Periplanata americana, the predominant cockroach species in India, are often found in close association with human beings widely distributed in domestic as well as peridomestic surroundings, hospital environments and catering establishments. They have omnivorous feeding habits feeding on sewage, food wastes and other filth including faecal matter. Hence it is likely that they acquire and harbour human pathogens in their intestine as well as on body surfaces. The pathogens may subsequently be transmitted to human environments either mechanically or through faecal matter raising concerns on health in humans (Le Guyader et al., 1989). Rivault et al., (1994) noticed a positive association between the bacterial fauna of an environment and the diversity of bacteria carried by the cockroaches. It has been noticed that cockroaches captured from hospitals carry potential nosocomial bacterial pathogens including E. coli, Klebsiella spp., Proteus spp., P. aeruginosa, Enterococcus spp., Enterobacter spp. and Staphylococcus spp. In spite of the potential of this insect to carry and disseminate pathogenic organisms, the intestinal flora of this insect is not adequately explored particularly in India. A study focussing on the bacterial pathogens in the intestinal contents of cockroach population belonging to P. americana collected from different sources viz. hospitals, domestic environment, restaurants, markets may help to establish the diversity of pathogens carried by them in their intestinal tract.

Listeria spp. are Gram positive bacteria with ubiquitous distribution in the environment. L. monocytogenes and L. ivanovii are the two important
pathogenic species of this genus. *L. monocytogenes* is a food-borne human pathogen responsible for listeriosis particularly in pregnant women and immunocompromised individuals while *L. ivanovii* is an animal pathogen. Though the other species of the genus *Listeria* viz. *L. seeligeri, L. welshimeri, L. innocua, L. grayi* are generally considered as non pathogens, there are occasional reports of infection with these species particularly in the immunocompromised. Owing to the omnipresence and filth feeding habits of *P. americana* it is likely that they can be a possible carrier of *Listeria* species. Despite the potential of *L. monocytogenes* to cause human infection, the probable role of cockroaches as an intestinal carrier of this bacterial species has not been studied in detail and the literature regarding its isolation from the insect is very scanty. Therefore, it is felt appropriate to search the potential role of cockroaches to carry *Listeria* species in their intestine along with other bacterial pathogens.

The ability of bacteria to cause disease in a susceptible host is referred to as its pathogenicity and is determined by various virulence factors acting at different stages of infection. Identifying the virulence factors is therefore important in understanding bacterial pathogenesis which is done either by biochemical approaches or through genetic screening. Bacterial virulence factors include membrane associated proteins, polysaccharide capsules, secretory proteins, cell wall and outer membrane components. Of the different virulence factors of *L. monocytogenes*, haemolytic activity is classically considered as one of the major virulence markers. The haemolysin, listeriolysin O, is encoded by the haemolysin gene *hlyA* facilitates the escape of *L. monocytogenes* from the internalization vesicle into the cytoplasm initiating its cell-to-cell spread (Cossart et al., 1989). *L. monocytogenes* also secretes two phospholipases viz.
phosphatidylinositol-phospholipase C (PI-PLC) and phosphatidylcholine-phospholipase C (PC-PLC) encoded by \textit{plcA} and \textit{plcB} genes respectively, acting in synergy with LLO in lysing primary and secondary vacuoles (Smith \textit{et al.}, 1995). Though \textit{L. monocytogenes} is the only recognized human pathogen of \textit{Listeria} species, occasional reports of bacteraemia with the non pathogenic \textit{L. innocua} and \textit{L. grayi} in immunocompromised human beings have been recorded (Perrin \textit{et al.}, 2003; Rapose \textit{et al.}, 2008; Salimnia \textit{et al.}, 2010). Since \textit{L. monocytogenes} shows significant strain to strain variation in virulence and pathogenicity (Liu \textit{et al.}, 2003a) and references regarding the virulence related studies of \textit{L. innocua} and \textit{L. grayi} are rare, a detailed study in this aspect appears to be appropriate to get an insight into the virulence potential of these \textit{Listeria} species isolated from cockroaches.

Certain bacteria form biofilm communities of sessile organisms enclosed in an amorphous extracellular matrix primarily of polysaccharide materials. Formation of these sessile communities and their inherent resistance to antimicrobial agents including biocides and antibiotics are the cause of many persistent and chronic bacterial infections. The potential for biofilm development of \textit{L. monocytogenes} on various kinds of surfaces is well documented and increased resistance of sessile \textit{L. monocytogenes} cells to higher concentrations of sanitizing agents in comparison with their planktonic counterparts has been demonstrated (Mah and O'Toole, 2001; Robbins \textit{et al.}, 2005). Though extensive work has been done regarding the biofilm formation of \textit{L. monocytogenes} and its susceptibility to different disinfectants, documents related to that of \textit{L. innocua} and \textit{L. grayi} are rare. Factors such as temperature, pH, sugar and salt, the application of which is common in the food processing units have been demonstrated to have an impact on the adhesion and biofilm formation of \textit{L. monocytogenes} (Moretro
and Langsrud, 2004). A study aimed to search the biofilm potential of Listeria species isolated from the intestinal contents of P. americana and various factors influencing its biofilm potential would be pertinent as far as hospital environment or food processing industry is concerned.

Understanding the factors having impact on the survival and growth of L. monocytogenes is essential for the effective management of L. monocytogenes control measures. L. monocytogenes, a food-borne human pathogen, is capable of surviving unfavourable environmental conditions both in the natural as well as within food-processing environments (Sauders and Wiedmann, 2007). The prime factors influencing the survival and growth of L. monocytogenes in food are temperature and pH. The tolerance of Listeria species to food preservatives such as sodium chloride and lysozyme also affects its survival in food. L. monocytogenes has a variety of genetically encoded survival mechanisms to withstand environmental stresses such as heat, salt and acidic conditions. As the infection is acquired through ingestion, this bacterial species has to resist various conditions prevailing in the alimentary tract including the effect of bile. The survival of Listeria isolates at room temperature under dry conditions is relevant as far as hospital environment is concerned where chances of infection in immunocompromised patients is relatively high. Therefore a study focussing the influence of various factors on the growth and survival of Listeria isolates viz. L. grayi, L. innocua, L. monocytogenes has been included as a part of the present study.

Though the role of inanimate objects in the dissemination of bacterial pathogen is often difficult to evaluate, considerable significance has been attached recently to its role in disease transmission. It has been observed that Gram positive bacteria such as Enterococcus spp., S. aureus, or S. pyogenes
and Gram negative species viz. *Acinetobacter* spp., *E. coli*, *Klebsiella* spp., *P. aeruginosa* and *S. marcescens* can survive on inanimate surfaces for prolonged periods. Control measures for preventing the dissemination of microorganisms involve improved contact precautions, cleaning and disinfection practices. A study focussing on the susceptibility or resistance of various nosocomial pathogens as well as *Listeria* species isolated from *P. americana* towards various disinfectants is imperative in an epidemiological point of view.

The resistance property of *L. monocytogenes* and other *Listeria* species to heavy metals may be considered as a contributing factor to the establishment and persistence of these bacterial species in various environments. Owing to the omnipresence and omnivorous feeding habits of *P. americana*, it is likely that the bacteria carried by it in their intestine may show resistance to heavy metals. The plasmid mediated heavy metal resistance in bacteria is having importance in environmental as well as clinical point of view as it also causes selection pressure in bacteria contributing to the maintenance and spread of antibiotic resistance along with biocide tolerance. Therefore, a study on the resistance of *Listeria* isolates to various heavy metals and searching the possible role of plasmid in mediating heavy metal resistance is relevant as far as the isolates from an all pervading insect such as *P. americana* is concerned.

Although resistance to antimicrobials is an inevitable consequence of the evolutionary adaptation of microbes, its use and misuse has driven a rapid emergence of resistance in pathogenic and non-pathogenic bacteria (Silbergeld *et al.*, 2008). Earlier, concern over resistance was restricted only to clinically relevant microorganisms. However, recently antibiotic resistance among bacteria becomes so common that a pool of resistance is
emerging in non pathogenic organisms found in humans, animals and in the environment. The intestinal ecosystem has been proposed as the most probable site where different bacterial species co-exist and therefore the digestive tract has been considered as the privileged site for the acquisition of transferable genetic elements such as plasmids and transposons. The genus *Listeria* was believed to be uniformly susceptible to antibiotics active against Gram positive bacteria. However, emergence of resistance in this bacterial species has been reported worldwide and acquisition of resistant genes from other bacterial species particularly enterococci has been attributed for the emergence of resistance. Anti-biogram pattern of various bacterial pathogens isolated from cockroaches studied earlier, variation in susceptibility pattern among isolates from different geographical areas has been observed. A data based on the anti-biogram of the bacterial isolates from *P. americana* in the proposed study may be useful in epidemiological analysis tracing the source of infection by comparing it with the anti-biogram pattern of other organisms in various human infections.

In view of the emergence of *Listeria* spp. as potential pathogens associated with food borne and hospital infections, the study has been emphasized on the isolation, identification and characterization of *Listeria* species isolated from *P. americana*.

### 1.1. OBJECTIVES OF THE STUDY

1. To analyse the bacterial pathogens including *Listeria* species in the intestine of *Periplanata americana* cockroach populations captured from various sources viz. hospitals, domestic environments, markets and restaurants.
2. To determine the predominant virulence factors of *Listeria* species with special emphasis on biofilm formation.

3. To study the effect of various physicochemical factors including disinfectants on the survival and growth of the bacterial isolates with special reference to *Listeria* species.

4. To determine the antibiotic resistance of the nosocomial pathogens as well as *Listeria* species isolated from *P. americana* and to search the possible role of plasmid in the transfer of resistance determinants.