INTRODUCTION

*Bacillus anthracis* is the etiologic agent that causes anthrax a common disease of livestock and occasionally of humans, which derives its name from the Greek word for coal (anthrax), because of its ability to cause black, coal-like cutaneous eschars (Riedel, 2005). The common name of this pathogen includes "anthrax" and "anthrax bacterium" and it belongs to the *Bacillus cereus* group of strains (Tourasse et al., 2008). *B. anthracis* is a Gram-positive, endospores-forming, rod-shaped bacterium, with a width of 1–1.5 μm and a length of 3–5μm (Mariappan et al., 2012). It can be grown in an ordinary nutrient medium under aerobic or anaerobic conditions. Under ideal conditions, *B. anthracis* organisms are surrounded by a gel-like layer called capsule and the capsule is made up of poly-D-Glutamic acid. This coating protects it from the immune systems of a host animal, and makes it virulent (Fouet, 2009).

Structure of *Bacillus anthracis*

The single chromosome found in the *B. anthracis* genome is a circular, 5,227,293 bp DNA molecule. The vegetative *B. anthracis* cells are Gram-positive therefore they contain an extensive peptidoglycan layer, lipoteichoic acids, and crystalline cell surface proteins (S-layer proteins) (Read et al., 2003). *B. anthracis* differs from other Gram-positive bacteria in that it does not contain teichoic acids and the S-layer proteins are not glycosylated (Sára et al., 2000). Cell wall polysaccharides function in anchoring the protective S-layer to the cell wall. The cell wall polysaccharides are composed of galactose (Gal), N-acetylglucosamine (GlcNAc), and N-acetylmannose (ManNAc) in a 3:2:1 ratio (Carlson et al., 2013). It is one of the few bacteria known to synthesize a protein capsule (poly-D-gamma-glutamic acid). Like *Bordetella pertussis*, it forms a calmodulin-dependent adenylate cyclase exotoxin known as (edema factor), along with lethal factor. The slime layer is a polymer of amino acids (D-
glutamate), unlike most other bacteria which have polysaccharide capsules (Hauschildt et al., 1995). The cells excrete the capsule for protection and virulence. The capsule and the S-layer are compatible, but they can both be formed independently (without the presence of the other) (Bottone, 2010).

**Surface proteins of Bacillus anthracis**

Surface proteins of Gram-positive bacteria perform critical biological functions that are required for the colonization of host tissues, the evasion of immune defenses, and the acquisition of nutrients; they promote bacterial adhesion to specific tissues and invasion of host cells; and provide resistance to phagocytic killing (Nobbs et al., 2009). It often plays an essential role in virulence and also plays a crucial role in the pathogenesis of *B. anthracis*. One group of surface proteins that is commonly found in gram-positive bacteria consist of proteins that are attached to the peptidoglycan by a family of proteins known as sortases (Marraffini et al., 2006). Such surface proteins contain an N-terminal signal peptide and a C-terminal sorting signal that contains the motif LPXTG (or a slight variation thereof) that is recognized by the sortase (Zink et al., 2005). Sortases are transpeptidases that are anchored in the membrane of the bacteria via an N-terminal hydrophobic leader peptide. They cleave the substrate protein after the threonine residue of the LPXTG motif and, through a nucleophilic attack of the amino group of the lipid II peptidoglycan precursor, link the protein to the cell wall (wu et al., 2010). Loss of sortase activity results in improper localization of the LPXTG-containing surface proteins with resulting loss of function and can lead to an attenuation of virulence (ahn et al., 2008).

**Sortase for Anti-Infective Therapy**

Sortase, a prominent virulence factor in Gram-positive pathogens, acts as a target for the development of anti-infectives. The inhibition of virulence factors as a therapy for bacterial infections still represents a theory that requires experimental testing and proof-of-
principle (Grigg et al., 2007). To date, the search for inhibitors of sortases has involved natural, synthetic, and high-throughput screening methodologies (Alksne et al., 2008). Sortase, a major virulence factor responsible for the covalent attachment of surface proteins to the cell wall, is a target candidate for the treatment of Gram positive infections (Scott et al., 2006). Compounds with reasonable inhibition, specificity, and mechanisms of inactivation have been uncovered. In a continuous struggle to stay ahead of the ever-changing drug resistance traits of pathogenic bacteria, the selective inhibition of virulence factors may prove to be an effective therapeutic strategy that may either augment or perhaps even supplant the more traditional approaches (Barczak et al., 2009). Inhibition of the cell wall anchoring of surface proteins emerges as a possible target for the development of therapeutics against these pathogens (Mazmanian et al., 2010).

Class of Sortase Enzymes

Sortases have been classified into six groups (sortase classes A–D) on the basis of sequence homology, the substrate for sortase cleavage and the nucleophile accepted by the sortase and also sometimes class E and F enzymes whose members have been shown experimentally to have distinct functions (Marraffini et al., 2006). Class A enzymes, SrtA is responsible for the cell wall anchoring of proteins that are involved in bacterial adhesion, immune evasion, internalization, or function as receptors for phage binding (Proft et al., 2009). Class B SrtB enzymes anchor proteins to the cell wall envelope that are specifically involved in iron acquisition (Mazmanian et al., 2002). Class C SrtC enzymes assemble pili on the surface of Gram-positive bacteria (Hendrickx et al., 2011), whereas class D sortases anchor proteins to cell wall peptidoglycan as bacilli or streptomyces engage in sporulation, a developmental program that generates dissimilarly sized daughter and mother cells (Marraffini et al., 2007).
Figure 1: Phylogenetic tree showing the relationships among the six classes of sortases from Gram-positive bacteria

Instead of a class A enzyme, some high G + C bacterial species may use a class E enzyme as their housekeeping sortase (Scott et al., 2006). Comparative genome analyses suggest that class E enzymes recognize an LAXTG sorting signal, instead of the canonical LPXTG motif processed by class A enzymes (Spirig et al., 2011). In Streptomyces coelicolor, class E enzymes display chaplin surface proteins containing an LAXTG sorting signal, which presumably mediate aerial hyphae formation (Ton-That et al., 2004). Class F enzymes are present in S. coelicolor and other Actinobacteria, but their functions have yet to be explored (Spirig et al., 2011). Moreover, unlike class D enzymes, genes encoding class E enzymes are not genomically clustered near their substrates (Dramsi et al., 2008).

Sortase A enzymes
The first sortase was identified in *Staphylococcus aureus* by Olaf Schneewind and colleagues and named SrtA, for surface protein sorting A (PSkaar *et al.*, 2004). Sortase enzymes decorate the surfaces of Gram-positive bacteria with a diverse array of proteins that enable each microbe to effectively interact with its environment. They either ‘sort’ proteins to the cell surface by covalently joining them to the cell wall, or polymerize proteins to construct pili, multi-subunit hair-like fibres that extend from the cell surface to promote bacterial adhesion. SrtA substrate proteins contain a C-terminal sorting signal consisting of a conserved LPXTG motif followed by a hydrophobic domain and a positively charged tail (Cabanès *et al.*, 2002). After binding to its substrate, SrtA cleaves the peptide bond between the threonine and the Gly residues within the LPXTG motif and catalyses the formation of an amide bond between the carboxyl group of threonine and the amino-group of the pentaglycine cross-bridge in the peptidoglycan cell wall (Ton-That *et al.*, 1999). Nearly all bacteria in the Firmicutes subfamily contain a single class A sortase whose primary sequence is most closely related to the prototypical *Sa*-SrtA enzyme (Robson *et al.*, 2012).

Sortase class A (SrtA) enzymes are present in all Gram-positive bacteria and are often referred to as housekeeping sortases. Sortase class A substrates include surface proteins from the microbial surface components recognizing adhesive matrix molecules (MSCRAMM) family that have been implicated in the virulence of multiple Gram-positive bacterial species (Hendrickx *et al.*, 2011). All SrtA homologs contain appropriately positioned active site residues (SrtA residues H120 and C184) and transmembrane segments, and their genes are frequently clustered with genes encoding CWS containing proteins (Comfort *et al.*, 2004). Class A enzymes have attracted significant interest as potential drug targets because a number of clinically important pathogens use these sortases to display virulence factors and they are attenuated in their virulence if their SrtA gene is eliminated (*S. aureus*, *L. monocytogenes*, *Streptococcus pyogenes* and *Streptococcus pneumoniae* among others) (Frankel *et al.*, 2007).
Figure 2: (I) SrtA intended for cell wall anchoring are first initiated into the Sec pathway by an N-terminal signal peptide (LPXTG: X indicates potential points of inhibition in the “sorting pathway”). (II & III) Thioester-linked acyl-enzyme intermediate is formed between sortase and C-terminal threonine of surface protein. (IV) Acyl-enzyme occurs through a nucleophilic attack of the amino group of the pentaglycine of lipid II to generate lipid II-linked surface protein. (V) Cell wall incorporation through the progress of SrtA transpeptidation. The black tail represents the hydrophobic domain, followed by the positively charged tail of cell wall sorting signals.

SrtA harbours an N-terminal hydrophobic segment that functions as a signal peptide for secretion and as a stop transfer signal for membrane anchoring. Membrane localization of sortase was confirmed experimentally after immunoblot analysis of S. aureus sub-cellular fractions. The enzyme adopts a type II membrane topology, with the N terminus inside the cytoplasm and the C-terminal enzymatic portion located across the plasma membrane. SrtA is a founding member of this family of sortases (Hempel et al., 2010).

SrtA as “Universal Drug target”

SrtA catalyses the anchoring of surface proteins to the bacterial peptidoglycan and a cell wall sorting reaction, in which a surface protein with a sorting signal containing a
LPXTG motif, is cleaved between the Thr and Gly residue (Ton-That et al., 2000). It specifically works to establish an interaction between bacteria and host cells and is essential for pathogenesis. This makes SrtA, a potential suitable target for inhibition of gram positive pathogens, in order to treat bacterial infections (Egan et al., 2010). It serves as receptor structure for invading the phage protein interactions. By inhibiting the function of this enzyme it would be possible to target bacterial virulence without targeting its viability directly (Supuran et al., 2001). By inhibiting bacterial virulence it might be possible to treat infections with less selective pressure and therefore with a potentially decreased risk of resistance development (Fernebro, 2011).

**SrtA mechanism in *B. anthracis***

*B. anthracis* contains class A and D sortase enzymes that recognize closely related sorting signals; both BasH and BasI contain a LPNTA sorting signal that differs only slightly from the canonical LP[A/N/K]TG sorting signal present in proteins anchored by *B. anthracis* SrtA (Comfort et al., 2004). *B. anthracis* SrtA structure indicates that an N-terminal extension may affect how lipid II is recognized. Furthermore, SrtA is unable to anchor an LPETG peptide to m-DAP, to which the cell wall sorting signal proteins are bound (Aucher et al., 2011). In contrast to what is observed in other sortases, *B. anthracis* sortases may require additional protein components or larger portions of the lipid II to catalyze the transpeptidation reaction (Khare et al., 2011).

**Structure of SrtA**

NMR and X-ray crystallography of *S. aureus* sortase A revealed that the enzyme adopt a unique eight stranded β-barrel, which contains several short helices and loops. The active site is located in a hydrophobic depression formed by two β-strands, features that are preserved in SrtB from *S. aureus* and *B. anthracis* (Ilangoyan et al., 2001).
Two conserved residues, His120 and Arg197, are positioned in close proximity to the active site sulphhydril of Cys184. Calcium ions stimulate *S. aureus* SrtA activity 8-fold but have little or no impact on the activity of *S. aureus* SrtB or *B. anthracis* SrtA, SrtB, or SrtC (Zong *et al.*, 2004). Calcium concentrations required for stimulation of SrtA activity are found under physiological conditions in host tissues, and these ions are involved in structural rearrangements of a disordered loop covering the active site that enable substrate binding (Melvin *et al.*, 2011). Interestingly, sortase also forms dimers *in vitro*, a function that may stimulate catalysis. The SrtA enzyme from *B. anthracis* (*Ba*-SrtA) is 210 amino acids in length and consists of two parts, a non-polar N terminus that presumably embeds the protein...
in the membrane (residues Met1–Gly23) and a C-terminal catalytic region (residues Lys24–Lys210). The NMR structure of Ba-SrtA contains an N-terminal tail that forms numerous contacts to the active site histidine (His126). The tail is formed by residues that precede strand β1 and its positioning within the active site has not been previously observed in other sortase structures.

The N-terminal tail in Ba-SrtA may be required for later steps in the transpeptidation reaction involving lipid II. An understanding of the binding site pocket and substrate recognition mechanism by SrtA enzymes may serve to be beneficial in the rational development of sortase inhibitors. Ba-SrtA contains several unique active site features that include the presence of an N-terminal extension that contacts the catalytically essential Histidine and a large structurally disordered active site loop. The sorting signal binding pocket in Ba-SrtA is ordered and rigid in the apo-state and therefore presumably only needs to undergo minimal structural changes to recognize the sorting signal. B. anthracis encodes three sortase enzymes: Ba-SrtA, Ba-SrtB, and Ba-SrtC. The Ba-SrtB enzyme is involved in iron acquisition and anchors the heme-binding IsdC protein to the cell wall. Ba-SrtC, which is a SrtD-type enzyme, anchors two proteins required for proper spore formation. The Ba-SrtA enzyme is a SrtA type sortase that attaches seven proteins to the cell wall by joining the Threonine of the C-terminal LPXTG sorting signal to the amine group of meso-diaminopimelic acid (m-Dap) within lipid-II (Weiner et al., 2010). Ba-SrtA is a potential target for new therapeutics as it is required for B. anthracis survival and replication within macrophages, a presumed early step in the development of inhalation anthrax (Barczak et al., 2009). An understanding of the binding site pocket and substrate recognition mechanism of SrtA enzymes may serve to be beneficial in the rational development of sortase inhibitors. It facilitates the further development of these molecules into useful anti-infective agents to treat
infections caused by *S. aureus*, *B. anthracis*, and other Gram-positive pathogens (Hajishengallis *et al.*, 2008).

**Specificity of SrtA in B. anthracis**

Lytic enzymes like plyG, plyL from the phage, are particularly targeting this SrtA enzyme and inhibiting the *B. anthracis* (Missiakas *et al.*, 2005). This particular enzyme is additionally plays the vital role in biofilm formation and pili formation. Inhibition of SrtA from microbes will be deficient in microbial and biofilm formations (Hu *et al.*, 2010). In the year of 2002, Schuch R *et al.*, has reported “A bacteriolytic agent that detects and kills *Bacillus anthracis*” and exploited the inherent binding specificity and lytic action of bacteriophage enzymes called lysins for the rapid detection and killing of *B. anthracis*, which proved that γ phage derived plyG enzymes are having the ability to target the *B. anthracis* and kill it (Schuch *et al.*, 2002). Most *B. anthracis* strains are sensitive to γ phage, but most *B. cereus* and *B. thuringiensis* strains are resistant to the lytic action of γ phage. In the year of 2005 Davison *et al.*, has reported the “Identification of the Bacillus anthracis γ Phage Receptor” and from that plyG enzyme binding with surface protein namely SrtA is confirmed (Davison *et al.*, 2005).

**Antimicrobial peptides**

Antimicrobial peptides (AMPs) or host defence peptides are evolutionarily highly conserved components of the innate immune system and are produced by all complex organisms (Izadpanah *et al.*, 2005). Their importance in host defence is indicated in plants and insects which live in non-bacteria free environments without the ability to produce lymphocytes and antibodies. In humans and other mammals, the significance of peptides in host defence are especially demonstrated by the low risk of infection in the cornea of the eye where they serve as a first line of defence like they do throughout the human body, e.g. in
epithelia cells of human colon mucosa (Lahov et al., 1996) and at the skin surface due to sweat glands peptide secretion (Murakami et al., 2002).

**Figure 4:** SrtA mechanism is inhibited by means of plyG enzyme and here SrtA plays the receptor for phage proteins.

They are also found in large amounts in granulocytes where they are part of degranulation (Destoumieux et al., 2000). AMPs are polypeptides of usually 9-50 amino acids of which the majority of them, due to the positively charged amino acids arginine and lysine, are cationic with an overall charge of +2 to +9 (Teixeira et al., 2012). Hydrophobic
residues contribute to ≥30% of the peptide which gives the peptides an amphipathic nature with the clustering of cationic and hydrophobic amino acids into distinct domains (Dathe et al., 1999). Many antimicrobial peptides have a wide range of activities and they often have broad spectrum antimicrobial activity and some even kills multi drug resistant bacteria at low concentrations (Marr et al., 2006). They were originally known for their antifungal, antiviral, antiparasitic and antibacterial properties (Hancock et al., 2000). Most antimicrobial peptides disrupt the bacterial cell wall by forming pores (Figure 3) and therefore many AMPs show highest activity against gram-positive bacteria (Hancock et al., 2000). Some AMPs target lipid II or other cell wall biosynthetic processes to disturb peptidoglycan synthesis and translocation, other targets are DNA, transcription, translation, replication and essential enzymatic activity (Nguyen et al., 2011). There is evidence showing that antimicrobial peptides are not only involved in direct antimicrobial action but also serve as immunomodulatory peptides, functioning as chemokines and/or inducing chemokine production, inhibiting LPS induced pro-inflammatory cytokine production, promoting wound healing, and modulating the responses cells of the adaptive immune response (Lai et al., 2009). Due to the importance of anti microbial peptides, researchers had much interest in developing the AMP from the source of plant, bacteriophages and via synthetic approaches also (Meng et al., 2010). Peptides isolated from bacteriophages will serve as best anti microbial agents because of high virulence property and application of targeting host and not humans (Amiche et al., 2011).

**Sortase Inhibitors**

Inhibitors of sortase should be useful for the characterization of this fascinating enzyme. However, can such inhibitors affect the outcome of human or animal infection with *S. aureus*. 
Figure 5: Mechanism of SrtA inhibitors blocking the interactions between SrtA and LPXTG binding motif in Gram positive pathogen.

The reported research clears the virulence factors with SrtA mutants provide a correlate for the contribution of SrtA to disease, and to be hopeful that inhibitors of the sortase reaction may display therapeutic effects (Marraffini et al., 2006). Moreover, as sortase is a universal virulence factor of gram-positive pathogens, compounds that inhibit the enzyme’s activity could constitute antimicrobial agents for the treatment of many diseases, such as enterococcal and pneumococcal infections (Kreikemeyer et al., 2011).

Sortases anchor surface proteins to the cell wall of Gram-positive pathogens through recognition of specific motif sequences. Loss of sortase leads to large reductions in virulence, which identifies sortase as a target for the development of antibacterials (Maresso et al., 2007). The first search for sortase inhibitors occurred even before the enzyme was identified. Methane-thiosulfonates such as MTSET and (2 sulfonatoethyl) methane-thiosulfonate inhibit sortase in vivo and in vitro, with MTSET achieving complete inhibition. The mercurial p-hydroxymercuribenzoic acid could also inhibit sortase. All of these compounds react with the catalytic Cys184 and prevent formation of acyl intermediates (Marraffini et al., 2006).
In contrast, sulfhydryl alkylating agents like iodoacetamide, N-ethylmaleimide, or iodoacetic acid have not inhibited sortase. While these reagents proved useful to elucidate the catalytic mechanism of the enzyme, non-discriminate interactions of thiol-reactive molecules renders these compounds useless for therapeutic studies because of their associated toxicity in mammalian organisms. The first inhibitors of the cell wall sorting reaction were discovered even before sortase was identified (Mazmanian et al., 2001). At that time, it was known for S. aureus that surface proteins are linked through an amide bond to the amino group of pentaglycine cross bridges (Marraffini et al., 2006). The similarity of this mechanism to the transpeptidation reaction of cell wall biosynthesis led to the investigation of antibiotics as inhibitors of the sorting pathway. Penicillin, an inhibitor of the transpeptidation reaction of cell wall synthesis, had no effect on the generation of mature anchored surface proteins; however, vancomycin and melenomycin reduced surface protein anchoring (Ton-That et al., 2004). As vancomycin and melenomycin both target steps in the lipid II biosynthesis cycle, it was presumed that lipid II may be a substrate for the sorting reaction. Interestingly, compounds that are reactive with sulfhydryl groups, such as methanethiosulfonate, i.e., [2-trimethylammonium] ethyl] methanethiosulfonate, were also inhibitors (>2 log units) of surface protein anchoring (Maesso et al., 2008). Suree et al., has synthesized a chemical library of rhodannine, pyridazinone, pyrazolethione and tested for activity against the Staphylococcus aureus and Bacillus anthracis. Additionally, he suggested that these derivatives may have the effect of surviving as the common SrtA inhibitors for gram positive pathogens (Suree et al., 2009).

Role of SrtA in microbial and Biofilm formation

Bacteria can live in two disparate ways: as single, free-floating cells (planktonic) or in sessile aggregates, so-called biofilms where the bacteria live in organized communities. The production of a biofilm originates with the initial adherence of the bacteria to a surface
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(Fischer, 2003). Here, the bacteria are embedded within a self-produced matrix of extracellular polymeric substance (EPS), which mainly contain polysaccharides, nucleic acids, lipids and proteins. Biofilms consist mostly of EPS, 90%, whereas the cells account for only 10% (Flemming et al., 2010). The formation of a biofilm gives the bacteria several advantages: it immobilizes the cells while maintaining a comfortable architecture allowing the cells to communicate; it creates a reservoir of nutrients from lysed cells including DNA, which makes horizontal gene transfer more likely to occur of most clinical importance, it also protects the cells from the surrounding such as host immune defence, many antibiotics, ultraviolet radiation and oxidizing or charged biocides (Ishino et al., 2013). The biofilm mode of life is a central infection mechanism and is recognized as the causing or exacerbating feature in many medical infections including dental caries, nosocomial infections, pneumonia, cystic fibrosis, urinary tract infections, and infections related to catheters and medical implants (Lebeaux et al., 2013). According to the US National Institutes of Health, biofilms are medically important and they estimate biofilms to account for 80% of human bacterial infections (Huigens et al., 2007).

Some of the advantages of biofilms are that they aid to overcome immune system and create resistance towards antibiotics (Hall-Stoodley et al., 2004). When the bacteria are organized in a biofilm they are up to 1,000 times less susceptible to antimicrobial agents compared to the planktonic state (Ramage et al., 2004). A lower metabolic/slow growing rate (persisters), difficult penetration of biofilm matrix, up regulation of efflux pumps and stress response regulons are often mentioned as the reason. The clinical consequences are that biofilm infections often develop into a chronic infection which is difficult to eradicate (Vlastarakos et al., 2007). Due to the lack of discovery of new and effective antibiotics, and because biofilms are regarded as a deterrent for chronic infections and decrease of antibiotic susceptibility, many alternative strategies have been focusing on reducing biofilm formation.
in infections and preventively incorporate antibiotics into in-dwelling medical devices (Fernbro, 2011). There are numerous ways to inhibit biofilm formation but when it comes to an infection where the biofilm has already formed, quorum sensing might turn out to be the most strategic advantageous mechanism to inhibit (Cegelski et al., 2008). Recently, researchers have identified the importance of SrtA in biofilm formation. Deletion or mutation in SrtA gene leads to absence of biofilm growth. So that, SrtA enzyme is considered to be an important target for the inhibition of biofilm formation (Muñoz-Elías et al., 2008).

**SrtA in target for Biofilm Inhibition**

Microbial life in the natural environment commonly exists as biofilms and rarely as planktonic growth. Biofilms develop when microorganisms attach to a surface and encase the community of cells in an extracellular environment. It is also apparent that bacteria within the population behave as a cooperative and coordinated community of cells (Liu et al., 2007). Biofilm development causes immense problems for industrial water systems, human health and for the shipping and food industries. Biofilm related issues are often intensified by the inherent nature of biofilms and their resistance to antimicrobials (Rosche et al., 2009). The recent emergence and spread of multi-resistant micro-organisms and refractory biofilm-induced infections have prompted an intense search for novel antibiotics that inhibit pathogenic microorganisms through novel targets (Scheie et al., 2004). Infections once being easy to cure with antimicrobials are now becoming difficult, and sometimes even impossible to treat due to multidrug resistance (Towner, 2009). The antimicrobial used today were developed and generally tested against planktonic microorganisms in the laboratory, disregarding the increased resistance against antimicrobials in microorganisms living in biofilms (Neut et al., 2007). Recently, the role of the sortase gene (SrtA) in monospecies biofilm development and observed that inactivation of SrtA caused a shrink in biofilm formation. Genes encoding three putative sortase-dependent proteins are found to be up-
regulated in biofilms versus planktonic cells and mutations in these genes resulted in reduced biofilm biomass (Lévesque et al., 2007). So, that researcher is targeting this particular enzyme for inhibition of biofilm formation in the gram positive bacteria.

**Drug delivery systems in Microbial inhibition**

Anti-bacterial drugs work by exploiting differences between mammalian and microbial physiologic processes. Since bacteria, unlike mammals, have cell walls, many anti-bacterial agents work by binding to components of the cell wall or inhibiting the synthesis of the cell wall (Finberg et al., 2012). The predictable view of antibiotic resistance is one where bacteria display extensively reduced susceptibility to antimicrobials in laboratory tests by mechanisms such as altered drug uptake, altered drug target and drug inactivation (Smith, 2005). Whilst these mechanism undoubtedly make a major contribution to antibiotic failure in the clinic, the phenomenon of clinical failure in spite of sensitivity in laboratory test is also well recognized (Wu et al., 2009). Growth as a biofilm, more or less always leads to a considerable shrink in vulnerability to antimicrobial agents compared with culture grown in suspension and, whilst there is no usually agreed mechanism for the resistance of biofilm bacteria (Freebairn et al., 2013). A number of elements in the process of biofilm formation have been studied as targets for novel drug delivery technologies. These consist of highlight towards targeting the bacterial attachment and biofilm development as well as incorporation of antimicrobials - again to prevent colonization. Electrical approaches have been used either to release antimicrobial from device surface or to drive antimicrobial through the biofilm (Smith, 2005). Similarly, focused on biofilms include aerosolized delivery of antibiotics to the polymer-based vehicles and enhance the drug activity (Tamilvanan et al., 2008).

Many polymer-based carrier systems have also been proposed, including those based on biodegradable polymers such as poly (lactide-co-glycolide) as well as thermo reversible hydro gels. Cyclodextrins (CDs) are cyclic oligosaccharides made up of D-(+) glucopyranose
units attached by α- (1, 4) glucosidic bonds, containing a relatively hydrophobic central cavity and hydrophilic outer surface. The commonly available forms of these ring-shaped molecules are α, β and γ-CDs having 6, 7 and 8 glucose units, respectively (Shanmugapriya et al., 2014). In addition to their well-known effects on drug solubility and dissolution, bioavailability, safety, and stability, their use as excipient in drug formulation makes cyclodextrins as important drug delivery polymer or drug delivery vehicles (Brewster et al., 2007).