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Review Article

The Nature and Prevention from *Streptococcus pneumoniae* (Pneumococcus) Infection Causing Pneumonia and Other Pneumococcal Diseases

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Abstract

Streptococcus pneumoniae resides asymptomatically (non-invasive) in healthy carriers by colonizing in the respiratory tract, sinuses, and nasal cavity. However, in the case of susceptible elderly, young children, and immunocompromised patients due to their weak immune systems. *S. pneumoniae* became highly invasive and pathogenic causing pneumonia and other pneumococcal diseases. *S. pneumoniae has multiple* virulence factors that capable to adhere and invade host cells and tissue. In addition, *S. pneumoniae* is capable to escape host immune defenses to survive. These serious proprieties of S. *pneumoniae* infection causing epidemiological concern due to its highly impact on world public health with estimated over one million death per year. Continue understanding *S. pneumoniae's* virulence factors, host immune responses, and pneumococcal diseases prevention, in addition to continue improving diagnostics test methods, pneumococcal diseases treatments, and vaccines will enhance regulations and infection prevention from this serious pathogenic microbe.

Keywords: *Streptococcus Pneumoniae; Pneumococcus; Non-Invasive; Invasive; Pneumococcal Diseases; Virulence Factors; Diagnostics; Antibiotic Resistance; Penicillin-Binding Proteins; Vaccines*

Introduction

Streptococcus pneumoniae, or *pneumococcus,* is a member of the genus *Streptococcus,* a gram-positive bacteria lancet-shaped, usually found in pairs (diplococci), non-spore former and non-motile. It is facultative anaerobic, alpha-hemolytic meaning partially lysis red blood cells-RBCs on blood agar plate under aerobic conditions, and beta-hemolytic meaning completely lysis red blood cells-RBCs on blood agar plate under anaerobic conditions (Figure 1). *S. pneumoniae* is a significant human pathogenic bacterium that has a highly genetic diversity with over 90 known serotypes, and small minority of these serotypes causing pneumococcal diseases. *S. pneumoniae i*s normally colonized asymptomatically in the upper respiratory tract flora, but it can become pathogenic under the right conditions, typically when the immune system of the host is suppressed. *S. pneumoniae* infection cause many types of illnesses, including lung infection (pneumonia), ear and sinuous infection which can manifest as a middle ear infection (otitis media or sinusitis), and blood stream infection (bacteremia). In addition, this bacterium has the capability to infect and spread to the central nervous system around the brain and spinal cord infection (meningitis) which is considered a serious infection and required immediate medical attention. *S. pneumoniae* infection spread through coughing, sneezing, and close contact with infected person causing initial symptoms include fever, cough, shortness of breath, chest pain, stiff neck, confusion and disorientation. In the case of severe infection *S. pneumoniae* could cause hearing loss, brain damage, and death. The infection with *S. pneumoniae* is usually occurs in dry, and cold months specially when the host airway secretions are high, plus occurs

in conjunction with viral infections of the upper respiratory tract, such as influenza or Covid19. *S. pneumoniae* like some other pathogenic bacteria produce toxins that are harmful to infected host, plus has several cell surface proteins that play a vital role in its pathogenesis. These virulence factors hindering the host's immune system response specially for children's, elderly, and immunocompromised patients that have weak immune response. The prevention from *S. pneumoniae* infection is mainly by vaccinations, and in the treatment from the infection is mainly by antibiotics. Diagnosis of *S. pneumoniae* infection is based on bacteria isolation and identification from patient's blood, cerebrospinal fluid, middle ear fluid, joint fluid, or peritoneal fluid.

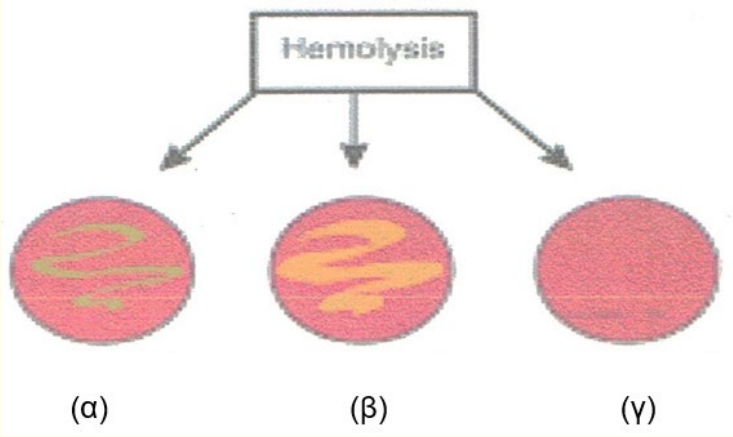

Figure 1: *Alpha-hemolysis (α) is often observed in Streptococcus pneumoniae. It is partial destruction of red blood cells resulted in greenish discoloration around the colony on blood agar. Beta-hemolysis (β) is complete lysis of red blood cells showing clear zone around the colony, Gamma-hemolysis (γ) is no visible hemolysis or change in the blood agar.*

World Health Organization (WHO) included *S. pneumoniae* as one of serious pathogens infection due to the rising rates of penicillin, and other antibiotics resistance in treatment. Currently, vaccines are the best method to protect hosts against the infections by the most common types of invasive *S. pneumococcus*. Despite significant successes in traditional vaccination, the capacity of *S. pneumococcus* to evolve in the face of the selective pressure of anti-capsular immunity are challenges in immunization programs. Recently, some alternative vaccination methods such as conjugate vaccine that targets most identified serotypes of *S. pneumoniae* are under evaluation. This conjugate vaccine contains bacteria surface proteins/polysaccharide combination and expected to be with better efficacy than existing traditional vaccines. In general, continue extending the knowledge on *S. pneumoniae* cell biology, mechanism of infection, and associated host immune response to this bacterium infection are important factors to improve pneumococcal disease prevention, diagnostics of the infection, and treatment from the infection. Continue such research will reduce life threating pneumococcal diseases of pneumonia, septicemia and meningitis that are caused by invasive *S. pneumoniae.*

Streptococcus pneumoniae cell structure

S. pneumoniae is a gram-positive spherical bacterium, usually found in pairs (diplococci) or in chain cocci, non-spore former, and non-motile. It is alpha-hemolytic bacterium under aerobic condition, facultative anaerobic, causes pneumococcal infections of the upper respiratory tract leading into pneumonia, as well as other infections such as meningitis, brain abscess, otitis media, endocarditis, conjunctivitis, osteo-myelitis**,** cellulitis, septic arthritis, peritonitis, pericarditis, and acute sinusitis (Figure 2). Cell structures of *S.*

pneumoniae labeling of capsule, cell wall, cell membrane, cytoplasm, and chromosome (DNA). *S. pneumoniae* has a complex cell wall that plays key roles in cell shape, maintenance, growth and cell division, The major virulence determinant of *S. pneumoniae* is the capsule polysaccharide that is resistant to opsonophagocytic by host innate immune cells response [1]. Virulence factors of *S. pneumoniae* that contribute to pathogenicity are in cellular structures, These virulence factors that are harbor in cellular structures include the polysaccharide *c*apsule which consist of a variety of antigens, The bacterium cell wall peptidoglycan where surface adhesins molecules to host cells, and are pneumococcal serine-rich repeat protein (PsrP), neuraminidase (NanA), enolase (Eno), pneumococcal adhesion/virulence (PavA), hyaluronate lyase (Hyl), autolysin (LytA), pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), pneumococcal iron acquisition A (PiaA), pneumococcal iron uptake A (PiuA), pneumococcal surface adhesin A (PsaA), and pili are located. In addition to these surface adhesion molecules the cell periplasmic/intracellular space is the location for secreted IgA protease enzyme into outer environment, and also where pneumolysin (Ply) is released upon cell lysis (Figure 3). *S. pneumoniae* genome has a closed circular DNA structure contains between 2.0 and 2.1 million base pairs and its core set of 1553 genes, this in addition to 154 genes contribute to virulence, and 176 genes to maintain a non-invasive phenotype [2]. *S. pneumoniae* is a highly recombinogenic bacterium that is responsible to the high burden of human pneumococcal diseases globally. Recombinogenic is a genetic recombination process which exogenous DNA is acquired and incorporated into bacterium genome [3]. This recombinogenic is a key evolutionary mechanism in *S. pneumoniae* for the adaptation to selective pressures and mutate to acquire genetic variations capable to escape the host innate and adaptive immune responses [3]. Also, these acquired genetic variations assist *S. pneumoniae* to escape clinical interventions, by vaccinations for infection prevention and by antibiotics therapy for infection treatment [4]. In summary, *S. pneumoniae's* virulence factors thrives because its ability to acquire new genetic material via recombination as well as via transformation.

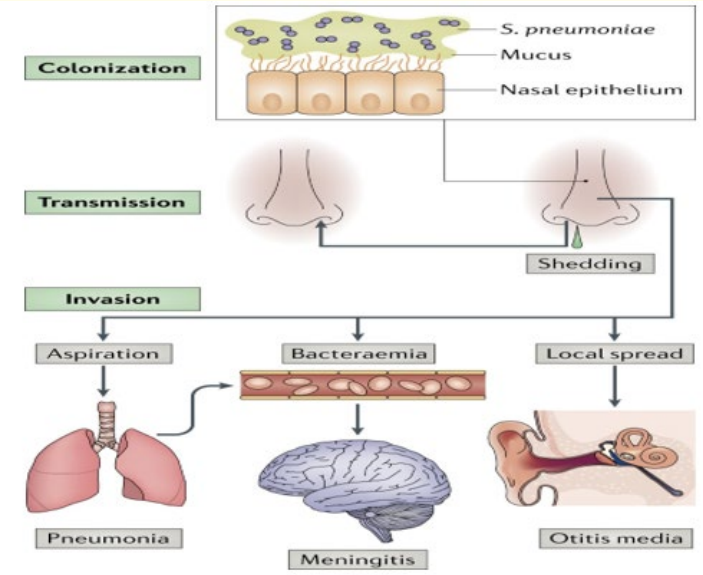

Figure 2: *Streptococcus pneumoniae: colonization, transmission, and invasion in susceptible host causing serious pneumococcal disease such as pneumonia, meningitis, and otitis media.*

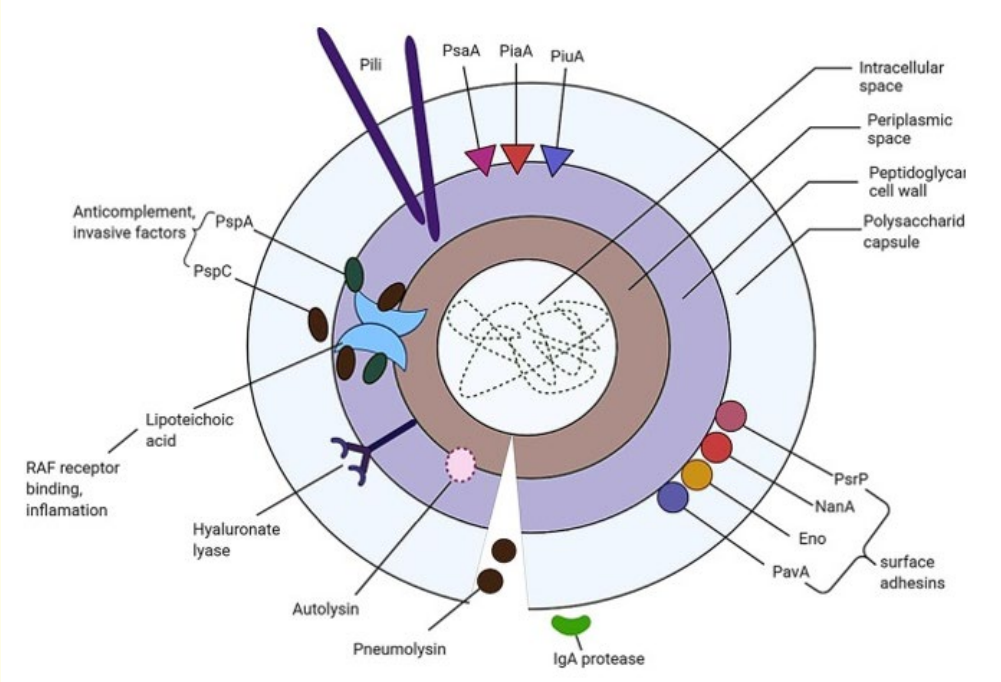

Figure 3: *Virulence factors of Streptococcus pneumoniae are variety of surface proteins, and toxins that are main factors in this bacterium pathogenicity. Major virulence factors include: Pneumococcal surface adhesin A (PsaA), Pneumococcal surface protein C (PspC), Pneumococcal iron acquisition A (PiaA), Pneumococcal iron uptake A (PiuA), pneumococcal iron transporter (PitA), Pneumolysin, autolysin, IgA protease, capsule, and pili.*

Streptococcus pneumoniae virulence factors

S. pneumoniae, produce adhesion molecules and toxins that are harmful to the host. Plus, it has several surface proteins and physical structures, that are playing vital roles in its pathogenesis. These virulence factors include: Polysaccharide capsule, cell wall components, pneumolysin, autolysin, pneumococcal surface proteins, immunoglobulin A1 protease enzyme, hydrogen peroxide (H_2O_2), pili, biofilms, and has DNA pathogenicity islands.

Polysaccharide capsule*:* It is the most important virulence factor in infection initiation by allowing invasive S. *pneumoniae* cells to adhere to host cells [5], while providing protection from host's immune systems [6]. This extracellular polysaccharide capsule inhibits phagocytosis of invasive *S. pneumoniae* by host innate immune cells. Also, prevent the recognition of these invasive *S. pneumoniae* cells by host immune cells receptors, and by complement factors. It is important to highlight that most serotypes of *S. pneumoniae* cells are characterized by this pathogenic extracellular polysaccharide that is located on the outer coat of the bacterium cell wall. This extracellular polysaccharide capsule on invasive *S. pneumoniae* cells play important rule in virulence enhancement by its ability to undergo capsule serotype switching [7]. The mutation in *S. pneumoniae* genes (*cps*) for the synthesis of this extracellular polysaccharide capsule promote these serotypes switching [8]. The role of invasive *S. pneumoniae* extracellular polysaccharide capsule in pathogenesis is due to its negative net charge. This negative net charge is due to the acidic polysaccharides and phosphates in this capsule chemical structure. This negative net charge of invasive *S. pneumoniae* extracellular polysaccharide capsule gives this pathogen the ability to escape host immune response by avoiding being trapped by host mucus layers and phagocytic cells such as macrophages due to electrostatic repulsion [9].

Cell wall components: *S. pneumoniae* both invasive, and non-invasive are gram-positive with a thick cell wall. This thick cell wall gives the bacterium cell shape and protection. The main components in this bacterium cell wall are; peptidoglycan, wall teichoic acids (WTAs), and lipoteichoic acids (LTAs). The peptidoglycan chemical structure is made of the alternating glycan chains *N*-acetylglucosamine (*GlcNac*) and *N*-acetylmuramic (*MurNac)* acids crosslinked by peptide chains. Both polysaccharide capsule and cell-surface proteins of *S. pneumoniae* cells are linked to the peptidoglycan chemical structure, and the wall teichoic acids (WTAs) are covalently attached to peptidoglycan, while lipoteichoic acids (LTAs) are non-covalently attached to the bacterium cell cytoplasmic membrane with a lipid anchor (Figure 4). Peptidoglycan chains of *S. pneumoniae* cells can undergo secondary modifications by mutation such as deacetylation of *N*-acetylglucosamine (*GlcNac*) and O-acetylation of *N*-acetylmuramic (*MurNac*) to make *S. pneumoniaes* cell resistant to host lysozyme enzyme [10]. In addition, both wall teichoic acid (WTA,) and lipoteichoic acids (LTAs) chemical structures have phosphorylcholine (PCho) residues that serve as anchors to choline-binding proteins (CBPs). This choline-binding proteins (CBPs) play crucial role for invasive *S. pneumoniae/* host cells interactions to evade the host immune response from binding to the host complement factors resulted in inhibiting complements activation, and allowing invasive *S. pneumoniae* bacteria to survive within the host body [11]. Choline-binding proteins (CBPs) key factors include Pneumococcal surface proteins A (PspA) and C (PspC). Both are virulence factors that help the *S. pneumoniae* to evade the host immune system [12]. In summary, *S. pneumoniae* cell wall chemical structures of peptidoglycan, wall teichoic acids (WTA), and lipoteichoic acids (LTA), are pathogen-associated molecular patterns (PAMPs), and also causing inflammatory response to the host.

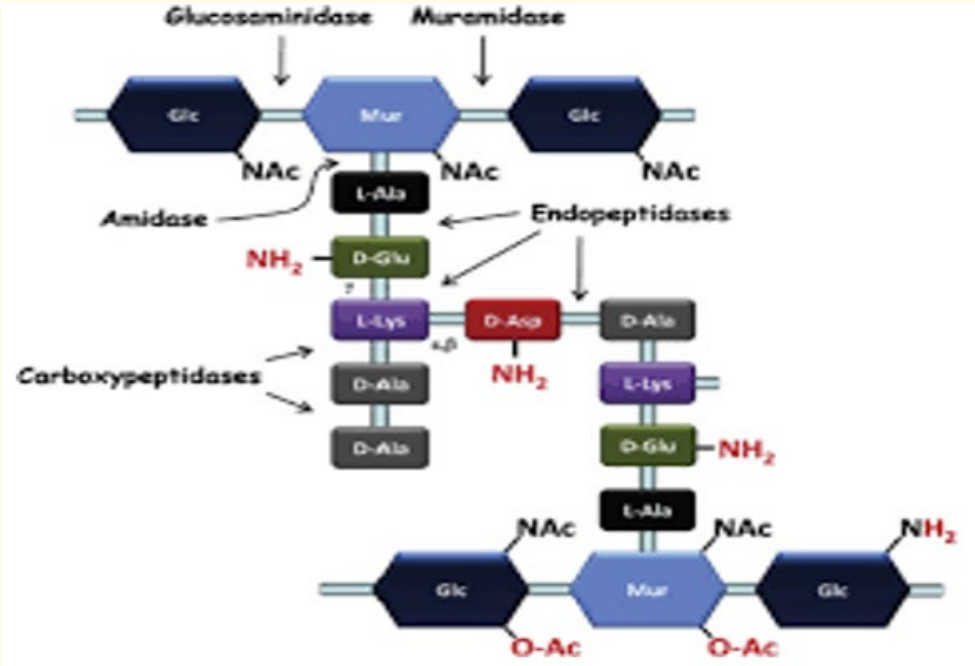

Figure 4: *S. pneumonia cell wall is a complex chemical structure consist of and N-acetylglucosamine GlcNAc, and N-acetylmuramic (MurNAc) residues in glycan chains of peptidoglycan. In addition, there are direct and indirect peptide chains cross-links. This diagram shown the two sites for proteolytic enzymes endopeptidase and carboxypeptidase with mechanism to hydrolyze peptides cross links and kill the bacterium.*

Pneumolysin: Is a microbial toxin present in the cytoplasm of some gram-positive bacteria including *S. pneumoniae.* This toxin released as the result of the invasive *S. pneumoniae* bacteria lysis of in host cells. The released toxin pneumolysin binds to host cells membranes containing cholesterol and forms pores which later lead into host cell lysis and death. Also, the released pneumolysin interfere with host immune system [13] by negatively regulate complement system, reduce phagocytosis by innate immune cells, negativity regulate cytokine/chemokine secretion, and cause host cell's DNA damage by inducing double-stranded DNA breaks leads into increase *S. pneumoniae* virulence in infection specially to elderly that normally experiencing a compilation of DNA damage and telomere shortening from aging [14].

Autolysin: Is endogenous microbial enzyme with a function to autolyze invasive *S. pneumoniae* cell wall peptidoglycan specially when the bacterium reached the stationary phase of growth. The autolysis of invasive *S. pneumoniae* peptidoglycan by endogenous autolysin result in releasing bacteria cells components including the microbial toxin pneumolysin [15]. Releasing pneumolysin promote host nasopharyngeal cells to change into nasopharyngeal carcinoma (NPC). Nasopharyngeal carcinoma (NPC) is a type of cancer that starts in the host nasopharynx.

Pneumococcal surface proteins*:* Invasive *S. pneumoniae* has multiple surface proteins that plays important role in enhancing its pathogenicity by acting as adherent to host cells and hinder the host's immune response via complement system [16]. These pneumococcal surface proteins are classified into four groups of choline-binding proteins (CBPs), lipoproteins, non-classical proteins, and proteins that have C-terminal LPXTG amino acids motif (Leu-Pro-Xs-Thr-Gly,). This sequence LPXTG is in the amino group of peptidoglycan cross-bridge, that can be cleaved by specific enzyme sortase. This enzyme sortase cleaves LPXTG motif in bacterium cell wall peptidoglycan structure facilitate and enhance the adhesion of Gram-positive (G^+) bacteria such as *S. pneumoniae* to host cells.

These pneumococcal surface proteins group in some details are:

a. Choline-binding proteins (CBPs): Are family of surface proteins found on *S. pneumoniae* surface and contribute to this bacterial pathogen virulence. These CBPs attached to phosphor choline (PCho) located on *S. pneumoniae's* cell wall and play a role in *S. pneumoniae* adhesion to host cells [17]. In addition, these CBPs proteins block host complement factors that is responsible to activate immunoglobulins for the elimination of *S. pneumoniae* infection [18]. There are more than 10 choline-binding proteins (CBPs) for *S. pneumoniae* including the most important ones such as pneumococcal surface protein A (PspA)**,** pneumococcal surface protein C (PspC), and the *pneumococcal* autolysin LytA. These pneumococcal surface proteins share the same choline-binding anchors but have different surface-exposed regions. All are in direct contact to host cells surface with diverse functions. The pneumococcal surface protein A (PspA) has high net negative charge property that block host complement binding and prevents opsonization of *S. pneumoniae* cells by host innate immune cells such as macrophages or neutrophil. In addition, this PspA bind to iron free apo-lactoferrin and protect *S. pneumoniae* cells from the bactericidal activity of apo-lactoferrin [19]. The pneumococcal surface protein C (PspC) is highly polymorphic peptide that promotes its adherence to the polymeric immunoglobulin receptor [20], and facilitate the colonization of *S. pneumoniae* into the host nasopharynx. In addition, this PspC prevent the formation of the host complement C3b from binding to its factor H protein essential to regulates the complement immune system, and protect host cells and tissues from damage. PspC also interfere with opsonization of *S. pneumoniae* bacterium by macrophage or neutrophil [21]. The pneumococcal surface protein autolysin (LytA) is one of S. *pneumoniae* lytic enzymes [22] hydrolyze bacterial cell wall peptidoglycan by cleaving the *N*-acetyl-muramoyl-l-alanine bond causing cell lysis releasing *S. pneumoniae* antigens of pneumolysin, peptidoglycan, and teichoic acids that are harmful to host cells [23]. Releasing these harmful antigens from *S. pneumoniae* cells inhibit the production of host cell signal cytokine interleukin12 (IL12), which in turn blocks the activation of phagocytes such as macrophages and neutrophils [24]. Other choline-binding proteins (CBPs) in *S. pneumoniae* as virulence factors are CbpF, CbpD, CbpG, CbpI, CbpJ, CbpK, CbpL, CbpM, and CbpN [25].

b. Lipoproteins: There are about 50 lipoproteins that have been characterized, and are necessary to transport nutrients and metabolites in/out of *S. pneumoniae* cells. The four main lipoproteins in *S. pneumoniae* are pneumococcal surface adhesin A (PsaA), pneumococcal iron acquisition A (PiaA), pneumococcal iron uptake A (PiuA), and pneumococcal iron transporter A (PitA). These four lipoproteins are metal-binding proteins that joined to ATP-binding cassette to move a variety of substances such as amino acids, lipids, and metallic ions across *S. pneumoniae* cell membranes using energy from ATP hydrolysis into ADP, and AMP [26]. Such transport mechanism support *S. pneumoniae* cells growth, enhances its virulence, and invasive factors to infect host cells. The pneumococcal surface adhesin A (PsaA) enhance bacterium attachment to host cells, and enhance magnesium transport into host cells essential for invasive *S. pneumoniae* protein synthesis, energy production, and DNA stability inside host cells. These functions allow invasive *S. pneumoniae* to adhere to host cells and acquire nutrients that are essential for *S. pneumoniae* survival [27]. Other three lipoproteins of pneumococcal iron acquisition A (PiaA), pneumococcal iron uptake A (PiuA), and pneumococcal iron transporter A (PitA), also, regulate iron-uptake, enhance *S. pneumoniae* growth and enhance virulence factors for *S. pneumoniae* pathogenicity [28].

c. Non-classical proteins (NCSPs): These are other *S. pneumoniae's* surface proteins that have no membrane-anchoring motif, nor have leader peptide sequences. These non-classical proteins (NCSPs) are, also. known by the name moonlighting proteins due to their multiple functions [29]. There are two non-classical surface proteins (NCSPs). These are pneumococcal adherence and virulence factor A (PavA), and glycolytic enzymes (Enolase and GAPDH). The Pneumococcal adherence and virulence factor A (PavA) assist in the adherence of invasive *S. pneumonia* cells to host cells fibronectin. Some researchers demonstrated that pneumococcal adherence and virulence factor A (PavA), plays potential function in host immune system for *S. pneumoniae* evasion, and for cytokine production by host dendritic cells. The glycolytic enzymes (Enolase and GAPDH) are plasminogen-binding proteins. The Enolase is free enzyme protein located on the surface of *S. pneumoniae* [30]. It is proteolytic enzyme that is necessary for the pathogeny of *S. pneumoniae*, and to facilitate the invasive *S. pneumonia* to escape from host complement immune system [31]. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) is also enzyme protein located on the surface and in the cytoplasm of *S. pneumoniae* cell. The glycolytic enzyme GAPDH strongly bind to host cells plasminogen due to its higher affinity for plasmin. This higher affinity enhances the adhesion of invasive *S. pneumoniae* to host cells. GAPDH also, play important a role in iron acquisition for bacterial growth due to its ability to bind to host iron rich hemoglobin and heme [32]. In general, both Enolase and GADPH enzymes play important roles as well in host cell invasion and in avoiding host immune response.

Immunoglobulin A1 (IgA1) protease: It is protease enzyme produced by *S. pneumoniae* to cleave the host secreted antibody IgA1 into deactivated fragments [33]. The function of host secreted antibody IgA1 is to protect host cells from *S. pneumoniae* capsule from binding to host cells to cause the infection, and to facilitate host immune cells such as phagocytes to recognize, and neutralize bacterial infection via opsonization and via engulfment mechanism. *S. pneumoniae* is capable to disrupt this host neutralization mechanism by the production of IgA1 protease enzyme which cleaves host secreted IgA1 antibody hindering its protective function at the host mucosal surfaces where *S. pneumoniae* initiate colonization in host cells [34].

Hydrogen peroxide: *S. pneumoniae* secretes hydrogen peroxide (H_2O_2) causing damage to host cell's DNA [35]. In addition, H_2O_2 has bactericidal effects and *S. pneumoniae* secreted H_2O_2 to limit the growth of other pathogenic bacteria that might compete with *S. pneumoniae* for host cells infection [36].

Pili: It is a hair-like protein structure that extend from the cell surface of *S. pneumoniae.* This pili generally extends from other pathogenic and non-pathogenic bacteria. Pilli function is for the attachment and colonization of invasive *S. pneumoniae* to host epithelial cells within the nasopharynx and lungs located on hosts cell surface. Pili helps *S. pneumoniae* to avoid phagocytosis mechanism by host innate immune cells [37].

Biofilms: Are clusters of bacteria attached to a solid surface, and forming a slimy layer called biofilm. Biofilm matrix consists of proteins, and polysaccharide that is the major cause of bacterial infections. It protects bacteria from antibiotics efficacy and from other treatments. Biofilm enhance *S. pneumoniae* virulence, and assist the bacterium to escape from host immune responses [38]. This biofilm matrix is responsible for the contamination of invasive medical devices by *S. pneumoniae*' or by other biofilm forming bacteria causing second infection to hospitalized patients, in the case these invasive medical devices did not sterilize properly [39].

Pathogenicity islands (PAIs): are genetic elements located in the chromosome (DNA) of the majority of pathogenic bacteria, and are normally absent from non-pathogenic bacteria even from related strain of the same species. Pathogenicity islands (PAIs) in invasive *S. pneumoniae* DNA are coded for iron-uptake systems for survival, or coded for proteins expression involve in the attachment of *S. pneumoniae* to host cells [40]. These pathogenicity islands (PAIs) genes can be acquired through transformation process where the bacteria uptake free DNA from the environment and incorporated it into its chromosome (DNA). This acquired free DNA might lead into the addition of virulence factor, or antibiotic resistance within the population of *S. pneumoniae* strains. This phenomenon in *S. pneumoniae* strains considered to be a key mechanism for the genetic diversity in the bacteria pathogenicity, and antibiotics resistant. In summary these Pathogenicity islands (PAIs) are capable to promote new virulence factors or antibiotic resistant into the invasive S. *pneumoniae* [41].

All these highlighted virulence factors in the invasive *S. pneumoniae* can be acquired, and genetically expressed and translated into proteins (antigens) when non-invasive *S. pneumoniae* switch into invasive and causing pulmonary diseases to vulnerable populations of young children, elderly, and immunocompromised patients.

Streptococcus pneumoniae infection and host immune response

Invasive *S. pneumoniae* express and produce virulence factors to neutralize host immune response, and to enhance pathogen growth for infection. In most cases the host immune response is capable to recognize and clear bacterial infection from the body before causing pneumococcal diseases and symptoms. The first line of host defense is the innate immune response followed by the adaptive immune response as a second line of defense.

Innate immune response: Is a non-specific immune response cells, using cell receptors that recognize invaders with non-specific foreign particles to trigger host immune response, and to eliminate these invaders of nonspecific foreign particles that might be harmful to the host body. There are different host non-specific cells with different mechanisms that are related to innate immune responses against *S. pneumoniae* infection. These non-specific immune cells include *m*ucosa, and respiratory epithelial cells that are involves in innate immune response producing mucus and antimicrobial peptides to trap, neutralizes, and remove pathogen debris from the host surface cells such as lung cells (Figure 5). Other innate immune response cells are phagocytes cells such as neutrophils, and macrophages circulated in host blood as part of white blood cells as a barrier for host tissues and organs for the protection from *S. pneumoniae* invasion. These neutrophils, and macrophages cells plays important function in both innate immunity and also in adaptive immunity. In innate immune response, neutrophil cells produce granules (defensins, and enzyme lysosomes) that are capable to kill the invasive bacterium by breaking down its cell wall structure [42]. While, macrophage cells in addition to its function as phagocytic cells engulf and kill the invasive pathogen, they are also via cytokines signaling other immune cells for host immune response activation [43]. Major innate immune cells that are circulated in host blood are macrophage cells, mast cells, natural killer cells, dendritic cells, monocyte cells, and granulocytes cells of neutrophil, eosinophils and basophils. Macrophage cells in addition to its function in innate immunity, it is also associated in adaptive immunity response with B-cells, and T-cells (Figure 6).

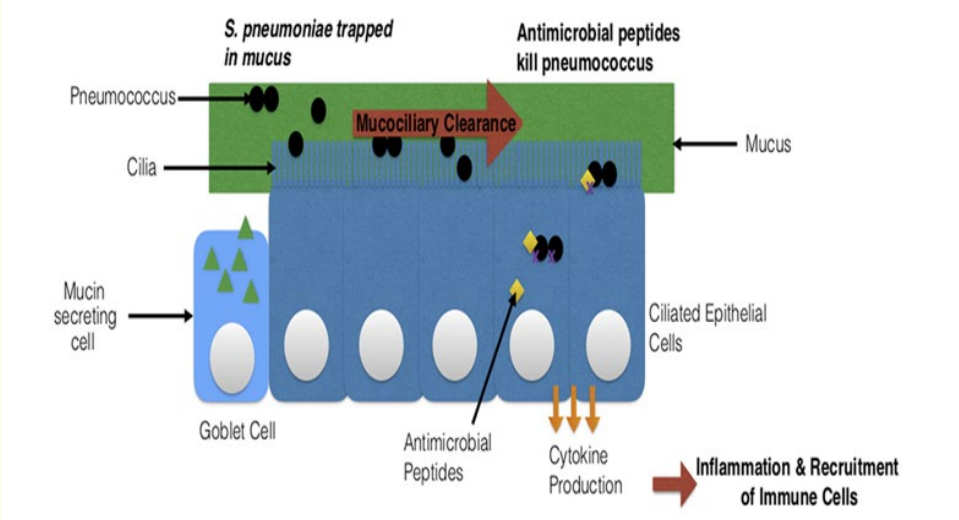

Figure 5: *Innate immunity for the interaction of S. pneumoniae' to host epithelial cells inhibition are based on goblet cells and ciliated epithelial cells. The cilia on the epithelial cells together with the mucus produced by goblet cells clear S. pneumoniae invasion via mucociliary clearance, and antimicrobial peptides secreted epithelial cells that directly kill S. pneumoniae. Host epithelial cells also produce cytokines which leads to a state of inflammation and the recruitment of other host immune cells.*

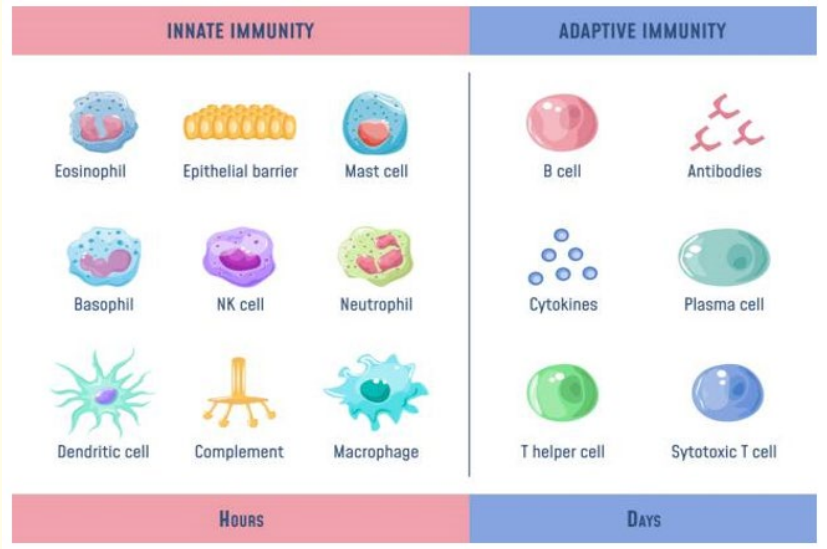

Figure 6: *Innate immune cells are eosinophil, epithelial cells, mast cell, basophil, natural killer, neutrophil cells, dendritic cells, complement, and macrophage cells. While passive immune cells are B-cells include secretory antibodies and plasma cells, and T-cells include helper T-cells, and cytotoxic T-cells.*

Adaptive immune response: In this immune response some host cells recognize only specific antigens expressed on the surface of the pathogen microbial cells. This adaptive immune response can be classified into humoral immunity, and cell-mediated munity. Humoral immunity involves B- cells that are activated by pathogen's (bacterial) specific antigens into plasma cells to produce antibodies specific to the invaded pathogen's (bacterial) specific antigens [44]. B-cells mature in the host bone marrow into plasma cells, and programed to produce these antigen specific antibodies such as blood circulated IgM, IgG antibodies and the secretory IgA antibody against *S. pneumonia* (antigens) *virulence* factors (Figure 7). The secretory IgA antibody is normally colonized in mucosal areas in the host nose and saliva to control *S. pneumoniae* invasion. In cell mediated immunity T-cells are involved in the activation to other immune cells to kill invaded pathogenic bacterial cells [45]. T-cells matured in host thymus cells into helper T-cells ($CD4^+$), and cytotoxic T-cells ($CD8^+$). In the case of *S. pneumoniae* infection, Antigen-Presenting Cells (APCs) such as macrophage process the pathogen virulence factors (antigens) into peptides and present these peptides to paired with the major histocompatibility complex (MHC II) in presenting these peptides to native helper T cells ($CD4^+$) for the stimulation of host immune response. Upon the activation of native helper T-cells ($CD4^+$) it differentiates into helper T-cells (Th1), and helper T-cells (Th2) cells. Helper T-cells (Th1) stimulate cellular-mediated immune response to produce cytokines such as interferon-gamma (IFN-γ), that activate and recruit other host immune cells such as macrophages. While helper T-cells (Th2) release the interleukin 4 (IL-4) cytokine that interact with B cells to produce specific antibodies against *S. pneumoniae* infection (Figure 8). In addition, the activated T and B cells are differentiated into memory B-cells and T-cells that can be used to provide immune response in the case the host reoccurring the same infection by the same *S. pneumoniae* strain years later. It is important to highlight that Infants, and elderly populations are highly susceptible to *S. pneumoniae* infection due to, Infants experience weak immune cell responses to foreign antigens because of multiple factors including their exposure to non-maternal antigens was restricted prior to birth, and for their inability to protect themselves with their own antibody generation before reaching to the age of two years. Elderly population experience weak immune cell responses to *S. pneumoniae* infection due to aging factors. Aging causes the reduction in antibodies production, poor immunoglobulin class switching from IgM to IgG antibody, and poor immune cells maturation. In general, weak immune cells response for both infants and elderly groups explained their high-risk for *S. pneumoniae* infection, and for pneumococcal diseases.

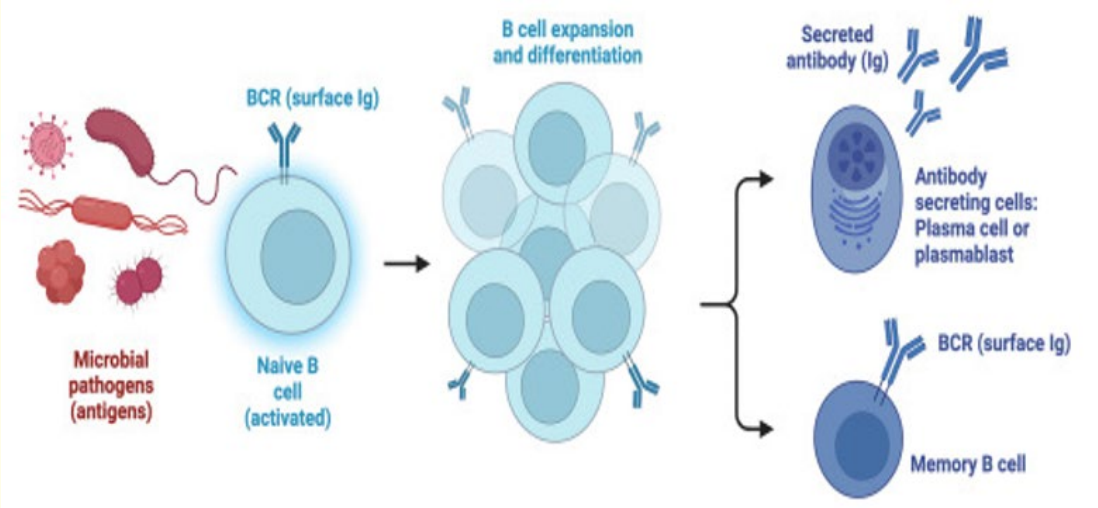

Figure 7: *B-cell receptors (BCRs) encounters S. pneumonia antigens to bind to its specific BCRs triggering and signaling cascade of B-cells proliferation and differentiation into a plasma cell to secrete antibodies, and memory B-cell with function to provide immune response in the case the host reoccurring the same infection by the same S. pneumoniae strain years later.*

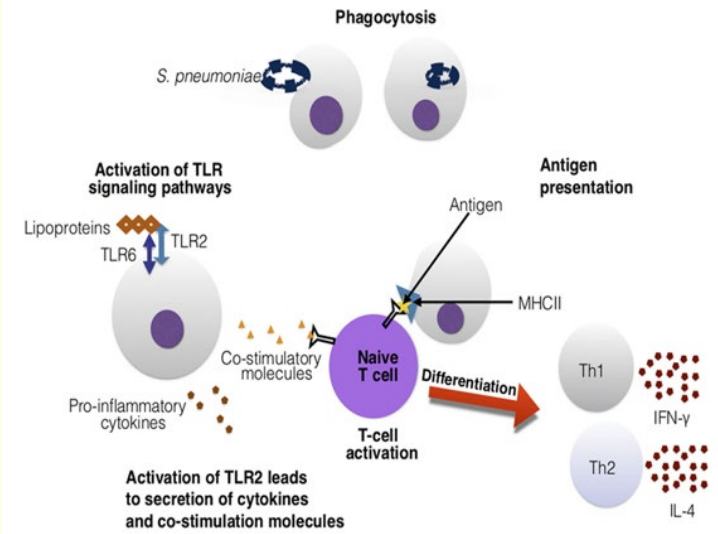

Figure 8: *Toll-like receptors (TLRs) on Antigen-Presenting cell (APC) recognize the S. pneumoniae's antigen (e.g. lipoproteins), and secretes cytokines that are essential to activate native helper T-cells ($CD4^+$). Activated native helper T-cell ($CD4^+$) bind to Antigen-Presenting cell (APC) present peptides from processed antigen (e.g. lipoproteins) with major histocompatibility complex (MHCII) to activated native helper T-cells. Native T-helper cells ($CD4^+$) recognition of the antigen/ MHCII complex differentiate into CD4 helper T-cells (Th1 and Th2). The top diagram is innate immune response which cell receptor on e.g. Macrophages cell surface recognize non-specific foreign particles for neutralization via phagocytosis.*

Streptococcus pneumoniae mechanism of infection and symptoms

S. pneumoniae is normally colonize in the mucosa of the upper respiratory tract (URT) as non-invasive bacteria. Carrier person shed *S. pneumoniae* in nasal secretions and thereby transmit bacteria to others. Major risk factor of infection is associated with the development of invasive pneumococcal disease (IPD) in a child of age less than five years old, adults over 60 years old, and immunosuppress patients. Infection by *S. pneumoniae* as a second infection has been identified as a predominant pneumonia cases developed after virus infection by influenza or Covid19. In this secondary infection case by *S. pneumoniae* can spread from the nasopharynx directly via the airway to the lower respiratory tract causing pneumonia. Also, this second infection can transfer into the sinuses or middle ears causing sinus infection or otitis media respectively. In addition, invasive *S. pneumoniae* can penetrate the host epithelial cell surface causing local infection or causing blood infection (bacteremia). Blood infection with invasive *S. pneumoniae* could lead into the infection of membranes covering the brain and spinal cord causing meningitis.

Symptoms in early *S. pneumoniae* infection are usually takes one to three days and includes, fever, **c**hills, nausea, and vomiting. Symptoms from later infection depend on the infected part in the body. In the case of lung infection cause pneumococcal pneumonia with symptoms include chest pain, cough, fever or chills, and rapid or difficult breathing. In the case of bloodstream infection cause pneumococcal bacteremia with symptoms include chills, fever, and low alertness. In the case of the lining of the brain and spinal cord infection cause pneumococcal meningitis symptoms include confusion, fever, headache, stiff neck, and photophobia (eyes being more sensitive to light). In addition to these serious symptoms, there are mild infections as well such as ear infections cause acute otitis media with symptoms include red, swollen ear drum, ear pain, fever, and sleepiness. Other mild infection is sinus infection cause mild symptoms include bad breath, cough, facial pain or pressure, headache, runny or stuffy nose, sore throat, and mucus dripping down the throat (nasal drip).

*Streptococcus pneumoniae d*iagnostics test methods

Diagnostics include patient history, physical examination, and relevant imaging. This is in addition to Gram-stained of sputum sample for microscopic detection of predominance Gram-positive (G+) cocci bacteria cells in pairs. This is in addition to the detection of high count of white blood cells, and epithelial cells in the sample. In the case of fluids sample such as blood, cerebrospinal, middle ear, or joints, samples should be collected under sterile conditions for Gram- staining test to identify, and confirm the microbe causing the infection, and disease symptoms. It is important to highlight that blood culture and other sterile body fluid should be performed for patients at high risk for bacteremia, meningitis, and other microbial infection causing community-acquired pneumonia (CAP). In addition to Gram- staining test for the detection, *S. pneumoniae* antigen detection tests should be applied as confirmatory test for the detection of a common cell wall antigens such as teichoic acid (C-polysaccharide) in the sample- urine as an example [46]. This antigen detection test is based on primary antibody to capture S. *pneumoniae* serotype (C-polysaccharide), followed by secondary fluorescent tagged antibody for the detection of the infection. This *S. pneumoniae* antigen detection test method is useful for the early identification of pneumococcal pneumonia and meningitis. Other rapid test method for the diagnostic of S. *pneumoniae* infection is polymerase chain reaction (PCR). This PCR test method is usually recommended for confirmation after positive test results from both Gram-staining and antigen test methods. Polymerase chain reaction (PCR) for *S. pneumoniae* infection is based on the detection of virulence factor pneumolysin (PLY) DNA [47]. This virulence factor Pneumolysin (PLY) is a cytotoxic protein usually found in the cytosol of *S. pneumoniae* strains, and also released from bacterial cells during cells growth. In addition to these highlighted laboratories testing for the diagnostic of *S. pneumoniae* infection, physicians are usually conduct chest X-ray to examine the lung for the presence of infection, and to monitor inflammation for treatment.

Streptococcus pneumoniae infection prevention

General health practices such as covering mouth, nose when coughing or sneezing, washing hands frequently with soap and water can help from the spread of pathogenic microorganisms' such as *S. pneumoniae*, and infection. There are two science methods for preventing and treatment from S. *pneumoniae* (pneumococcal) infection. These are vaccines for the prevention from infection, and antibiotics for treatment after the infection. In the case of prevention method, there are two types of inactivated vaccines that protect the person from *S. pneumoniae* infection. These are the pneumococcal polysaccharide vaccine (PPV23), and multiple pneumococcal conjugate vaccines (PCVs). The pneumococcal polysaccharide vaccine (PPV23) is a purified capsular polysaccharide of *S. pneumoniae* cell wall containing antigens from 23 different serotypes from different *S. pneumoniae* strains to protect against these 23 *S. pneumoniae* strains (serotypes). This pneumococcal polysaccharide vaccine (PPV23) offers T-cells independent immunity by directly recognizing host B-cells to differentiate into plasma cells to produce antibodies specific against the *S. pneumoniae* 23 polysaccharide antigens (Figure 9). PPV23 vaccine is given intramuscular or subcutaneous injection in one dose (0.5 ml) yearly to adults at the age of 65 and older [48]. Pneumococcal conjugate vaccines (PCVs) are capsular polysaccharide from *S. pneumoniae* coupled to a carrier protein for allowing a stronger immune response compared to a polysaccharide PPV23 vaccine. These pneumococcal conjugated vaccines (PCVs) are T-cell-dependent promote interaction between host B-cells and T-cells (Figure 10), resulting in stronger, and longer-lasting immune response comparing to pneumococcal polysaccharide vaccine (PPV23) that offers T-cells independent immunity. These pneumococcal conjugated vaccines (PCVs) such as (PCV13) are routinely given to children in a single dose (0.5 ml) intramuscularly injection, and is used to protect against 13 serotypes of *S. pneumoniae* strains [49].

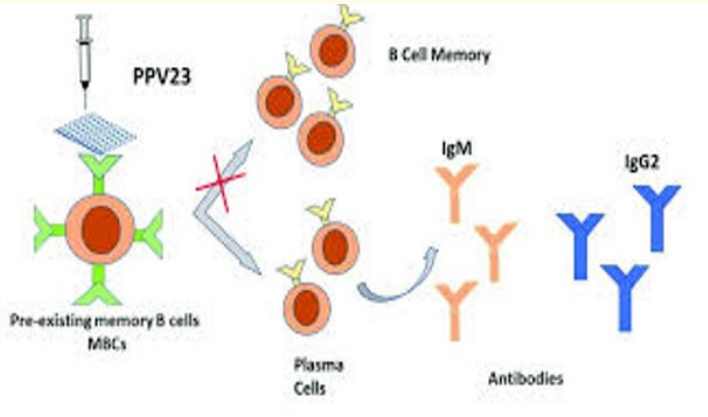

Figure 9: *T-cells independent mechanism of PPV 23 vaccine: The pneumococcal polysaccharide vaccine PPV23 stimulate B-cells memory cells towards differentiation into antibody-producing plasma cells to produce antibodies IgM, and IgG.*

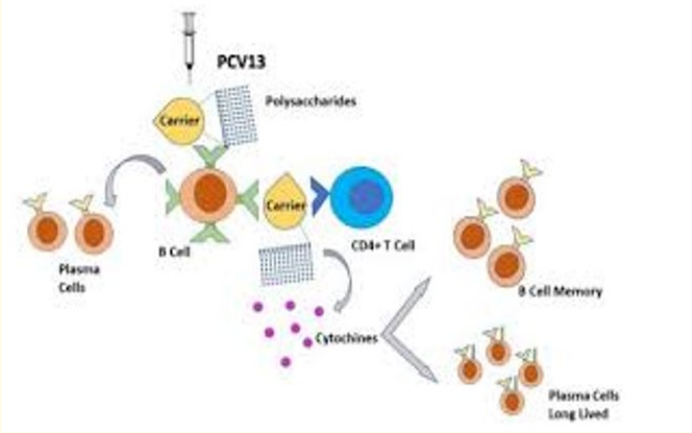

Figure 10: *T- cells dependent mechanism of the PCV13 vaccine: The polysaccharide portion in pneumococcal conjugate vaccines (PCVs) stimulates the production of plasma cells to produce IgG antibody, while the carrier harmful protein portion in a pneumococcal conjugate vaccine (PCVs) activate helper T-cells (CD4+) to induce memory B cell to provide immune response in the case the host reoccurring the same infection by the same S. pneumoniae strain years later.*

Streptococcus pneumoniae infection treatment

Antibiotics are essential treatments from pneumococcal diseases to kill or hindering the growth of *S. pneumoniae* infection. Currently antibiotics resistant *S. pneumoniae* strains to penicillin, erythromycin, tetracycline, and chloramphenicol are emerged and spread worldwide. These emerged antibiotics resistant *S. pneumoniae* is due to the bacterium acquires multiple antibiotic resistance genes *via* transformation, and mutations. This emerging antibiotics-resistant *S. pneumoniae* creating public health crises worldwide specially for children less than 5 years and elderly over 65 years old. It is estimated that overt one million children worldwide die of pneumococcal disease every year [50]. These harder pneumococcal diseases treatment by antibiotics due to the emerging antibiotics resistant *S.*

pneumoniae led to strongly relying on vaccines to provide protection prior the infection, and before developing pneumococcal diseases symptoms. World Health Organization (WHO) recommends routinely pneumococcal vaccination specially for children, elderly for protection from the infection, and for the potential to slowdown emerging *S. pneumoniae* antibiotic resistance. This vaccination protocol will help in reducing the number of hospitalizations and deaths from pneumococcal diseases [51].

Discussion

Streptococcus pneumoniae is a Gram-positive, non-motile, non-spore former spherical bacterium. It is a significant pathogen to human, usually spread from direct person-to-person contact via respiratory droplets, and is the main cause of pneumonia and meningitis for children, elderly and for immunocompromised patients. Also, it is the second infection after virus infection for such as influenza or Covid 19 illness. Pneumococcal diseases are caused by Invasive *S. pneumoniae* strains. There are many types of pneumococcal infections other than pneumonia, there are meningitis, sepsis, osteomyelitis, bronchitis, rhinitis (inflammation of the nose), acute sinusitis, otitis media, conjunctivitis (inflammation in the outer membrane of the eyeball and the inner eyelid), septic arthritis (infection in a joint), endocarditis (inflammation of the heart's inner lining), peritonitis (Inflammation of tissue that lines the abdominal wall), pericarditis (Inflammation in the sac that surrounds the heart), cellulitis (infection in the skin and underlying tissues), and brain abscess. The non-invasive S. *pneumoniae* strains *are* normally resides asymptomatically in healthy carriers colonized in the respiratory tract, sinuses, and nasal cavity. In the case of susceptible individuals such as children, elderly, and immunocompromised patient, the encapsulated bacterium of *S. pneumoniae* became invasive (pathogenic), and spread in the host body causing these highlighted pneumococcal diseases. The encapsulated bacterium of *S. pneumoniae* is due to the presence of capsular polysaccharide (CPS) attached to the peptidoglycan on the bacterium cell wall. This capsular polysaccharide (CPS) is a virulence factor, and also serve as a critical defense mechanism against the host immune system. This capsule consists of high-molecular-weight of repeating oligosaccharide units attached by covalent bonds to the bacterium cell wall peptidoglycan. Encapsulated *S. pneumoniae* strains are vary according to the capsule chemical composition and the quantity of these capsular polysaccharide (CPS) on the bacterium cell wall. These variations in the chemical structure of capsular polysaccharide (CPS) are serotypes among different *S. pneumoniae* strains that influence the bacterium pathogenicity. In addition to the capsular polysaccharide (CPS) *S. pneumoniae* has other major virulence factors, such as pneumolysin, neuraminidases, cell-surface proteins (PspA, PspC, LytA), and the metal-ion-binding proteins (PsaA, PiaA, PiuA). All these virulence factors and others plays specific roles in the host respiratory colonization and pneumococcal diseases. Also, these virulence factors hindering the host's immune system activation against bacterial invasion and clearance from the host body. These pneumococcal diseases caused by *S. pneumoniae* are worldwide public health nightmare due to continue emerging antibiotics resistant of invasive *S. pneumoniae* strains.

There are two types of pneumococcal vaccines. The first type is polysaccharide vaccine, (PPV23) was licensed in 1977. This PPV23 vaccine contains polysaccharide coating from several types of *S. pneumonia* bacteria, and is used to vaccinate adults over 65 years old, and children over 2 years old with certain risks. Because children less than 2 years old don't develop very good immune response to this polysaccharide vaccine (PPV23), second type of pneumococcal vaccines called pneumococcal conjugate vaccines (PCVs) were developed by conjugating pneumococcal polysaccharides to a harmless protein as helper carrier. These developed pneumococcal conjugate vaccines (PCVs) elicits a strong T-cell dependent response, leading into long-term immunological memory and protection, comparing to the T-cell independent response polysaccharide vaccine (PPV23). These PCVs vaccines demonstrated to be significantly better immune response to young children comparing to polysaccharide vaccine (PPV23) that showed less effective specially for children. In addition, these pneumococcal conjugate vaccines (PCVs) showed effectiveness for various age groups of people. Current challenges facing vaccination program are the presence of over 100 serotypes of invasive *S. pneumonia* strains causing pneumococcal diseases, and the current developed pneumococcal vaccines does not protect against all these 100 serotypes. Current developed vaccines protect only the majority of common *S. pneumoniae* strains causing pneumococcal diseases. It is important to highlight that the developed polysaccharide vaccine (PPV23) protects only against about 23 serotypes of *S. pneumonia* strains, while developed conjugate vaccines (PCVs) protects against 13

serotypes by vaccination using PCV13 vaccine, against 15 serotypes by using PCV15 vaccine, against 20 serotypes by using PCV20 vaccine, and against 21 serotypes by using PCV21 vaccine.

Antibiotics drugs are still the main pneumococcal diseases treatment from S. *pneumoniae* infections. Until 1970s, all invasive *S. pneumoniae* strains causing pneumococcal diseases were sensitive to most commonly used antibiotics, including penicillin's, macrolides, clindamycin, cephalosporins, rifampin, vancomycin, and trimethoprim-sulfamethoxazole. This changed in the beginning of 1990s when antibiotics resistant strains of invasive *S. pneumoniae* were emerged against penicillin and against other commonly used antibiotics. These antibiotics resistance *S. pneumoniae* ae varies greatly among countries, states, and even counties. These emerged antibiotics resistant *S. pneumoniae,* because this bacterium is capable to acquire multiple antibiotic resistance genes *via* transformation and evolution due to the increase in antibiotics use. Bacteria generally use variety of mechanisms to survive antibiotics treatment (antibiotics resistant). These mechanisms are such as the bacterium change its protein's structure (receptor) which the antibiotics target, or produce enzymes that break down/modify antibiotics structure to become less efficient, or the bacterial pathogen change its cell membranes structure to become less permeable to antibiotics for efficacy. As an example, *S. pneumoniae* developed resistance to the antibiotic penicillin or cephalosporins via genetic mutations to alter the structure of its penicillin-binding proteins (PBPs) expressed on its cell wall as penicillin receptor. This altered structure of bacteria penicillin-binding proteins (PBPs) leads into the inhibition of penicillin binding to bacteria modified PBPs hindering the antibiotic ability to inhibit the *S. pneumoniae* cell wall synthesis [52]. *S. pneumoniae* resistant to antibiotic penicillin are often resistant to multiple additional classes of antibiotics. Due to emerged *S. pneumoniae* resistant to antibiotics, laboratory testing for antimicrobial susceptibility [53] became critical test for the selection of effective antibiotic(s) against *S. pneumoniae* infections, and also to determine the minimum inhibitory concentration (MIC) to be used effectively in antibiotic therapeutic strategy. This antimicrobial susceptibility assay test method for the selection of efficient antibiotic(s) for pneumococcal diseases treatment became currently the gold standard in clinical practice.

Conclusion

There is worldwide health care burden from invasive *S. pneumoniae* infections. This is due to the ability of *S. pneumoniae* to acquire new genetic materials *via* transformation and recombination, resulted in less efficacy of developed vaccines, and antibiotics. These are challenges require continue developing effective vaccines and antibiotics for better protection from this serious invasive *S. pneumoniae* infection.

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