the ability to bypass new host's defences and virulence [13].

The organised or well-arranged in vitro process that bacteria will undergo alongside some biochemical reactions to establish diseases in humans and lower animals is referred to as bacterial pathogenesis [14]. The idea that pathogenic microbes are endowed with certain components that confer upon them the capacity for virulence is the central theme of the virulence factor concept. It is important to state that virulence factor has been widely defined by various authorities with different related views, leading to high dose of controversies around the concept of virulence factor [13].

The steps involved in the process of pathogenesis are numerous, sequential and usually begins with the transmission of the infectious agent (bacterial) to the host, which is followed by colonization of the site. After the colonization of tissue sites, the bacteria persistently adhere to colonization site before invading the host system. If the bacteria survive the host immune system, then the pathogen will establish its specific disease on the host.

Steps involved in pathogenesis of bacteria include;

### 2.1. Transmission

Transmission has to provide insight on the route through which pathogens must gain access to the host. Bacterial pathogens may access the body of an intact host by various routes which are not limited to the urinary or genital tracts, respiratory and gastrointestinal tracts. In other words, bacterial pathogens may directly enter tissues through the bites of venomous or non-venomous insects, and also through skin tissue abrasions during wounds, accidents or surgery.

### 2.2. Colonization

After transmission, colonization which is the initial stage of microbial infection takes place. The common ports of entry into the human and veterinary hosts are the urogenital tract, gastrointestinal tract, the respiratory tract and the eye. Bacterial pathogens which attack these sites must adhere to tissues of hosts before exhibition of potentials to enable them bypass the defensive mechanisms especially on body surfaces.

### 2.3. Attachment

Attachment is an essential initial colonization followed by penetration through tissues. Attachment is seemingly essential to avoid the innate host defensive mechanisms which are not limited to the enzyme effects and mechanical flushing which can effectively remove bacterial pathogens that are non-adherent.

However, for the attachment of bacterial pathogens to be successful, the bacterial pathogens must;

i. Be able to access some essential nutrients such as iron for growth.
ii Possess fimbriae or pili. The bacterial pathogens(s) must have one or more common attachment or adherence strategies which are fimbriae and monomeric protein adhesins.
iii. The fimbriae or pili receptors of most bacterial pathogens are usually made of carbohydrate residues of glycoproteins or glycolipids.

Attachment is rather a more fragile, highly specific binding, often mediated by adhesins, can be blocked by antibodies, often specific for host tissue type/location. The attachment organelle such as pilli could be made of monomeric protein adhesins, where the attachment is mediated by cell surface proteins, tighter binding to host cell may recognize proteins on host cell surface or may follow looser fimbrial attachment.

### 2.4. Invasion

Invasion by bacterial pathogens has been described as the inward penetration of host cells and tissues, which are often enhanced by a battery of molecules, which are collectively called invasinsons. Invasinsons as cell surface or internal proteins can bind specifically with the receptors of the intact host cells target host cells molecules (receptors). Penetration therefore remains a critical aspect of the initial phase of cellular invasion by bacterial pathogens to gain access into an intact host.

Many of these intracellular bacterial pathogens are not phagocytised by immune responses and equally escape from vesicles into the cell cytoplasm where they gain adequate nutritional balance and multiply rapidly before spreading to adjacent cells. Notably, it is important to mention that most bacterial pathogens in nature invade their hosts through penetration, and that has become indispensable for them to show pathogenicity. Typical examples of this group of bacteria that uses penetration as an initial weapon include but not limited to bacteria of the family of enterobacteriaceae which include *Escherichia coli*, *Salmonella shigella*, *Edwardsiella*, *Klebsiella* and *Proteus* etc.

Interestingly, there are few Gram-positive bacteria which do not need to attach to mucosal surfaces of the host in order to exert virulence and pathogenicity. Invariably, these bacterial pathogens have no need of penetration in order to show their pathogenic potentials on a host tissue. *Clostridium tetani and Corynebacterium diphtheria* which are causative agents of tetanus and diphtheria respectively, are typical examples of bacteria pathogens which do not require penetration to attack or exert pathogenicity [15]. Bacteria that do not require penetration before pathogenicity are able to reach their targets by production of gene-mediated toxins which mediate tissue damage [16].

## 3. Tissue injuries by bacterial pathogens

Tissue injuries are a major pathological impact of bacterial pathogenesis on the hosts. Bacteria that cause tissue injury are able to perfect such tissue process by some distinct mechanisms involving: the production of exotoxins, endotoxins, non-specific immunity and specific humoral and cell mediated immunity [17]. Popoff [17] reported that toxins are intrinsic pathogenicity tools released by certain physiological group of bacteria that negotiate interactions of these groups of bacterial

pathogens and their hosts. Notably, toxins from bacteria became the premier set of secondary metabolites of bacteria which led to bacterial diseases in human. Endotoxins are metabolites of Gram-negative bacteria which are responsible for inflammatory responses within a host while exotoxins are metabolites which are produced by Gram-positive bacteria. They exert their influence at distances away from the initial point of the bacterial colonization [17].

However, in terms of exotoxins, the virulence factors are injected into the cell where the bacterium is attached by specific secretion types, such as the type III secretion system, and their activity is restricted to the attacked cell [17].

Alternatively, exotoxins have equally been succinctly defined as the metabolites whichare released by bacteria during their exponential growth phase.

Notably, it must be clearly mentioned that exotoxins are produced by virulent bacterial strains while the non-virulent strains of bacteria are not genetically coded to produce exotoxins. Bacterial exotoxins have been recognised, among the most poisonous toxins produced by microorganisms and very high activities of these exotoxins at extreme dilutions confirms the potency of exotoxins of bacterial pathogens. There are also wide reports on the specificity of exotoxins of some bacterial pathogens. For instance, the exotoxins of *Clostridium tetani* which is botulinum toxin can never be produced by any other organism even in the presence of favourable conditions for horizontal gene transfers in a community of related bacterial pathogens except *Clostridium tetani*.

Interestingly, some wider ranges of bacterial exotoxins are not extremely specific in terms of their targets, as they attack wider ranges of tissues to cause tissue damage. These include the exotoxins of Streptococci and Staphylococci etc. These non-specific toxins are structurally related to phospholipases. In terms of damage to tissues, some of these exotoxins which may eventually lead to the death of the host after extensive damage to tissues and organs are referred to as lethal toxins. Examples of lethal toxins in nature include tuberculosis toxins, anthrax toxins and *Clostridium tetani* toxins etc.

On the other related version, endotoxins on their own are invariably associated with and produced by Gram-negative bacterial pathogens which invade tissues without penetration approach. In addition, the chemistry of endotoxins suggests that they are structurally related to the lipopolysaccharide (LPS). In comparison with exotoxins, bacterial endotoxins are less potent in their actions and also less specific. This is because their mode of action is non-enzymatic while the exotoxins often act enzymatically.

In terms of behaviour of endotoxins in the presence of heat, they (endotoxins) are said to be heat stable (boiling for 30 min does not destabilize endotoxin), but can be reduced in the presence of oxidizing agents such as superoxide, peroxide and hypochlorite. Another major difference between endotoxins and exotoxins is that all known endotoxins in nature are strongly antigenic, never transformed into toxoids. However, the exotoxins are easily converted to toxoids, which make it possible for Modern Biotechnology to produce toxoids which have been extensively used as vaccines to protect populations at risks of most infectious diseases.

The level of toxicity of endotoxins which can be measured using lethal dose 50 ($LD_{50}$) approach using some well-established isothermic models such as Langmiur and Fruendlich models is directly proportional to the lipid component (Lipid A), immunogenicity (antigenicity) and polysaccharide components.

There is need to mention the central roles and positions of O polysaccharide as a typical endotoxins known in clinical practices. The cell wall related O polysaccharide is a lipopolysaccharide which has been found to be a major factor of virulence in Gram-negative bacteria. Genetically, the central role of O polysaccharide is to confer smoothness on cell walls of certain strains of bacteria such as the S-strain of Streptococci. However, this smoothness invariably has been reported or rather hypothesised to be helpful for the bacteria to evade phagocytic engulfment by phagocytes [18].

Alternatively, O polysaccharide as an antigenic component of the cell wall of bacterial pathogens is the principal cause of antigenic variation in pathogens. This antigenic variation in bacterial strains enable them to overcome immune systems if the lymphocytes are not initially primed by the antigens of the attacking pathogen.

There is equally need to x-ray the central roles of the interactions of neutrophils towards inflammatory reactions and injuries to the tissues of bacterial infected hosts. Most known chronic infections are associated with sustained influx of neutrophils. Kruger et al. [19] held the opinion that chronic inflammatory diseases are known to be sustained through overdose of neutrophil constituent which are never limited to chronic obstructive pulmonary disease, nephritis, and cardiovascular diseases. Notably, emphasis are now laid on the battery or sequences of reactions which have enhanced the procedures of tissue degradation, lipid mediators and complement fragments. These reactions are often followed by the travel of neutrophils into tissues of hosts that are diseased. Christoffersson et al. [20] held the opinion that in these sequential protocols, neutrophils that are transmigrated often release their toxic components to aid further damage to tissue injuries through an immune compromised strategy [10].

## 4. Other critical virulence factor in bacterial pathogenesis

Apart from the use of bacterial toxins, modulins and agressin etc to exhibit virulence and pathogenicity, there are other notable virulence factors which have been widely reported in establishment of bacterial diseases in human and veterinary cases (Fig.1).

Notably, capsule is one of the virulent factors for exhibition of virulence by bacteria in mammals including man. Kozel et al. [21] opined that most of the known and well-researched pathogenic bacteria which are capsulated utilize the capsule to exhibit virulence and also make attempts with capsules to evade phagocytosis. Some of these bacteria with polysaccharide capsules including *Neisseria meningiitidis, Streptococcus pneumonia*, *Haemophilus influenza*, and *Klebsiella* are prokaryotes with well–established polysaccharide capsules for virulence and evasion of immune responses of an intact host.

### 4.1. Pigments

Pigments in bacterial pathogens have become a well-known virulence factor in clinical medicine both in the cases of humans and lower animals. Nosanchuk and Casadevall [22] previously held the opinion that production of bacterial pigments is linked to virulence in most known bacterial pathogens.

It is worthy to note that some pigments which are structurally related to melanin in terms of chemistry are known to protect the invading pathogen against a variety of host defensive mechanisms which are not limited to immunological responses such as phagocytosis, free radical fluxes and defensins [12].

### 4.2. Enzymes

Enzymes have been widely reported as a critical virulence component for bacterial pathogens. Succinctly put, enzymes are proteins which catalyse biochemical reactions without taking part or being consumed in the reaction. Thus it is obvious that the processes of bacterial pathogenicity are non-spontaneous.

The enzymes which are known to be a major influence in bacterial virulence factors are generally active against host components and show their ability to cause disease in the damage of host tissues [16]. Phospholipases, proteases and neuraminidases are among the wide range of known enzymatic virulence factors in pathogenic bacteriology and immunopathology [23]. The mechanisms of operation of enzymes have not been fully understood but as cells are damaged, the damage and degradation of tissues into minute fragments provide nutrients to

invading bacteria. *Staphylococcus saprophyticus* strains from various sources including urinary tract have been reported to have virulence associated genes encoding for proteins, D-serine deaminase and urease enzymes [24].

### 4.3. Iron acquisition within tissues

In tissues of humans, iron remains indispensable for the growth and metabolism of microorganisms including bacteria. However iron is not readily available for pathogens in human tissues which are essential for microbial growth and metabolism. This restriction in iron availability plays a major role in host defence against most Gram-negative and Gram-positive bacteria. In humans, the rate at which the human body access iron is fully determined by iron-adsorbing macromolecules such as lactoferin, transferrin and ferritin etc [12]. The close relationship that exist between iron acquisition and virulence among bacterial isolates implicated in disease conditions in humans is illustrated by the direct relationship between iron overload levels and infectious diseases as previously shown by Weinberg and Bral [25] using animal models that received different doses of iron and final monitoring of physical manifestation of diseases and death [12].

Ratledge [26] and Litwin and Calderwood [27] independently documented the available scientific and natural means through which bacteria access iron from host's tissues. These mechanisms are not limited to compounds that mob-up irons or those that adhere specifically to iron, which of course are esterified to lactoferin, transferrin, ferritin and ferrous iron transporters. Siderophores are commonly found in bacterial pathogens that survive within the host and serve as biomolecules which assist them in assessing iron molecules. Ratledge [26] opined that siderophores is transcriptionally regulated by the level of iron in microbial cells by negative repressor molecules, principally Fur (ferric uptake regulation), functional homolog of DtxR, IdeR (iron-dependent transcriptional repressor) in *E. coli*, diphtheria and Mycobacterium, respectively.

## 5. Quorum sensing in bacterial pathogenesis

The central role of quorum sensing is very critical to the understanding of bacterial pathogenesis. Quorum sensing is just a regulatory mechanism for genes towards controlling of microbial population or bio-load. For a group of bacteria to elicit some potentials, including virulence and pathogenicity, they must be able to attain a particular threshold or population. Often in pathogenesis, attacking bacterial isolates communicate within the tissue using chemical responses through the help of a biomolecule called acylated homoserine lactone (mainly for Gram-negative bacteria). Once a minimum threshold required to exhibit virulence is achieved, the bacterial isolates will elicit such physiological functions on the host. The process of quorum sensing has effectively co-ordinated virulence and bio-film formation in tissues and antibiotic resistance within the genes of microbial pathogens. Lee et al. [28] recently reported the deficiency of quorum sensing potentials in two strains of *Pseudomonas aeruginosa* named $QSD^3$ and $QSD^7$. This deficiency of quorum sensing in these two strains led to the decrease in the concentrations of pyocyanin, pyooverdine, rhanmolipid (a biosurfactant). Lee et al. [28] also reported that the two strains deficient in quorum sensing potentials have higher abilities to produce biofilms than wild types, and also have higher ability to resist antibiotics than the wild type pathogens. Interestingly, Peng et al. [29] reported novel finding where quorum sensing and bio-film formation and expression of virulence genes were inhibited by a biomolecule called rutin in *Escherichia coli* of avian origin.

## 6. A look at the concept of pathogenicity islands (PAI) amongst bacterial pathogens

The process by which short segments of oligonucleotide, which are called genes are transferred from alien genomes into the host genomes is known as horizontal gene transfer whereas in lateral gene transfer, gene transfers occur within the host genomes. Due to the fact that horizontally transferred genes have their alien origin, such regions are known as genomic islands.

Hacker and co-workers were the premier scientists that observed the natural phenomenon called pathogenicity islands (PAIs). At the first instance they tried to annotate or assign the function of genomic region within *Escherichia coli* housing group of genes that can be deleted from in a simultaneous fashion [30]. Later on, other independent researchers found more clusters of genes with different functions in a complete genome.

Pathogenicity islands are therefore, special islands of genes or sets of nucleotides or extra chromosomal units which are acquired by horizontal gene transfer within pathogens in a microbial community or biofilm (Fig. 2). These pathogenicity islands often occupy about 10–220 kilo base in a gene region or segment, and they usually exist in codons. However, when these codons are decoded, they confers into the pathogens the virulence factors which is a principal factor in determining establishment of infectious diseases in human and veterinary cases [31]. It has also been identified that PAI play a major role in the evolution of microorganisms including bacterial pathogens [31].

Interestingly, early workers in molecular biology have identified that a particular species of bacteria may have more than one pathogenicity island. For instance, over four pathogenicity islands have been identified in various strains of *Salmonella* species and most other Gram-negative bacterial isolates. However, in nature there are only a few of PAI's in Gram-positive bacterial pathogens [31].

Generally, the proteins that express and work in co-operation with most virulence-associated genes in PAI's can include but are not limited to the following categories: (a) Adhesins, which are cell-surface components that facilitate bacteria adherence to eukaryotic cells; (b) type III and IV secretion systems, which in terms of structural chemistry are needle-like structures (c) invasins, which are proteins secreted to assist pathogens to invade eukaryotic cells and tissues at the epithelium; (d.) toxins, which can be exotoxins, enterotoxins; and iron uptake systems [31].

## 7. Advance methods in diagnosis/investigations of bacterial pathogenicity

In the beginning of the history of Microbiology, it was clearly documented that Microbiology as a field has very close association with the microscope the way hands and gloves are close and related. The first attempt of human visualization of a bacterium was reported in the 19th century when Van Leeuwenhoek took cognisance of bacteria he got from his teeth, and was able to describe the shapes of certain bacteria isolates, and his findings were acceptable by the authorities at that time in London. However, it was still in the 1880's that Robert Koch was able to develop culture media and the idea of pure cultures was conceptualised and propagated.

Robert Koch was a major personnel who combated the demonic concept of diseases, thereby proving the reality of the Germs theory of diseases. In the presence of well-accepted concept of the germ theory of diseases in 19th century, Medicine as a field entered into a revolution encounter that opened up the scientific chapter of chemotherapy and search for chemotherapeutic agents.

Klemm and Dougan [32] reported that the development of next-generation sequencing as a cost-effective technology which holds a lot of promises in facilitating the analyses of bacterial population structure, and function in Clinical Medicine and Pathogenic Bacteriology. Although next generation technologies for investigations into pathogenic bacteriology had just commenced, these approaches have made great scientific and economic inputs such as in the advancement of gene studies. In 1985, Carl Woese in an experimental breakthrough was able to amplify the genes of microorganisms in a process referred to as

polymerase chain reaction (PCR). In this era, Polymerase Chain Reaction which utilizes pure isolates from solid agar started. Thus, pure cultures which have been characterized to generic level were identified using site specific primers. This actually assisted in identifying cultured microorganisms to the strain levels but this did not overcome studying the non-culturable organisms which will never appear in culture media 100% amidst efforts to resuscitate the VIBNOC (Viable but non-culturable) organisms [33]. In overcoming the VIBNOC challenges during investigations in bacterial pathogenesis, next generation tools employed include but not limited to proteomics, sequencing and phylogenetic tree analyses. They have several success stories on the use of advanced biological tools in the study of microbial pathogenesis. McCarthy et al. [34] reported the successful use of illumina whole genome sequencing to de novo, assembly and annotation to study the conservation of virulence genes across strains of *Pseudomonas aeruginosa* in Septicaemic conditions.

### 7.1. Proteomics

Proteomics is basically the study of proteins in terms of chemistry, structures, expression and function in all levels of life including plants, animals and microorganisms. But in this present review, attention is paid to proteomics as it concerns man and bacterial pathogens. In addition to the significant accomplishments of genomics and bioinformatics. Proteomics as a systemic analyses of all expressed cellular proteins has come into reality in this post genomic era, leading to an extensive grasp of bacterial interaction in tissues with all immunological responses of the host. One important goal of proteomics is obviously to characterize and study proteins expressed by two cells that are not similar in genotype and phenotype. The two-dimensional polyacrylamide gel electrophoresis (2D PAGE) often linked with mass spectrometry is currently the most widely used hyphenated technology for proteomics analyses (Fig. 3).

A major advantage of 2D PAGE is its high reproducibility which makes it a tool of choice during investigation of multiple samples. Above all, the 2D PAGE is adequate as a proteomic tool for identifying and correlating differences at the peptide levels of proteins. In terms of diagnosis, unknown bacterial isolates is often identified using the 2D PAGE by systematic study of spectra at the visual display units, and comparing the spectra with existing spectra in the proteomic database. This helps to match the spectra of the unknown to related spectra with the percentage level of relatedness duly emphasised by the software.

### 7.2. Sequencing and phylogenetic analyses

Sequenced-based analysis often requires that clones with phylogenetic anchors which are indispensable for taxonomy-based function be completely amplified [35]. Once the degrees of variations within and between genomes are well ascertained, the next line of action is to build a phylogenetic tree or dendogram. These phylogenetic trees underscore the variation and relatedness in genes, and effectively assist in decision making with respect to evolutionary relationship and trend analyses. This can be done using several different tree-building methods or dendogram programmes, including maximum likelihood, Bayesian inferences, or neighbour-joining. Advances in web-based, automated phylogeny building from sequence to tree are also being made. Improved phylogenetic analyses have enabled studies on the distribution of bacterial lineages in the environment, community level physiological profiling (CLPP), and equally have advanced the identification of emerging infectious bacterial strains and diseases with well-established genotypes, serovars and biovars [36,37].

## 8. Conclusion

In order to hold a full grasp of the process of pathogenesis, there is the need to understand the concepts of normal flora, pathogens, opportunistic infection, factors that interact to establish diseases, and basic immunology. The virulence factors used by bacterial pathogens to capture the hosts must be well-understood as it is a principal factor driving research and development in the area of bacterial pathogenesis. The knowledge of bacterial pathogenesis is adequate to design research works towards vaccine production in the area of Medical Biotechnology. In the presence of wide range of culture independent approaches in studying bacterial pathogenesis, scientists are expected to investigate bacterial diseases with causative agents that are not yet culturable.

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