Introduction
1. INTRODUCTION

Infectious diseases are the leading cause for the increase in death rate worldwide. The causative antimicrobial resistant pathogens threaten public health worldwide as they reduce the effectiveness of treatment and increases morbidity, mortality, and health care costs. The problem of antibiotic resistance is a complex one which involves the interaction of multiple factors, which depend on the particular organism, the drug being used, and the environment. Despite the introduction of newer and most efficient antibiotics, a number of infectious diseases are still broadly endemic because of continual and large supply of the causative infectious agents that keep the diseases active and alive. One of the reasons could be our late recognition of the nature and role of biofilms and they are defiance to antimicrobial agents and the controlling curative medicines are yet to be developed.

Biofilms are the rationale for over 80 percent of microbial infections and treatment related infections in the body. Biofilm is a community of microorganisms encased in self synthesized extracellular matrix and is adherent to solid biologic or non-biologic surface. Many biofilms are sufficiently thick to be visible to the naked eye. So these microbial communities were among the first to be studied by the late experts developing science of microbiology. The father of microbiology Anton Van Leeuwenhoek himself laid foundation for the study on these biofilms. He scraped the plaque biofilm from his teeth and observed the so called “animalculi”, components of this complex microbial community with his primitive microscope. Examples of biofilms are great in number, ranging from the plaque on our teeth, the slippery slime on river stones, and the gel-like film on the inside of pot holding water for a week, in the gut linings of animals etc. The organisms’ which prefer biofilm modes of life are primarily responsible for a variety of persistent infections that respond poorly to conventional antibiotic chemotherapy. The failure of conventional culture
techniques to predict antibiotic susceptibilities of these communities explains the part of our letdown to eradicate biofilm-related infections.

Biofilm-related infections are substantial but are largely in the unaddressed area of need in the treatment and management of infectious and chronic diseases in medical field. New approaches are required to diagnose and treat these persistent infections effectively that are unmanageable to conventional antimicrobial therapies. Prevention or control of biofilm mediated diseases cannot be achieved by reliance only on current methods and models. The future treatment should target at small groups of organisms, single species or at key virulence factors they produce. Hence undoubtedly, understanding bacteria in biofilms is a step ahead in preparing for the future.

Though biofilm formation is a mode of primitive survival mechanism, all the organisms are not forming biofilms. It is also clear that microorganisms undergo profound changes during their transition from planktonic (free-swimming) organisms to cells that are part of a complex, surface-attached community. These changes are reflected in the new phenotypic characteristics developed by biofilm bacteria and occur in response to a variety of environmental signals. Therefore it should be studied morphologically, biochemically and should be concentrated on the genetic characters of phenotypically distinct populations within biofilms and not the planktonic microbial population because the later one may help in the quick identification, diagnosis and management of infections caused by these biofilm communities.

Biofilms are associated with both biotic and abiotic surfaces. Organisms which have biofilm forming abilities adhere to a privileged site where they build protective structures. Once established, biofilms are too difficult to eliminate from those surfaces. Formation of a biofilm begins with the attachment of free-floating microorganisms to the surface. This attachment and development is more rapid in the surfaces, containing sufficient moisture and nutrient supply. Tissue surface such as teeth and intestinal mucosa which are constantly bathed in a rich aqueous medium
rapidly develop a complex aggregation of these biofilm microorganisms. Microbes readily form biofilm on abiotic surfaces like medical devices such as urinary catheters, contact lenses, prosthetic heart valves, central venous catheters, intrauterine device, dental unit, water lines etc., resulting in acute and chronic diseases.

Better understanding of the biofilm formation and development may help in intervene and disrupt their establishment. Biofilm development occurs in a number of stages which include surface conditioning where a clean surface contacts with water and organic molecules adhere to surface. These organics neutralize the surface charge which may otherwise be repulsive to an approaching bacterium. Next stage is adhesion of "pioneer" bacteria in which planktonic bacteria attach themselves by electrostatic attraction and physical forces. Bacterial adhesin, the microbial surface component recognizes adhesive matrix molecules like protein, glycoprotein or polysaccharide receptors on the attaching surfaces and other cell types. Genes encoding these adhesions can be used for the quick identification and differentiation of biofilm forming organisms.

After attachment, the participating bacteria synthesizes extracellular polymers which consist of charged and neutral polysaccharide groups that not only cement the cell permanently to the adhering surface but also act as an ionic exchange system for trapping and concentrating trace nutrients from the circulating fluid. As nutrients accumulate, the pioneer cells reproduce. The daughter cells then produce their own exopolymers, greatly increasing the volume of ion exchange surface. Then soon a thriving colony of bacteria is established. In a matured biofilm, most of the volume (75 – 95 %) is occupied by the loosely organized polysaccharide matrix filled with water. This watery slime makes biofilm — covered surfaces gelatinous and slippery, which attracts secondary colonizers. They metabolize waste from the primary colonizers.
The exact structure of any biofilm is probably a unique feature of the single environment in which it develops and the microflora producing it. Exopolysaccharides play a crucial role not only in the formation of biofilms, resistance to antimicrobials but also enhances virulence of the pathogens by playing an immunoprotective role. They are the large components of bacterial biofilms, their contribution to biofilm structure and function has been examined for limited number of pathogens. Numbers of studies are also available related to the existence of biofilm specific polysaccharides. They also behave as chemical weapons in order to defend the producing organisms against disinfectants and antibiotics, phagocytes and our immune system. They also provide effective resistance for biofilm cells against large molecules such as antimicrobial proteins like lysozyme, complement, antimicrobial peptides, defensin and against their analogs. This negatively charged exopolysaccharide is effective in protecting cells from positively charged aminoglycoside antibiotics by restricting their permeation, possibly through binding. Genes encoding exopolysaccharide plays an important role in safeguarding the bacterial population from antibacterial agents, adverse environmental factors, phagocytosis, and immune response. That's why these exoploysaccharide and encoding genes are promising targets for early identification and drug development aimed at combating these infections.

Most bacteria, and virtually all of the problematic multi-resistant nosocomial strains, have been shown to produce biofilms under the appropriate circumstances. According to a recent public announcement from the National Institutes of Health, "more than 60% of all microbial infections are caused by biofilms". Nowadays these biofilm forming organisms are intensively studied in clinical settings due to their increased resistance against most antibiotics which leads to chronic, untreatable infections and epidemics. Even strains that may be highly susceptible to antibiotics when grown in planktonic cultures may be quite resistant when grown in a biofilm. Further the biofilm mode-of-growth represents a major risk factor since they play a triple role in the spread of antibiotic resistance. First, the treatment of biofilm-related infections requires long-term (and often recurrent) antibiotic
therapy, exposing colonizing bacteria to prolonged antibiotic selection pressure. Second, biofilm physiology enables embedded bacteria to survive antibiotic exposure long enough to acquire specific resistance to the drug. Subinhibitory antibiotic concentrations induce the production of biofilm matrix and further promote biofilm survival. Finally, the high cell density, increased genetic competence and accumulated mobile genetic elements within biofilms provide an ideal stage for efficient horizontal gene transfer. Clearly, these biofilms, with high overwhelming numbers of morbidity and mortality, warrants means of current antibiotic therapy improvement. So the development of effective agents in these areas will serve as an important medical need and should find markets to be financially rewarding.

Apart from a number of side effects, the antibiotic treatment often leads to the bacterial imbalance which may invite secondary infections with other organisms. Infections caused by multidrug resistant bacteria cannot be treated by antibiotics combined with the most negligible discovery rate of novel antibiotics which forced the exploration of alternative avenues. Consequently there is an urgent need to develop novel therapeutic agents with effective delivery system to treat the newly forming and already established biofilms.

In selecting antimicrobial agents for the prevention, treatment and control of any diseases, the factors like (1) Specificity, (2) Efficacy, (3) Substantivity, (4) Safety, and (5) Stability need to be considered. Using these criteria, several antibiotics and antiseptics have been evaluated in recent years for controlling biofilm related diseases and device associated infections. But the outcome has not fulfilled the expectations. Hence a detailed study on developing a novel type antimicrobial agents controlling biofilm is the need of the hour.

Biofilm bacteria may be 150 – 3000 times more resistant to free chlorine than free-floating bacteria. In order to destroy the cell responsible for forming the biofilm, the disinfectant must first react with the surrounding polysaccharide network. The cells themselves are not actually more resistant,
rather they have a protective shield around them. The disinfectants oxidizing power can be used up before it reaches the cell. In fact, the biofilm bacteria often produce more exopolymers after biocide treatment to protect themselves further. Moreover these disinfectants are active against actively dividing and metabolizing organisms where as biofilm community comprises mostly sessile organisms. Thus the use of disinfectants is a questionable one.

The prognosis does not look good for the immediate future. Too little is known about persisters to suggest ways to eradicate them. Knowing where to look for the cause of biofilm resistance, however, is a good place to start. Genes responsible for persistence can be identified and used as a target for drug discovery. Any inhibitor of a factor that causes persistence could then be combined with conventional methods to eradicate a biofilm. This approach could decrease the probability of colonization. This analysis of options suggests that the development of a universal antibiofilm therapy, possibly on the basis of targeting of persister proteins, is a long term project, yet a possible simple solution to biofilm infection that follows directly from the dynamics of in vitro biofilm eradication.

Some antimicrobial agents are extremely irritant and toxic and there is much interest in finding ways to formulate new types of safe and cost-effective biocidal materials.

Phage therapy could replace the use of antibiotics and other antimicrobial agents since they specifically attack the target organisms. Bacteriophages endolysins specifically degrade the peptidoglycon of their host cell wall, thus lysing the bacteria. A number of studies previously conducted revealed the therapeutic potential of phages against pathogenic organisms. In addition, the cost of developing a new phage system is cheaper than that of developing new antibiotics. The phage system is a self replicating system which requires one time or limited application. Although phages have been proposed as a means of destroying or controlling biofilms, the technology for this has not yet been successfully developed. Therefore in the present study an attempt has been made to eliminate biofilm of the selected
isolates using specific phages for \textit{in vitro} comparison with other agents developed.

New sources, especially natural products from plants, are being investigated for the control of many types of acute and chronic disease causing microbes. Plant wealth by virtue of their antimicrobial potential can be greatly harnessed for their therapeutic potential and medicinal efficacy to control infections caused by biofilm forming organisms. The higher plants have been the source of medicinal agents since the early days. Consequently the history of medicinal plants dates back to the origin of human civilization on earth. People have used plants for millennia and vast information of the medicinal uses of plants has, therefore accumulated especially in the tropical parts of the world. Also in recent years, owing to the fear of side effects of common drugs over the counter medicines, there has been huge upsurge in people preferring to use more and more natural plant product for preventing and treating serious ailments worldwide. Biologically active compounds have made several plants useful for man since ancient times as spices or medicines, and they still continue to be an inspiration and important source of new medicines for pharmaceutical industry. Plant-derived compounds are known to have a variety of beneficial effect on the human body. India is sitting at the top of gold mine of biodiversity and the vast traditional knowledge of utilizing those makes an ideal place for pharmaceutical research. Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that, on average, two or three antibiotics derived from microorganisms are launched each year. Second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics.

The biodiversity of medicinal plants of India makes them rich sources of leading compounds for the development of novel therapeutic drugs. Thus,
it is imperative to search for more useful phytochemicals before we lose our herbal resources with the upward march of industrialization.

Nanotechnology is the science and manipulation of matter in the range of 1–100 nm. Nanotechnology has moved quickly from the realm of science fiction into clinical research. Pharmaceutical and biotechnology companies and government agencies are beginning to explore and test a variety of applications of nanotechnology in medicine (or nanomedicine). Industry analysts expect that within the next five years, nanotechnology will augment diagnostic testing and drug delivery. In ten years, it may be used in artificial biological structures for tissue repair and remodeling. Silver antimicrobial agents have been pursued as an alternative strategy for reducing bacterial adhesion and to prevent biofilm formation. Since the organisms undergo simultaneous mutation in every critical function within a single generation, the resistance development against silver is very rare. Numbers of reports were available on synthesizing nanoparticles using silver compounds. Previous studies have shown that antimicrobial formulations in the form of nanoparticles could be used as effective bactericidal materials.

Nanocomposites prepared with silver particles are recognized to have potential applications and to create surfaces resistant to bacterial adhesion and colonization; several methods of incorporating silver on medical devices have been described. Microlatice also become an optimal vehicle to deliver active agents to the affected site over an extended time. Nanoparticles tenaciously adhere to dozens of surfaces but not to each other. So they can be applied to control biofilm formation on medical devices. There is also an evidence for a long lasting biological activity of silver nanoparticles. Antimicrobial silver nanoparticles simultaneously attack multiple sites within the cell to inactivate physiological functions. This technology can be applied to control biofilms of the targeted organisms. To date the effectiveness of silver compounds releasing nanoparticles against established or forming biofilms remains unclear.
Hence, in the present work, an attempt is made to characterize the biofilm forming clinical isolates and their in *vitro* susceptibility and biofilm formation inhibition by developing novel agents.