ASM Sc. J., 18, 2023

https://doi.org/10.32802/asmscj.2023.1235

Advances in Understanding the Pathogenesis: Bacterial Surface Glycans as The Virulence Factor and Role in Pathogen Bacteria

C. Ghazaei[1]* and S. Amjad[2]

[1]*Department of Microbiology, University of Mohaghegh Ardabili, Ardabil, Iran, P.O. Box 179*

[2]*Department of Zoology, University of Narowal, Punjab, Pakistan*

Bacterial glycans are essential components of bacterial walls and crucially important cell surface antigens such as lipopolysaccharide and polysaccharide capsules, which act as virulence-causing factors. Polysaccharide capsules in bacteria are naturally composed of recurring oligosaccharides and lipopolysaccharides. LPS is composed of domains that are hydrophobic in nature known as lipid A or endotoxin, which is a nonrepeating oligosaccharide "core", and an O-antigen. Interaction of glycan-receptor is critical in pattern recognition of microorganisms and regulatory signals that perform regular immune cell activities. The true glycan structure is important for collaboration with the immune system of host, as demonstrated by microorganisms that produce polycarbohydrates that mimic host particles in order to evade the immunity of host. Certain bacteria circumvent antibody defences by molecularly mimicking the glycan structures of host and masquerade as "self" to avoid identification by immune system. Phages are the most prevalent biological entities on our planet, and they evolved glycan-binding proteins (GBPs) to bind to bacterial glycans. These proteins are in charge of the breakdown of antibodies and are impervious to harsh conditions where bacteria live for example, the gut of mammals, water, and soil. In order to increase contact with bacterial glycans, the phages manufacture multimeric proteins.

Keywords: Molecular mimicry; Bacterial-Glycans; Glycan receptor; GBPs

I. INTRODUCTION

Human beings are in constant interaction with microbes throughout their lives. The total number of bacteria colonised by the mucosal surface exceeds that of our somatic and germ cells (Hooper & Gordon, 2001). As a result, determination of the virulence causing factor is critical for understanding the disease causing abilities of bacteria and their relationship with the host, which might also provide a new target in treatment and antibody development (Wu *et al.,* 2008). Bacterial glycans are crucial components of the bacterial cell walls and essential surface antigens including lipopolysaccharides and capsular antigens in Gram-negative bacteria; they are linked by lipid anchors (diacylglycerophosphates or lipid A) (Schmidt *et al*., 2003). By establishing commensal bacteria and pathogens in their specialised environment, these glycans behave as 'personal business cards'.

The role of glycans in structural integrity maintenance, protein localisation, and host response induction; has been clarified. These carbohydrate conjugates have been thoroughly studied as virulence factors (Schmidt *et al*., 2003). Virulence factors are microbial compounds that are responsible for disease development. In a complicated environment, higher species of organisms have developed immunity against invasions of microbes in order to prevent the spread and to lessen the damage to their tissues and cells. The interaction of Glycan-receptors is vital in the pattern recognition of microorganisms and regulatory signals that execute regular immune cell activity. Most of the bacteria cause disease because they have developed to express their receptors as well as sugars in ways which either

*Corresponding author's e-mail: ciamakghazaei@yahoo.com

imitate or inhibit the glycan-based immunological capabilities of the host (Nizet & Esko, 2009). One of the most prevalent biological entities on Earth are phages, and phages have developed GBPs to bond with sugar covered bacteria (Brüssow *et. al.*, 2004; Casjens & Molineux, 2012; Suttle, 2005). These glycan-binding proteins (GBPs) aid in the phage's attachment with bacteria (Singh *et al.*, 2011). Phage populations grow by causing harm to bacteria. The infectious particles of phages are released in the extracellular matrix at the termination of the lytic cycle. Throughout the cycle, phages break the bacterial wall two times during the cycle by the secretion of an enzyme that allows incorporation of their DNA inside the host's cell, and once the cycle is complete, they release their infectious particles into the space (Latka *et al.*, 2017). (VALs) Virion-associated lysins or VALs are responsible for the penetration of DNA of virus into the host's cell. Following VALs action, the tailtube of phage contracts, injecting the DNA of virus into the host's cell (Moak & Molineux, 2004). The other enzyme known as endolysin is manufactured in protoplasm and is responsible for the lysis of bacteria, but it requires one more enzyme named holin to function properly. Holins are oligomerised in the membrane of bacteria, and they generate punctures in the bacterial wall, enabling endolysin to escape. Endolysins rapidly degrade the peptidoglycan polymer of dead host bacteria, causing immediate osmotic lysis and the release of phage offspring (Catalão *et. al.*, 2013; Young, 2013).

A. Bacterial Surface Glycans- The Virulence-Causing Factors Polysaccharide Capsules

Capsules made up of polysaccharides, found in prokaryotes are primarily composed of repetitive oligosaccharide units commonly found on the bacterial surface. These capsules are formed of a single polysaccharide. They are found outside Gram-negative bacteria's exterior film and in the PG layer of Gram-positive cells (Moxon & Kroll, 1990). Generally, the host can generate strong antibody responses against bacterial polycarbohydrate capsules; however, this capacity is weakened at the extremes of the age, making newborn children and the elderly specifically susceptible to virulent infections caused by encapsulated pathogens. Carbohydrates occur in nature in the form of polymers instead of existing as monomers. Polysaccharides are regarded as the most abundant polymeric sugar in this particular scenario. These polymers feature a minimum of ten simple units of sugars and are composed of thousand or more basic sugars, bound together by glycosidic bonds to achieve sub-atomic masses of up to 100 million amu (Chawla *et al.*, 2009). The capsular polysaccharide, which is found in the bacterial cell's peripheral layer, serves as an intermediary between the organism and nature. The capsules composed of polysaccharides have been identified as important virulence-causing factors for various pathogenic bacteria as a result of these collaborations. The capsular polysaccharides are mostly present in the gram-negative strains, likely, E. coli, P. aeruginosa, N. meningitidis, H. influenzae, K. pneumoniae, and Salmonella. These capsular polycarbohydrates were also seen in many gram-positive microorganisms, for example, B. megaterium (can produce a polypeptide and polysaccharide capsule), Streptococcus pyogenes (could produce a hyaluronic acid capsule), Staphylococcus epidermidis, S.agalactiae, and S. pneumoniae. Aside from this, a capsular polycarbohydrate was also found in yeast, specifically C. neoformans, which has a capsule just like that of bacteria.

Most of the time, it is hard to distinguish between capsular polysaccharide or CPS and "O"-antigenic moieties of LPS, because capsular polysaccharide is observed to be connected to lipopolysaccharides (Whitfield, 1988). The capsule can be identified using either serological techniques or India ink recolouring in which the material of capsule acts as an antigen and is then, mixed with specific anti-capsular serum. The capsule appears enlarged under a microscope due to an increased refractivity. The Quellung response is the name given to this amazing phenomenon. Capsular polysaccharides contain >95% water and thus are hydrated particles. These molecules are covalently attached to the surface of bacterial cells, linked to phospholipids or lipid-A particles, whereas in some instances, CPS was shown to be directly related to the cell with no layer grapple (Whitfield, 1988; Whitfield & Valvano, 1993).

The capsular polysaccharide could be a hetropolymer or a homopolymer composed of repetitive units of monosaccharides connected with glycosidic bonds (Roberts, 1996). Two monosaccharide units might be connected in

numerous geometries, resulting in a wide structural variation in the CPS between species, but chemically similar capsular polycarbohydrate might be generated by a completely different species of bacteria also. It has been discovered that specific CPS or K-antigens are linked to specific illnesses. The true cause of neonatal meningitis is the K1 antigen of E. coli, a homopolymer of a N-acetylneuraminic acid that is 2,8-connected (Robbins *et al.*, 1974). The capsular polycarbohydrate of group B N. meningitidis is identical to the K1 polymer of E. coli (Grados & Ewing, 1970).

The CPS of bacteria can carry out a variety of different functions, including preventing drying out, adhesion, protection against inclusive host immunity, and protection from peculiar host immunity, and so interfering with particle diffusion across the cell surface (Whitfield, 1988; Whitfield & Valvano, 1993). The bacteria with capsules are capable of surviving outside the host cell, advancing the transfer of infectious microorganisms from one host to the next; this could be the result of the arrangement of hydrated gel present around bacterial surface, protecting it from drying up. CPS promotes the formation of biofilms because they tend to follow the bacteria to its surface or in conjunction with other cells, promoting the colonisation of bacteria (Costerton *et al.*, 1987). Throughout the course of an intrusive bacterial disease, interactions between the host's immune system and capsular polysaccharides might influence the consequences of the infection (Roberts, 1996; Roberts *et al.*, 1989) (Figure 1).

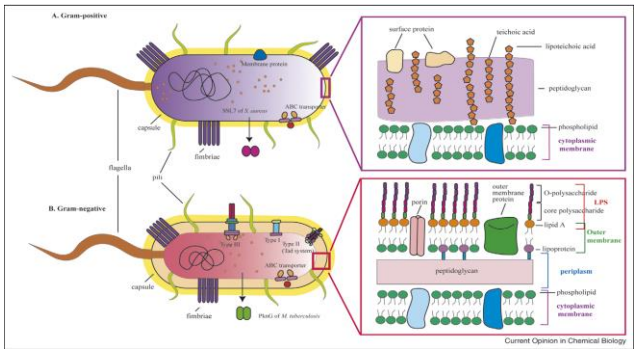

Figure 1. The schematic diagram shows the major virulence factors of pathogenic bacteria (A) Gram-positive, and (B) Gram-negative bacteria

B. Lipopolysaccharide

LPS is critical to the structural and functional coherence of the external layer of bacteria that are Gram-negative (Erridge *et al.*, 2002). Bacterial lipopolysaccharides (LPS) are composed of several hydrophobic domains known as lipid A or endotoxin, a nonrepetitive oligosaccharide core, and an O-antigen (distal polysaccharides) (Raetz & Whitfield, 2002).

C. O – Antigen

It is also called O polysaccharide, O antigen or bacterial O side chain. It is primarily a recurring unit of glycans, which forms a polymer. It is an oligosaccharide, firmly linked to the bacterial cell and exposed on the external surface of the LPS particle. The composition of the "O" polymer varies depending on the variety (Rittig *et al.*, 2003). Based upon the absence, existence or reduced form of the O antigen, lipopolysaccharides are of two different types; rough and smooth. Studies demonstrated that bacteria consisting of rough LPS become increasingly hydrophobic and consist of a more sensitive cell layer to hydrophobic anti-infection drugs (Tsujimoto *et al.*, 1999).

D. Core Polysaccharide

The oligosaccharide component that binds to lipid A is mostly found in the centre. 3-deoxy-D-mannooctulosonic corrosive (or ketodeoxyoctulosonate) and heptose are the sugars that are found most commonly. In most bacteria, on sugar components, like amino acids, phosphates, ethanolamine alternates, also exist in LPS centre. N. meningitidis's internal core is formed by heptose moieties and a 3-deoxy-D-manno2-octulosonic corrosive (Hershberger & Binkley, 1968).

E. Lipid A

In general, this portion is an extremely preserved part of lipopolysaccharides. Lipid A is basically a phosphorylated glucosamine disaccharide that has been enriched with a variety of lipids. These fatty acid polymers are hydrophobic and grab lipopolysaccharide and drag it inside the bacterial

layer, while the remainder of the lipopolysaccharide emerges from the cell surface. Lipid A portion is primarily responsible for the toxic nature of gram-negative bacterial cells. When bacteria undergo lysis under the effect of immune system, the layer fragments that contain lipid A are discharged into circulation, causing high fever, runs, and potentially fatal endotoxic stun (also known as septic stun) (Tzeng *et al.*, 2002).

F. LPS Activate the Immune System

LPS was recognised as PAMP (or pathogen associated molecular pattern) by natural immune system. They activated an immune response to kill microorganisms that had crossed the skin and mucosal epithelium's border guards. Invading bacteria release soluble LPS, peculiarly its Lipid A portion, which intercommunicates with the receptor, CD14 and initiates immunological signalling (Nizet & Esko, 2009). While it had long been assumed that the particle CD14 was necessary for the cells to respond to LPS; it was shown that it could not function as the only receptor molecule for lipopolysaccharides. CD14, as Glycosyl phosphatidylinositol (GPI)-bound protein, lacks a transmembrane region to promote signalling inside cells. Furthermore, it was discovered that human cells lacking CD14 could respond to lipopolysaccharides even in the absence of serum conditions lacking CD14 that was dissolvable (Akashi *et al.*, 2000). The component that binds with CD14 (an opsonic receptor) and TLR4 (membrane proteins Toll-like receptor 4) initiates the mechanism of immune signaling (Figure 2).

TLR4 belongs to an evolutionarily conserved class of receptors that may identify ligands originating from microorganisms (Nizet & Esko, 2009). Despite the absence of LPS in Gram-positive microbes, the receptor, TLR2 can detect PG or lipoteichoic acid derived from the walls of bacterial cells. The intracellular signalling of TLR is regulated by IL-1, a set of interleukin-1 IRAKs, receptor-associated kinases, which further connect with the TLR intracellular Toll/interleukin-1 receptor (TIR) domain; this mechanism needs the existence of adaptor protein (i.e., MyD88). The transcription factor NF-κB is activated by the signalling pathway and positively regulates promoters for the gene that encodes various inflammation promoting cytokines (e.g., IL-1 and TNF) and is relocated to the nucleus. Although the discovery of TLR4 of lipopolysaccharides is a fundamental component in activation of innate immunity of host, a hazardous sickness, named sepsis, could occur corresponding to overwhelming disease, when immune initiation patterns turn wild. Low circulatory strain, high fever, rapid pulse rate, unusual white platelet tests, and irregularity of multiple organ frameworks are the side effects that can cause lung failure, kidney failure and sometimes can lead to death.

In the 1890s, Pfeiffer, Richard discovered that a distilled thermostable unit of Gram-negative bacteria was adequate to cause sepsis in the experimental organisms; this unit was eventually identified as LPS and is now commonly referred to as bacterial "endotoxin." (Nizet & Esko, 2009). In 1960s, C3H/HeJ, a certain strain of mice was found to be resistant to the sepsis causing characteristics of lipopolysaccharide. During 1990s, a mutation found in this strain of mice was linked to a gene, TLR4, leading to the identification of TLR4 as the functional receptor in warm blooded species for lipopolysaccharides (Nizet & Esko, 2009). Several Gram-negative bacterial cells modify and adjust their lipopolysaccharide in order to thwart defence mechanisms in host (Nizet & Esko, 2009).

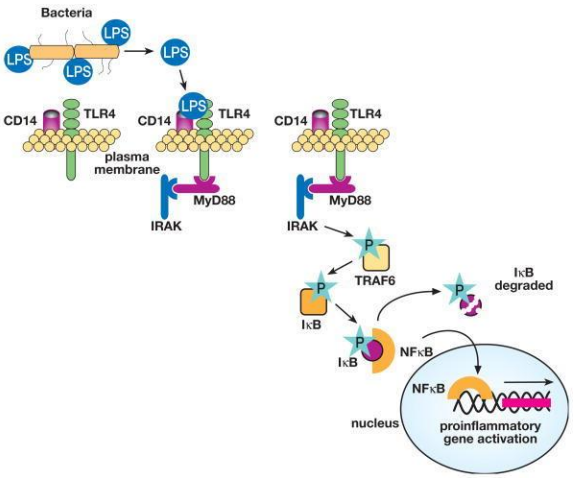

Figure 2. Immune signalling activation by bacterial lipopolysaccharides

The LPS on Gram-negative bacteria's cell wall is bound by TLR4 and CD14 (surface receptor). Interaction of LPS causes the main connector proteins namely, IRAK and MyD88 to be joined to the cytoplasm region of TLR4. This

complex triggers phosphorylation events chain via the kinase IκK and TRAF 6. Finally, the kinase, IκK phosphorylates an inhibitor IκB, attached to NF-κB (a transcription factor). After degradation, phosphorylated IκB releases NF-κB, which travels towards the core and triggers the transcription of the inflammation promoting gene. Components of cell wall of Gram-positive bacteria, including peptidoglycan as well as lipoteichoic acid, initiate comparative signal transduction pathways via TLR2 or TLR6 (Nizet & Esko, 2009).

II. IMMUNOLOGICAL RESPONSES TO BACTERIAL GLYCANS

A thorough investigation of bacterial infections has plainly determined the role of polycarbohydrates as damaging elements. The synthesis of capsule is indeed, essential for destructiveness in a variety of animal models of diseases. Polysaccharides cause harm by a variety of mechanisms, including anti-phagocytic and anti-bacteriolytic action, immune regulation, immune evasion, and biofilm formation (Comstock & Kasper, 2006).

A. Eluding Immune Detection

Bacterial strains that are polycarbohydrate deficient are destroyed frequently by the activity of PMNs (polymorphonuclear leukocytes) and regular serum in the unavailability of peculiar antibodies. Encapsulated bacteria, destroyed via serum and PMNs must be covered (opsonized) by antibodies as well as complement molecules. In the absence of particular anti-bodies, the deposition of complement on the surface of organism is moderated by association with C-lectin receptors or by the complement pathway (Kang *et al.*, 2006). Encapsulated bacteria usually contain structures composed of carbohydrates like sialic acids (e.g., N.meningitidis), which prevent complement activation in the absence of a specific antibody, allowing these bacteria to survive in the bloodstream. A major factor in successful vaccination is the evolution of peculiar antibodies via inoculation, with subsequent deposition of complement on an organism (Comstock & Kasper, 2006).

B. Immune Avoidance by Molecular Imitation

True glycan component is important for the specified collaboration with immune system of host, as demonstrated in the bacterial cells which produce polysaccharides which imitate host particles in order to avoid host immunity (Finne *et al.*, 1983). Certain bacteria that may bypass antibody defences personate as "self" via molecular imitation of glycan structures of host organism in order to elude immune identification (Nizet & Esko, 2009). One of the most common pathogens is GAS or group A streptococcus, which has a capsule of hyaluronan, which is non-immunogenic and is just like the non-sulfated glycosaminoglycan, that is abundant in the cartilage and skin of host (Nizet & Esko, 2009). The sialic acid capsules of N. meningitidis (meningococcus) are homopolymeric and illustrate host imitation that is based on capsule to evade the bacterial immune system. The meningococcal capsule of group C is a peculiar structure of bacteria composed of a sialic acid polymer that is α2-9-linked (Nizet & Esko, 2009). In contrast to group C meningococcal capsule, the meningococcal capsule of group B is formed of an α2-8-linked chains of sialic acid which is similar to a motif found on NCAMs or neural cell adhesion molecules found in human neural tissues. In the population of humans, the capsule of group C has been authenticated to be an effective vaccination antigen, but that of group B is non-immunogenic (Nizet & Esko, 2009).

C. Zwitterionic Polysaccharides and Immunity

ZPSs or zwitterionic polysaccharides of S. pneumonia type 1 and B. fragilis are polysaccharides with both a negative and a positive charge and provide an entirely different example of the fact that the fundamental theme of a polycarbohydrate is critical for its functioning. This unique category of bacterial polycarbohydrates has been shown to represent a remarkable instance of how starches induce immunological responses. Polysaccharides are antigens that are often T cell independent; thus, they don't initiate partners of T cells, which then trigger IgG exchange in B cells. They do not prompt immunologic memory (an insufficiency demonstrated by the inadequacy of supportive inoculation with natural polycarbohydrate antibodies).

Polycarbohydrates have been connected to the carriers of proteins in the formation of efficient vaccinations which activate the creation of B memory cells as well as IgG antibodies to help enrol T cell–independent antigens. The sugar in glycoconjugate antibodies presumably binds to B cells, which are polysaccharide-specific. After it, partner T cells are set in motion by the preparation and introduction of antigenic peptides of the compound by significant MHC II or histocompatibility class II particles. ZPSs have lately been found to be processed by the MHC II pathway (Cobb *et al.,* 2004), which was thought to be allocated for the proteins only. Inside the endosome of the antigen-showing cell (APC), the ZPSs are processed to a considerably smaller molecular size. ZPS is a nitric oxide (NO)-dependent mechanism that doesn't need glycosidases. The intelligence behind the interconnections of sensitive N2 or O2 species with zwitterionic polysaccharides has yet to be depicted. Apart from the Nitrogen and Oxygen dependent component that reduces atomic size, zwitterionic polysaccharides follow the MHC II pathway as conventional protein antigens before being conferred to the αβ T cell receptor in the setting of the MHC II molecule (Comstock & Kasper, 2006).

III. BACTERIAL GLYCANS ARE APPEALING TARGETS FOR PATHOGEN-SPECIFICITY

The bacteria are surrounded by a plethora of glycan structures, including their own wall. The walls of bacteria form a protective coating which protects it from its surroundings as well as from osmotic lysis. Bacterial wall is the most common target of bacterial antibiotics due to its significance in the survival of bacteria and its surface permeability (van Dam *et al.*, 2009). Antibiotics such as, vancomycin (Perkins, 1969), penicillin (Park & Strominger, 1957), and bacitracin (Storm & Strominger, 1973) all meddle with the biosynthesis of bacterial wall, demonstrating the appeal of the bacterial wall as a pharmacological focus for new treatments. The bacterial wall remains a potent agent because as it is protected by different surface approachable components which are connected to pathogenesis.

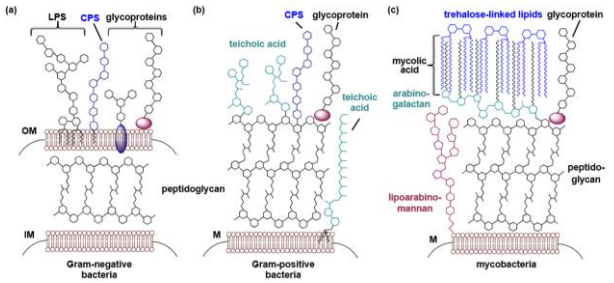

Figure 3. Bacterial walls are covered by an assortment of the glycan structures (a) Gram-negative microbes have both, an outer membrane (OM), an internal layer (IM). Peptidoglycan rigidifies the gap between the membranes. Capsular polysaccharide (CPS), lipopolysaccharide (LPS), and glycoproteins are present in the exterior film, (b) Gram-positive microbes have only one membrane (M), reinforced by a dense layer of PG. CPS, glycoproteins, and teichoic acids are located on the periphery of the cell, and (c) Mycobacteria are a type of gram-positive microbes with unique glycans present on cells, including mycolic acid, lipoarabinomannan, and trehalose-connected lipids.

An examination of architectures of bacterial glycans reveals only the monosaccharide building units of bacteria. A few of these are commonly found in bacterial cells, while others are exclusive to a few pathogenic bacteria (Dube *et al.*, 2011) For instance, every cell of bacteria is coated with peptidoglycan, which is a complex of repetitive units of N-acetylmuramic acid (MurNAc) and 1,4-connected N-acetylglucosamine (GlcNAc) cross connected by short peptides. Although GlcNAc is widely used in both prokaryotic and eukaryotic cells, MurNAc is primarily found in bacteria. As a result, an imaging specialist or a therapeutic specialist which targets MurNAc or PG would have an effect on all bacteria. Surprisingly, only the Gram-negative microbes combine lipopolysaccharide, which contains the unique monosaccharides like L-glycero-D-mannoheptose and Kdo or 3-deoxy-D-manno-oct-2-ulosonic corrosive (Cipolla *et al.*, 2011). As a result, only gram negative bacteria are affected by antimicrobials or structures. Gram-positive bacteria also contain teichoic acid.

These structures might be used to detect or eradicate all of the major kinds of bacteria, forming a wide ranging method. Rare carbohydrates, found on bacteria could impact the development of limited therapies and diagnosis, and are potentially appealing targets. N. meningitides, for example,

makes use of 2,4-diacetamido2,4,6-trideoxyhexose (DATDH) (Smedley *et al.*, 2005), P. aeruginosa introduces FucNAc residues (Alemka *et al.*, 2013), and B. fragilis appends 2acetamido-4-amino-2,4,6-trideoxy-galactose (AAT) (Howard *et al.*, 2009) into the polysaccharides present on the surface of its cell. These specific building blocks are present in only few species of bacteria (Dube *et al.*, 2011). Similarly, the glycoproteins of C. jejuni and H. pylori have the amino- and deoxymonosaccharides pseudaminic acid, bacillosamine, and, legionaminic acid (Schirm *et. al.*, 2003; Black *et al.*, 2000). These sugars are linked to pathogenic bacteria, (Goon *et. al.*, 2003; KNIREL *et al.*, 1986; Schirm *et al.*, 2003) no intelligence exist in the commensal bacteria of these monosaccharides that control the microbiome of human gut (e.g., Prevotella & Bacteroides; Lozupone *et al.*, 2012). Because specific sugars of bacteria are not used extensively, they are an intriguing target for specific intervention and investigation. Bacterial glycans have proven to be excellent targets as they are frequently linked to the health of bacteria and their pathogenicity. The cells of bacteria which have incorrectly synthesised PG are then osmotically lysed (Chatterjee & Young, 1972), those with modified lipopoysaccharides are exterminated by the immune system of the host (Chatterjee & Young, 1972), and microorganisms lacking the teichoic acids are debilitated in the infection of host and colonisation (Weidenmaier *et al.*, 2005). Comparably, mycobacteria that are trehalose-inadequate are not manageable (Woodruff *et al.*, 2004), and bacterial strains have impaired host cell official and colonisation problems (Alemka *et. al.,* 2013; Howard *et. al.*, 2009; Schirm *et. al.*, 2003; Smedley *et al.*, 2005). These views disclose that the glycans of bacteria have important functions. They are also linked to pathogenicity in some circumstances. The capability of targeting and observing these components could provide the squeeze needed to develop novel medications and diagnosing tests for the illnesses of bacteria.

A. The Interactions of Phages with The Glycan Receptors

Phages are the most prevalent biological entities existing on Earth, with around 1x1031 virions (Suttle, 2005). Preceding the illness for the precise detection of their hosts, phages encipher explicit acknowledgment factors which authorise them to distinguish among various strains of a single species (Casjens & Molineux, 2012). Evolution of phages over 3 billion years has allowed them to bind to sugar covered bacteria (Brüssow *et al.*, 2004). As a result, phage glycan-binding proteins (GBPs) have been developed that can traverse a wide spectrum of glycan variety while having binding capacities equivalent to or surpassing those of most antibodies (Singh *et al.*, 2011) (Figure 4).

Bacterial cells that dwelt in harsh environments such as the gut of mammals, water, and soil were detected by phages and developed the GBPs, making these GBPs responsible for the breakdown of antibodies and resistant to harsh environments. Because of their power, GBPs of phages are perfect for their use as "replacement antibodies" in detection and treatments. Furthermore, GBPs of phages are easily recombinantly expressed in E. coli as well as plants (Simpson *et al.*, 2015), implying a function for as comprehensible instruments for glycan investigation. Numerous GPs are competent enough to reduce oligosaccharides. (Born *et. al.*, 2014; Schulz *et al.*, 2010).

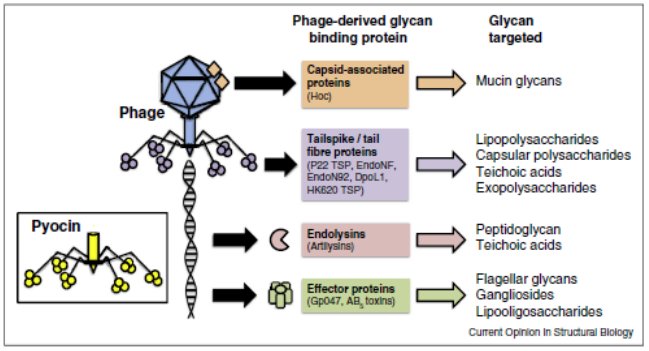

Figure 4. A review of several categories of phage-ciphered glycan restricting proteins or the GBPs that have been identified to date, as well as the glycans recognised by every class. Tail spike proteins are also known as stands for TSPs. Inset: pyocins, like headless phages also encrypt GBPs.

IV. TYPES OF PHAGE-ENCODED GBPS

A. Receptor-binding Proteins

Tail strands and TSPs, also known as RBPs or receptor binding proteins, are found on the tails and are responsible for the binding of phage to host's cell (Andres *et. al.,* 2010; Casjens & Molineux, 2012). To compensate for the protein's

low overall affinity for carbohydrates, phage develops a multimeric protein to boost the avidity of the interaction with carbohydrates (Casjens & Molineux, 2012; Dalziel *et. al.*, 2014; Schulz *et al.*, 2010).

B. Phage-Derived Enzymes Recognising/Degrading Bacterial Glycans

Phage-encoded enzymes destroy the cell wall of bacteria, the peptidoglycan (PG), and murein, revealing a possible surrogate category of antibacterial agents that maximise the differences (Nelson *et. al.*, 2012; Schmelcher *et al.*, 2012).

V. PEPTIDOGLYCAN-DEGRADING ENZYMES—A RISING CATEGORY OF ANTIBACTERIAL AGENTS

A. Biology and Heterogeneity

The phages having tails are universal viruses which peculiarly destroy the bacteria to invigorate them to proliferate. On termination of the reproductive cycle, the viruses cause lysis of host cells and release infectious particles into extracellular space. A phage breaks the cell wall twice throughout the lytic cycle, once for the infiltration of its DNA into cell of host and again at the cycle's termination to discharge the viral offsprings. In both steps, the phage's PG-degrading enzyme was administered. VALs or Virion-associated lysins and VALPHs or virion-associated peptidoglycan hydrolases are enzymes that allow viral DNA to enter the host cell (Latka *et al.*, 2017). VALs assault the host cell wall from the outside, increasing the local breakdown of murein to facilitate phage tail tube penetration and viral DNA insertion at the start of phage infection (Moak & Molineux, 2004). However, other VALs, such as those from phage phi11 of S.aureus and phage T7 of E.coli, have been shown to be unnecessary for the phage infection and may be beneficial under physiological conditions (Moak & Molineux, 2000; Rodríguez *et al.*, 2013). Endolysins are enzymes that are generated in the cytoplasmic regions of bacteria that are infected and are responsible for the lysis of the host's cell. Endolysins need the assistance of a phage-encoded protein, namely holin, to traverse across the membrane and access the cell wall, allowing them to traverse across the cell wall of bacteria at cycle's termination. Holins are oligomerised on membrane of bacteria. They often constitute of a tiny hydrophobic protein which forms deadly punctures in the bacterial wall and aids endolysin in escaping. Endolysins rapidly degrade the PG polymer of deceased host, causing abrupt osmotic lysis and the release of phage offsprings (Catalão *et. al.*, 2013; Young, 2013). VALs as well as endolysins have developed different enzymatic actions for the selective breakdown of peptidoglycan.

The PG polymer is formed of alternatively recurring GlcNAc or N-acetylglucosamine and MurNAc or N-acetylmuramic acid residues which are associated with one other via amide bond utilising the peptide stem's first amino acid (often L; alanine) (Vollmer *et al.*, 2008). VALs or endolysins are classified into several kinds based on the bonds they break: transglycosylase, lysozyme, amidase or endopeptidase, and glucosaminidase (Oliveira *et al.*, 2013). The glycosidic bonds in glycan chains are broken by the initial three classes: MurNAc is attached to the peptide stem by an amidase which hydrolyses the amide bond, and the peptide moiety is cleaved by endopeptidases. Endolysins can be flexile or spherical in shape (Oliveira *et al.*, 2012). In the initial example, the enzymes capable of causing lysis must confrom to an single ECD or enzymatic catalytic domain which is amenable for the breakdown of a certain peptidoglycan linkage. Mostly in conventional standard structure, 1 / 2 N-terminal enzymatic catalytic doamins are linked via a pliant connector to single or more than one cell-connecting zones, which are amenable for identifying certain antigenic determinants present on the cell wall. Such arrangement is established in the endolysins of mycobacteria infected with bacteriophages and Gram +ve bacteria (Nelson *et. al.*, 2012; Payne & Hatfull, 2012).

However, few instances with adaptable organisation have been discovered in Gram-negative bacteria, although their functional domains are oriented inverted (Walmagh *et al.,* 2012). Endolysins come in a wide diversity, with 24 distinct types of ECDs and 13 distinct types of CBDs, and 89 novel architectural linkages discovered (Oliveira *et al.*, 2013). The endolysin enzyme of phages will not disseminate and demolish the host in Gram +ve hosts, allowing phage offsprings to launch new rounds of infection. (Loessner *et al.*, 2002). Because the Gram -ve bacteria's exterior

membrane protects inner bond, the endolysins liberated at the termination of bacteriophage's lysis not obliged to badly affect non-lethal cells. VALs are intrinsically different and typically bigger with the size of 37–252 kDa than similar endolysins with the size of 15–40 kDa. In addition to murein cleavage, they may provide functional as well as structural properties to phage infection particles, and few are observed as small number of linked monomer in virion form. VALs from Gram +ve complex usually exhibit 2 different Enzymatic Catalytic Domains to breakdown the linkage, most probably in reaction to their hosts' dense linkage layer. There is a specific ECD, endopeptidase of the M23 family, that is most commonly found in VALs but is hardly ever seen in endolysins. Surprisingly, Such area is in charge of peptidoglycan lessening the activity of the bacteriolysins lysostaphin and enterolysin A, both of which act on actively developing cells (Khan *et. al.*, 2013; Thumm & Gotz, 1997). However, the CBDs usually lack in VALs (Rodríguez *et al.*, 2013).

The lack of presence of CBDs in VALs could be elaborated as they are frequently subdomains of bigger proteins or associate to virion forms that advance the VAL's presence to CW (Rodríguez *et al.*, 2013). VALs form a section of tail fibres, measuring tape proteins, and baseplates on a regular basis (e.g., phage TP901-1 of lactococcas, bacteriophage phiIPLA35 of S. aureus and phage T4 of E. coli, separately) (Nishima *et al.*, 2011) yet can additionally be interior proteins of capsid (e.g., in bacteriophage T7 of Escherchia coli and phiKMV of Pseudomonas aeruginosa) (Lavigne *et al.*, 2006). The lysis of VAL is accountable for the "lysis from without" process, which was initially seen in 1940 as a result of various phages puncturing (Delbrück, 1940). VALs from the lactococcal bacteriophages Tuc2009 and TP901-1, as well as the Bacillus subtilis bacteriophages SP-β and phi29, were found to destroy the bacterial CW in Gram-positive hosts. (Cohen *et. al.*, 2009; Stockdale *et al.*, 2013). Surprisingly, the VALs out of all of such bacteriophages contain at the least one Enzymatic Catalytic Domain from family peptidase / M23. This location appeared to help CW intrusion and bacteriophage genome transmission in lactococcal phages, particularly in stationary stage cells with an extensively cross-connected CW (Stockdale *et al.*, 2013). (Figure 5).

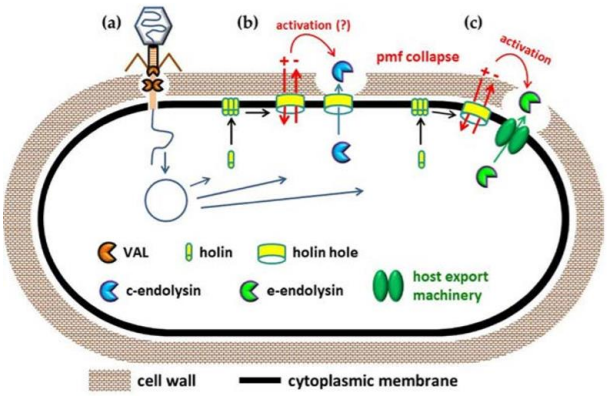

Figure 5. The activity of phage lytic proteins is set in a specific way (PLEs) (a) Virion-related lysins (VALs) aid infiltration of the phage tail cylinder and viral DNA entrance into the host cell cytoplasm by promoting adjacent absorption of the cell wall (CW) peptidoglycan. Tainted cells must lyse after phage genome expression to discharge the newly produced infection particles. This is due to endolysins' peptidoglycan-degrading activity, (b) most known endolysins (c-endolysins) access the CW compartment via holin channels, and (c) some, however, are sent out (e-endolysins) via host cell apparatuses (e.g., the bacterial Sec framework). The dissipation of the cytoplasmic film proton-motive force (pmf) by Holin is required for the activation of e-endolysins, and it may also enhance the lytic activity of c-endolysins.

VI. CONCLUSION

Virulence factors are microbial compounds that are responsible for disease manifestation. In bacteria, the cell wall contains several extra structures, including glycan, which is a key component of the cell wall, as well as crucial surface antigens such as capsules of polysaccharides and lipopolysaccharides, which act as virulence causing factors. Glycans are vital while it comes to maintaining the structural integrity of the bacterial cell wall. Glycan-receptor interaction is important for recognising microorganism patterns and also for regulating the signs that conduct the regular actions of immune cells. Many bacteria are capable of causing disease as they developed to show their sugars and sensory receptors in a way that masquerades host glycan-based immune activity. The capsule made of polysaccharides that lies outside the bacterial wall is a virulence-causing factor that has been proven by disease research of bacteria, demonstrating its importance as a

virulence-causing factor. This capsule is often composed of repetitive units of oligosaccharides. Polysaccharides cause harm through a variety of mechanisms, including antiphagocytic and anti-bacteriolytic action, immunological regulation, immune evasion, and biofilm formation. To avoid host immunity, these polysaccharides duplicate host particles. Many bacteria develop antibody protection by molecularly mimicking glycan structures of host and so avoid identification by immune system. Phage is one of the most prevalent entities on the planet, and it has evolved GBPs to bond with high sugar covered bacteria. The glycan binding proteins aid in the breakdown of antibodies and the development of resistance to the harsh circumstances in which bacteria survive.

The phage tail strands and tail spikes add RBPs or receptor binding proteins that are located on tails of virions and assist in grappling the phage to the cell of host for the purpose of initiating infection. Phage also produces a large number of multimeric proteins in order to enhance its affinity for the host cell. Phage produced enzymes that degraded the bacteria's surface glycoprotein. Peptidoglycan-Degrading enzymes allow viral DNA to penetrate the host cell. The other enzyme, endolysin, causes bacterial cell wall lysis, but in order to work properly, it requires another phage-derived enzyme named holing. Holin generates deadly holes in bacteria's cell walls and aids endolysin escape from the bacterial cell wall. Following the completion of the phage's reproductive cycle, lysis occurs, releasing the phage's progeny.

VII. ACKNOWLEDGEMENT

The author would like to extend his gratitude for the support provided.

VIII. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

IX. REFERENCES

Akashi, S, Ogata, H, Kirikae, F et al. 2000, 'Regulatory Roles for CD14 and Phosphatidylinositol in the Signaling via Toll-like Receptor 4-MD-2', Biochemical and Biophysical Research Communications, vol. 268, no.1, pp.172-177.

Alemka, A, Nothaft, H, Zheng, J et al. 2013, 'N-glycosylation of Campylobacter jejuni surface proteins promotes bacterial fitness', Infection and Immununity, vol. 81, no. 5, pp. 1674-1682.

Andres, D, Hanke, C, Baxa, U et al. 2010, 'Tailspike interactions with lipopolysaccharide effect DNA ejection from phage P22 particles in vitro', Journal of Biological Chemistry, vol. 285, no. 47, pp. 36768-36775.

Black, S, Shinefield, H & Fireman, B 2000, 'Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children', The Pediatric infectious disease journal, vol. 19, no. 3, pp.187-195.

Born, Y, Fieseler, L, Klumpp, J et al. 2014, 'The tail - associated depolymerase of Erwinia amylovora phage L1 mediates host cell adsorption and enzymatic capsule removal, which can enhance infection by other phage', Environmental Microbiology, vol. 16, no.7, pp. 2168-2180.

Brüssow, H, Canchaya, C & Hardt, WD 2004, 'Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion', Microbiology and molecular biology reviews, vol. 68, no. 3, pp. 560-602.

Casjens, SR & Molineux, IJ 2012, 'Short noncontractile tail machines: adsorption and DNA delivery by podoviruses', Viral Molecular Machines, pp. 143-179.

Catalão, MJ, Gil, F, Moniz-Pereira, J et al. 2013, 'Diversity in bacterial lysis systems: bacteriophages show the way', FEMS microbiology reviews, vol. 37, no. 4, pp. 554-571.

Chatterjee, AN & Young, FE 1972, 'Regulation of the bacterial cell wall: isolation and characterization of peptidoglycan mutants of Staphylococcus aureus', Journal of bacteriology, vol. 111, no. 1, pp. 220-230.

Chawla, PR, Bajaj, IB, Survase, SA et al, 2009, 'Microbial cellulose: fermentative production and applications', Food Technology & Biotechnology, vol. 47, no. 2.

Cipolla, L, Polissi, A, Airoldi, C et al. 2011, 'New targets for antibacterial design: Kdo biosynthesis and LPS machinery transport to the cell surface', Current medicinal chemistry, vol. 18, no. 6, pp. 830-852.

Cobb, BA, Wang, Q, Tzianabos, AO et al. 2004, 'Polysaccharide processing and presentation by the MHCII pathway', Cell, vol. 117, no. 5. pp. 677-687.

Cohen, DN, Sham, YY, Haugstad, GD et al. 2009, 'Shared catalysis in virus entry and bacterial cell wall depolymerization', Journal of molecular biology, vol. 387, no. 3, pp. 607-618.

Comstock, LE & Kasper, DL 2006, 'Bacterial glycans: key mediators of diverse host immune responses', Cell, vol. 126, no. 5, pp. 847-850.

Costerton, JW, Cheng, K, Geesey, GG et al. 1987, 'Bacterial biofilms in nature and disease', Annual Reviews in Microbiology, vol. 41, no. 1, pp. 435-464.

Dalziel, M, Crispin, M, Scanlan, CN et al. 2014, 'Emerging principles for the therapeutic exploitation of glycosylation', Science, vol. 343, no. 6166, pp. 1235681.

Delbrück, M 1940, 'The growth of bacteriophage and lysis of the host', The journal of general physiology, vol. 23, no. 5, pp. 643-660.

Dube, DH, Champasa, K & Wang, B 2011, 'Chemical tools to discover and target bacterial glycoproteins', Chemical Communications, vol. 47, no. 1, pp. 87-101.

Erridge, C, Bennett-Guerrero, E & Poxton, IR, 2002, Structure and function of lipopolysaccharides, Microbes and Infection, vol. 4, no. 8, pp. 837-851.

Finne, J, Leinonen, M & Mäkelä, PH 1983, 'Antigenic similarities between brain components and bacteria causing meningitis: implications for vaccine development and pathogenesis', The Lancet, vol. 322, no. 8346, pp. 355-357.

Goon, S, Kelly, JF, Logan, SM et al. 2003, 'Pseudaminic acid, the major modification on Campylobacter flagellin', is synthesized via the Cj1293 gene, Molecular microbiology, vol. 50, no. 2, pp: 659-671.

Grados, O & Ewing, WH 1970, 'Antigenic relationship between Escherichia coli and Neisseria meningitidis', The Journal of infectious diseases, pp. 100-103.

Hershberger, C & Binkley, S 1968, 'Chemistry and Metabolism of 3-Deoxy-d-mannooctulosonic Acid I, STEREOCHEMICAL DETERMINATION', Journal of Biological Chemistry, vol. 243, no. 7, pp. 1578-1584.

Hooper, LV Gordon, JI 2001, 'Glycans as legislators of host–microbial interactions: spanning the spectrum from symbiosis to pathogenicity', Glycobiology, vol. 11, no. 2, pp. 1R-10R.

Howard, SL, Jagannathan, A, Soo, EC et al. 2009, 'Campylobacter jejuni glycosylation island important in cell charge, legionaminic acid biosynthesis, and colonization of chickens', Infection and immunity, vol. 77, no. 6, pp. 2544-2556.

Kang, YS, Do, Y, Lee, HK et al. 2006, 'A dominant complement fixation pathway for pneumococcal polysaccharides initiated by SIGN-R1 interacting with C1q', Cell, vol. 125, no. 1, pp. 47-58.

Khan, H, Flint, S & Yu, PL 2013, 'Determination of the mode of action of enterolysin A, produced by E nterococcus faecalis B 9510', Journal of applied microbiology, vol. 115, no. 2, pp. 484-494.

Knirel, YA, Vinogradov, EV, Shashkov, AS et al. 1986, 'Somatic antigens of Pseudomonas aeruginosa: The structure of O - specific polysaccharide chains of P'. aeruginosa O10 (Lányi) lipopolysaccharides, European journal of biochemistry, vol. 157, no. 1, pp. 129-138.

Latka, A, Maciejewska, B, Majkowska-Skrobek, G et al. 2017, 'Bacteriophage-encoded virion-associated enzymes to overcome the carbohydrate barriers during the infection process', Appl Microbiol Biotechnol, vol. 101, no. 8, pp. 101, 3103-3119.

Lavigne, R, Noben, JP, Hertveldt, K et al., 2006, 'The structural proteome of Pseudomonas aeruginosa bacteriophage Φkmv', Microbiology, vol. 152, no. 2, pp. 529-534.

Loessner, MJ, Kramer, K, Ebel, F et al. 2002,C - terminal domains of Listeria monocytogenes bacteriophage murein hydrolases determine specific recognition and high - affinity binding to bacterial cell wall carbohydrate, Molecular microbiology, vol. 44, no. 2, pp. 335-349.

Lozupone, CA, Stombaugh, JI, Gordon, JI et al. 2012, 'Diversity, stability and resilience of the human gut microbiota', Nature, vol. 489, no. 7415, pp. 220-230.

Moak, M & Molineux, IJ 2000, 'Role of the Gp16 lytic transglycosylase motif in bacteriophage T7 virions at the initiation of infection', Molecular Microbiology, vol. 37, no. 2, pp. 345-355.

Moak, M & Molineux, IJ 2004, 'Peptidoglycan hydrolytic activities associated with bacteriophage virions', Molecular microbiology, vol. 51, no. 4, pp. 1169-1183.

Moxon, E & Kroll, J 1990, 'The role of bacterial polysaccharide capsules as virulence factors, In: Bacterial capsules, Bacterial capsules, pp. 65-85.

Nelson, DC, Schmelcher, M, Rodriguez-Rubio, L et al. 2012, 'Endolysins as antimicrobials', Advances in virus research, vol. 83, pp. 299-365.

Nishima, W, Kanamaru, S, Arisaka, F et al. 2011, 'Screw Motion Regulates Multiple Functions of T4 Phage Protein Gene Product 5 during Cell Puncturing, Journal of the American Chemical Society', vol. 133, no. 34, pp. 13571-13576.

Nizet, V & Esko, JD 2009, 'Bacterial and viral infections, In: Essentials of Glycobiology'. 2nd edition. Cold Spring Harbor Laboratory Press.

Oliveira, H, Azeredo, J, Lavigne, R et al. 2012, 'Bacteriophage endolysins as a response to emerging foodborne pathogens', Trends in Food Science & Technology, vol. 28, no. 2, pp. 103-115.

Oliveira, H, Melo, LD & Santos, SB 2013, 'Molecular aspects and comparative genomics of bacteriophage endolysins', Journal of virology, vol. 87, no. 8, pp. 4558-4570.

Park, JT & Strominger, JL 1957, 'Mode of action of penicillin', Biochemical basis for the mechanism of action of penicillin and for its selective toxicity, Science, vol. 125, no. 3238, pp. 99-101.

Payne, KM & Hatfull, GF 2012, 'Mycobacteriophage endolysins: diverse and modular enzymes with multiple catalytic activities', PLoS One, vol. 7, no. 3, pp. e34052.

Perkins, HR 1969, 'Specificity of combination between mucopeptide precursors and vancomycin or ristocetin', Biochemical Journal, vol. 111, no. 2, pp. 195-205.

Raetz, CR & Whitfield, C 2002. 'Lipopolysaccharide endotoxins', Annual review of biochemistry, vol. 71, pp. 635-700.

Rittig, MG, Kaufmann, A, Robins, A et al. 2003, 'Smooth and rough lipopolysaccharide phenotypes of Brucella induce different intracellular trafficking and cytokine/chemokine release in human monocytes', Journal of leukocyte biology, vol. 74, no. 6, pp. 1045-1055.

Robbins, JB, McCracken Jr, GH, Gotschlich, EC et al. 1974, 'Escherichia coli K1 capsular polysaccharide associated with neonatal meningitis', New England Journal of Medicine, vol. 290, no. 22, pp. 1216-1220.

Roberts, I, Saunders, F & Boulnois, G 1989, 'Bacterial capsules and interactions with complement and phagocytes', Portland Press Limited.

Roberts, IS 1996, 'The biochemistry and genetics of capsular polysaccharide production in bacteria', Annual Reviews in Microbiology, vol. 50, pp. 285-315.

Rodríguez, L, Quiles-Puchalt, N, Martínez Fernández, B et al. 2013, 'The Peptidoglycan Hydrolase of Staphylococcus aureus Bacteriophage ϕ11 Plays a Structural Role in the Viral Particle', Applied and environmental microbiology, vol. 79, no. 19, pp: 6187-6190.

Schirm, M, Soo, E, Aubry, A et al. 2003, 'Structural, genetic and functional characterization of the flagellin glycosylation process in Helicobacter pylori', Molecular microbiology, vol. 48, no. 6, pp. 1579-1592.

Schmelcher, M, Donovan, DM & Loessner, MJ 2012, 'Bacteriophage endolysins as novel antimicrobials', Future microbiology, vol. 7, no. 10, pp. 1147-1171.

Schmidt, MA, Riley, LW & Benz, I 2003, 'Sweet new world: glycoproteins in bacterial pathogens', Trends in microbiology, vol. 11, no. 12, pp. 554-561.

Schulz, EC, Schwarzer, D & Frank, M 2010, 'Structural Basis for the Recognition and Cleavage of Polysialic Acid by the Bacteriophage K1F Tailspike Protein EndoNF', Journal of Molecular Biology, vol. 397, no. 1, 341-351.

Simpson, DJ, Sacher, JC, Szymanski, CM 2015, 'Exploring the interactions between bacteriophage-encoded glycan binding proteins and carbohydrates', Current Opinion in Structural Biology, vol. 34, pp. 69-77.

Singh, A, Arutyunov, D, McDermott, MT et al. 2011, 'Specific detection of Campylobacter jejuni using the bacteriophage NCTC 12673 receptor binding protein as a probe', Analyst, vol. 136, no. 22, pp. 4780-4786.

Smedley, JG, Jewell, E, Roguskie, J et al. 2005, 'Influence of pilin glycosylation on Pseudomonas aeruginosa 1244 pilus function', Infection and immunity, vol. 73, no. 12, pp. 7922-7931.

Stockdale, SR, Mahony, J, Courtin, P et al. 2013, 'The lactococcal phages Tuc2009 and TP901-1 incorporate two alternate forms of their tail fiber into their virions for infection specialization', Journal of biological chemistry, vol. 288, no. 8, pp. 5581-5590.

Storm, DR & Strominger, JL 1973, 'Complex formation between bacitracin peptides and isoprenyl pyrophosphates the specificity of lipid-peptide interactions', Journal of Biological Chemistry, vol. 248, no. 11, pp. 3940-3945.

Suttle, CA 2005, 'Viruses in the sea', Nature, vol. 437, no. 7057, pp. 356-361.

Thumm, G & Gotz, F 1997, 'Studies on prolysostaphin processing and characterization of the lysostaphin immunity factor (Lif) of Staphylococcus simulans biovar staphylolyticus', Molecular Microbiology, vol. 23, no. 6, pp. 1251-1255.

Tsujimoto, H, Gotoh, N & Nishino, T 1999, 'Diffusion of macrolide antibiotics through the outer membrane of

Moraxella catarrhalis', Journal of Infection and Chemotherapy, vol. 5, no. 4, pp. 196-200.

Tzeng, YL, Datta, A, Kolli, VK et al. 2002, 'Endotoxin of Neisseria meningitidis composed only of intact lipid A: inactivation of the meningococcal 3-deoxy-D-manno-octulosonic acid transferase', Journal of bacteriology, vol. 184, no. 9, pp. 2379-2388.

Van Dam, V, Olrichs, N & Breukink, E 2009, 'Specific Labeling of Peptidoglycan Precursors as a Tool for Bacterial Cell Wall Studies', ChemBioChem, vol. 10, no. 4, pp. 617-624.

Vollmer, W, Blanot, D & De Pedro, MA 2008, 'Peptidoglycan structure and architecture', FEMS Microbiology Reviews, vol. 32, no. 2, pp. 149-167.

Walmagh, M, Briers, Y, Dos Santos, SB et al. 2012, 'Characterization of modular bacteriophage endolysins from Myoviridae phages OBP, 201φ2-1 and PVP-SE1', PLoS One, vol. 7, no. 5, pp. e36991.

Weidenmaier, C, Peschel, A, Xiong, YQ et al. 2005, 'Lack of wall teichoic acids in Staphylococcus aureus leads to reduced interactions with endothelial cells and to attenuated virulence in a rabbit model of endocarditis', Journal of infectious diseases, vol. 191, no. 10, pp. 1771-1777.

Whitfield, C 1988, 'Bacterial extracellular polysaccharides', Canadian Journal of Microbiology, vol. 34, no. 4, pp. 415-420.

Whitfield, C Valvano, MA 1993, 'Biosynthesis and expression of cell-surface polysaccharides in gram-negative bacteria', Advances in microbial physiology, vol. 35, pp. 135-246.

Wu, HJ, Wang, AH & Jennings, MP 2008, 'Discovery of virulence factors of pathogenic bacteria', Current opinion in chemical biology, vol. 12, no. 1., pp. 93-101.

Young, R 2013, 'Phage lysis: do we have the hole story yet?' Current opinion in microbiology, vol. 16, no. 6, pp. 790-797.