CHAPTER 2

REVIEW OF LITERATURE

2.1. Role of insects in disease transmission

Though insects comprise one of the most diverse taxa of life, little is known about the implication of insect associated bacteria on human health (Allen et al., 2009). The arthropod borne infections have an extensive distribution over the face of the globe and such diseases have produced much suffering, economic loss and death in human population. Majority of the arthropod borne bacterial diseases are transmitted by mosquitoes, lice, ticks, mites or fleas. Arthropods associated with human infection often serve as vectors for the pathogenic microorganisms. A vector is an organism capable of mechanically or biologically transferring a pathogen from one organism to another. Two groups of non biting insects most frequently screened for food-borne pathogens are houseflies and cockroaches (Ahmad et al., 2011). These insects have been implicated as mechanical or biological vectors for bacterial pathogens including Salmonella spp., Shigella spp., Aeromonas spp., Yersinia pseudotuberculosis, E. coli O157: H7 that can cause diseases in humans and animals (Zurek and Gorham, 2008).

2.2. The intestinal flora of insects

The gut of insects favours the development of microorganisms because of the extended surface of their intestinal lumen and the availability of nutrients. The bacterial flora of insect’s gut appears to be the fortuitous contamination of the environment and often reflects its feeding habits (Hunt and Charnley, 1981). A variety of human pathogens are found to be
harboured by flies and cockroaches dwelling in hospitals and other urban settings (Pai et al., 2004; Rahuma et al., 2005; Macovei and Zurek, 2006). Broderick et al., (2004) noticed the dominance of human commensal species such as Enterococcus spp. and members of the enterobacteriaceae in the gypsy moth midgut. Graczyk et al., (2001); Zurek and Gorham, (2008) highlighted the importance of Musca domestica and Blattella germanica as vectors of various animal and human pathogens more than their status as nuisance pests. Ahmad et al., (2011) observed enterococci as very common in the gut content of house flies and cockroaches suggesting this bacterial species as the predominant commensal in the insect’s intestine. In addition to being as intestinal carriers, these non biting flies are also involved in the mechanical transmission of various nosocomial pathogens with multiple resistance in hospital premises (Graczyk et al., 2001). Vazirianzadeh et al., (2008) finds agreement with Graczyk et al., (2001) isolating medically important bacteria from house flies including K. pnenumonae, P. aeruginosa, P. mirabilis, E. coli with multiple resistance.

2.3. Significance of Periplanata americana as a carrier of human pathogens

According to World Health Organisation report, cockroaches have been in existence for about 360 million years and during the intervening time the insect has gone very little structural change (Cochran, 1982). Blatella germanica, Periplanata americana and Blatta orientalis are the important cockroach species having health impact on human being (Baumholtz et al., 1997). Periplanata americana (the american cockroach) is widely distributed throughout the temperate, tropical and subtropical regions of the world. The ability of these insects to harbour bacteria on external and internal surfaces provides multiple means of pathogen transmission through direct contact
with surfaces or via defaecation and / or regurgitation (Pechal et al., 2007). This creates a public health concern if cockroaches inoculated with bacteria from outside migrate to indoors and transmit pathogens to areas such as hospitals or kitchens of houses or food establishments. Although person to person contact may be the important route of transmission of nosocomial infections, the possible role of cockroach in the dissemination of pathogenic microorganisms in the hospitals cannot be ruled out (Pai et al., 2004). Pai et al., (2004) observed cockroach infestation in more than 40% of hospitals under study and were found to be harbouring multi resistant bacteria and fungi. There have been reports of correlation between the reduction in the population of cockroaches and decrease in the incidence of infection in hospitals. Tarshis, (1962) noticed a decrease in the hospital acquired infectious hepatitis followed by pest- control measures.

2.4. Pathogens reported in *P. americana*

Various studies revealed the role of cockroaches as potential carriers of pathogens including bacteria, fungi, protozoans and helminthes (Burgess and Chetwyn, 1981; Cloarec et al., 1992; Pai et al., 2003). Prado et al., (2006) isolated coagulase negative staphylococci along with members of enterobacteriaceae and fungi from cockroaches captured from hospitals. Fotedar et al., (1989) in their study on cockroaches obtained from hospitals observed that 99% of these insects carried potential bacterial pathogens including *E. coli, Klebsiella spp.*, *Proteus spp.*, *P. aeruginosa*, and *S. aureus*. The insects caught from domestic environment were also not shown any difference in the pathogens carried (Pai et al., 2005). Elgderi et al., (2006) reported an array of different enterobacteriaceae members in the insects obtained from hospital and household environment. Carriage of bacteria resisting antibiotics in cockroach was reported by various previous studies.
Gliniewicz et al., (2003) indicated the possibility of cockroach acting a source for the methicillin resistant coagulase negative staphylococci in the hospital environment. Ahmad et al., (2011) in their study on insects prevalent in swine operations noticed the likely role of house flies and cockroach as reservoirs of antibiotic resistant and virulent enterococci. The report of Cotton et al., (2000) on beta – lactamase producing Klebsiella, Fotedar et al., (1991) on K. pneumoniae resisting multiple antibiotics, Elgderi et al., (2006) on multi resistant Pseudomonas spp. are a few reports of antibiotic resistance in bacteria carried by the cockroaches. The significance of these insects is not only limited as a carrier of potential bacterial pathogens but also as a vector of fungi and parasitic eggs and cysts. Tatfenga et al., (2005) identified the cockroach as a mechanical vector of different fungi viz. Candida spp., Aspergillus spp., Mucor spp., Rhizopus spp. and parasites viz. Enterobius vermicularis, Isospora belli, ova of Trichuris trichiura, ova of Ascaris lumbricoides, cysts of Balantidium coli and cysts of E. histolytica. Pai et al., (2003) demonstrated the cysts of E. histolytica in the digestive tract as well as on the cuticle of cockroaches. It has also been reported that cockroaches have the vector potential for the coccidian parasite Toxoplasma gondii (Chinchilla et al., 1994). Salehzadeh et al., (2007) emphasized the significance of these insects as carriers of many medically important fungi.

2.5. *P. americana* as a carrier of food borne pathogens

Food-borne bacterial diseases particularly with the bacterial spp. Salmonella spp., Shigella spp., V. cholerae, S. aureus and B. cereus is common in developing countries. Insanitary food handling often leads to food contamination by the pathogens. The dissemination of food pathogens, though to a lesser extent, could also occur through non biting insects such as
house flies or cockroaches which are living in close association with human
being. Oothuman et al., (1989); Paul et al., (1992) reported Shigella spp. and
Salmonella spp. in cockroaches captured from hospitals, restaurants and
houses indicating the importance of this insect as a carrier of food borne
pathogens. Mpuchane et al., (2006) also recorded the isolation of various
food spoilage bacteria such as Bacillus spp., Staphylococcus spp.,
Salmonella spp., and Shigella spp. in addition to other pathogens from this
insect. Tachbele et al., (2006) reported the isolation of E.coli O 157 along
with other food borne pathogens such as S. aureus, Salmonella, Shigella and
B. cereus from cockroaches caught from in hospitals, restaurants and
residences indicate the possibility of spreading these pathogens through this
insect in these environments. Recovery of drug resistant Salmonellae from
hospital cockroaches has also been reported by Fathpour et al., (2003).

2.6. Disease transmission in health care facilities

The acquisition of nosocomial pathogens in health care facilities is a
complex interaction between the host, pathogen and environment (Rutala and
Weber, 1987). Infections may be caused by a microorganism acquired from
another person in the hospital (cross-infection) or may be caused by the
patient’s own flora (endogenous infection) especially in severely
immunocompromised persons. Various related studies have also suggested a
possible link between the microorganisms present in the hospital environment
and hospital acquired infections (exogenous flora). Environmental sources of
exogenously acquired pathogens include inanimate objects in the hospital
environment or substances contaminated from another human source.
Dissemination of pathogens from an animate or inanimate reservoir to the
patient may occur by one or more of several different routes such as
airborne, contact, ingestion, infusion or through arthropod vectors.
The acquisition of infection in hospital through contact may be direct or indirect. Direct contact transmission occurs if there is a close physical contact between the source and the patient. On the other hand, disease transmission through indirect contact occurs through an intermediate object which is usually inanimate.

Some pathogens are hard enough to remain infectious on inanimate objects or fomites and can become vehicles for transmission of these pathogens. Kramer et al., (2006) observed most common nosocomial pathogens as surviving or persisting on surfaces for months and found that they can be a continuous source of transmission if no regular preventive disinfection is performed. The longer a nosocomial pathogen persists on a surface, the lengthier act as a source of infection. Jawad et al., (1998) noticed the exceptional ability of *A. baumannii*, a rapidly emerging pathogen in the health care setting, to survive on inanimate objects for prolonged periods. Gram negative bacteria have been described to persist longer than gram positive bacteria (Hirai, 1991). Bacteria such as *L. monocytogenes*, *P. aeruginosa*, *E. coli* were found to be surviving long under humid conditions (Wenzel, 1987; Williams et al., 2005; Vogel et al., 2010). Though disinfecting surfaces in the immediate environment of the patient have been described to reduce acquisition of certain nosocomial bacterial pathogens routine treatment of clean floors with various types of surface disinfectants have no influence on the incidence of nosocomial infection (Dharan et al., 1999; Hayden et al., 2006).

### 2.7. Common nosocomial bacterial pathogens

The bacteria commonly encountered in hospital acquired infections are *Staphylococcus* spp., *Enterococcus* spp., *P. aeruginosa*, *E. coli*,

2. 7. 1. Staphylococcus spp.

Despite the availability of numerous effective anti staphylococcal antibiotics, *S. aureus* has been continuing as a major cause of morbidity and mortality. *S. aureus* is a pluripotent pathogen causing disease through both toxin mediated and non toxin mediated mechanisms. This organism is responsible for both nosocomial and community-based infections that range from relatively minor skin and soft tissue infections to life threatening systemic infections (Lowy, 1998). The organism produces many virulence factors such as protease, coagulase, clumping factor, enterotoxins, exfoliative toxin, leukotoxin and haemolysins. Staphylococci are also having biofilm potential adhering to its target tissues mainly implants and other foreign body materials, resisting antimicrobial agents (Gotz, 2002). They are also implicated as causative agents of pneumonia, sepsis and toxic shock syndrome. Methicillin resistant *S. aureus* (MRSA) is a common cause of infection among hospitalized patients. Vancomycin is the option for treatment for these infections. But since the earliest report of vancomycin resistance by Hiramatsu *et al.*, (1997), resistance to this drug in *Staphylococcus* is increasingly being reported.

Coagulase negative staphylococci are long been considered as avirulquant. But its importance as human pathogens has been recognized only in the past years (Huebner and Goldmann, 1999). Of the different species, *S. epidermidis* is the most predominant human pathogen. This component of the normal human flora is found on the skin as well as in the oropharynx and vagina. It can persist on medical equipments for several months and are
increasingly being reported in catheter-related infections. *S. saprophyticus*, another coagulase negative *Staphylococcus* species is commonly associated with urinary tract infection.

### 2.7.2. *Enterococcus* spp.

Enterococci form the normal flora of the intestinal tract of human being and animals. Though they are not considered to be highly virulent, their intrinsic resistance and ability to acquire resistance to several broad-spectrum antibiotics allows them to cause super infections in patients already receiving antimicrobial therapy (Jones *et al.*, 1995; Desai *et al.*, 2001). Enterococci are known to acquire resistance to several classes of antibiotics either by mutation or through the transfer of plasmids and transposons (Murray, 1990). The enterococcal antimicrobial resistance particularly to vancomycin is a serious concern. *Enterococcus* species are most commonly implicated in urinary, gastrointestinal and pelvic infections (Murray, 1990; Desai *et al.*, 2001). Endocarditis, surgical wound infections are the other infections caused. Of the different species, *E. faecalis* and *E. faecium* are responsible for infections in human beings. Aggregation substance, surface carbohydrates or fibronectin binding moieties may facilitate adherence of the bacterium to host tissues. Extracellular toxins such as cytolysin can induce tissue damage.

### 2.7.3. *Escherichia coli*

Most *E. coli* strains are commensals found in the intestinal tract. Extra intestinal infections due to *E. coli* form a major cause of morbidity, mortality, and increased health care costs (Russo and Johnson, 2003). Various infections caused by this bacterial species include urinary tract infection (UTI), sepsis, meningitis, pneumonia, intra-abdominal infections,
diverse soft-tissue infections and osteomyelitis. Pathogenic strains of this organism may be distinguished from non-pathogenic by their virulence factors such as exotoxins, capsule and pilus.

2.7.4. *Klebsiella* spp.

The species *K. pneumoniae* accounts for a significant proportion of hospital acquired urinary infection, pneumonia, septicemia and soft tissue infection (Podschun and Ullmann, 1998). *K. pneumoniae* strains may be considered as opportunistic rather than true pathogens since they mostly affect debilitated patients, infants and the elderly. The extensive use of broad-spectrum antibiotics in patients has led to increased carriage of *Klebsiella* as well as the development of multidrug-resistant strains that produce extended spectrum beta-lactamase (ESBL). Factors implicated in the virulence of *K. pneumoniae* strains include the capsule, lipopolysaccharide, iron scavenging systems and fimbrial and non-fimbrial adhesins (Williams and Tomas, 1990).

2.7.5. *Citrobacter* spp.

*Citrobacter* spp. are commonly found in water, soil, food and the intestinal tracts of animals and humans (Gupta *et al*., 2003). This bacterial species cause a wide spectrum of infections in the urinary tract, blood, superficial wounds, skin, peritoneum and several other normally sterile sites. Very often, their hosts are found to be hospitalized and immunocompromised patients (Forbes *et al*., 2002; Kim *et al*., 2003). Of the different species, *C. freundii*, *C. diversus* and *C. amalonaticus* are linked to human disease (Samonis *et al*., 2009). Aminoglycosides, fluoroquinolones, carbepenems and third-or fourth-generation cephalosporins have the highest in vitro antimicrobial activity against *C. freundii* (Gupta *et al*., 2003).
2.7.6. **Enterobacter** spp.

Most epidemiologic aspects of *Enterobacter* infections reflect the opportunity for infection rather than the intrinsic virulence of the organism involved. These organisms are generally innately resistant to older antimicrobial agents and have the ability to rapidly develop resistance to newer agents. Multiple resistant strains have emerged in areas of high cephalosporin use within the hospital. Among the different species, *E. aerogenes*, *E. cloacae*, *E. agglomerans* and *E. sakazakii* are often implicated in human infections (Gaston, 1988; Haddy et al., 1991; Stenhouse, 1992). Of these species *E. aerogenes* and *E. cloacae* are by far the most frequently encountered in human infections (Karnad et al., 1987; Haddy et al., 1991).

2.7.7. **Serratia** spp.

*S. marcescens* has been recognised as a cause of hospital-acquired infection for the last few decades. It is a widely distributed saprophytic bacterium and often described as opportunistic pathogens. Many *S. marcescens* strains are resistant to multiple antibiotics (Hejazi and Falkiner, 1997; Alexandrakis et al., 2000). It has been observed that in *S. marcescens* LPS O-antigen plays an important role in both resistance to host defences and adherence (Palomar et al., 1993; Palomar et al., 1995; Lerouge and Vanderleyden, 2002).

2.7.8. **Proteus** spp.

Micro organisms belonging to *Proteus* spp. are widely distributed in natural environment and are often associated with opportunistic infections particularly in immunocompromised. *Proteus mirabilis* is the predominant species responsible for nosocomial infection related to kidney stones (Shah et al., 2002). Virulent factors of *Proteus* spp. include fimbriae, flagella, outer
membrane lipopolysaccharide, urease, haemolysins, IgA and IgG protease (Rozalski et al., 1997).

2.7.9. *Pseudomonas spp.*

*P. aeruginosa* is an opportunistic human pathogen responsible for severe nosocomial infections, life-threatening infections in immune compromised persons and chronic infections in cystic fibrosis patients (Koch and Hoiby, 1993; Hoiby, 2000). In the cystic fibrosis lung, *P. aeruginosa* grows primarily as biofilms (Hoiby et al., 1977; Singh et al., 2000). The virulence of this bacterium depends on a large number of cell-associated and extracellular factors. It has been observed that exotoxin A, elastase or alkaline proteases are essential for maximum virulence of *P. aeruginosa*. The ability to persist long in moist environments and on hospital equipments adds importance to this bacterium as a nosocomial pathogen. *Pseudomonas* is adaptive and easily develops antibiotic resistance. The low susceptibility is attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelope. Apart from *P. aeruginosa*, other *Pseudomonas* species viz. *P. putida*, *P. fluorescens*, *P. cepacia*, *P. pseudoalcaligenes*, *P. putrefaciens*, *P. stutzeri*, *P. maltophilia* are also reported to be responsible for the infectious process, though less frequent (Gilardi, 1972).

2.7.10. *Acinetobacter spp.*

*A. baumannii* is a rapidly emerging pathogen in the health care facilities where it causes infections that include bacteraemia, pneumonia, meningitis, urinary tract infections and wound infection. This bacterium is usually recovered from patients who are immunocompromised or have been
under prolonged hospitalization. It is resistant to many antimicrobial agents and therefore infection with this bacterial species is a challenge as far as treatment is concerned. The characteristic feature of \textit{A. baumanii} is its ability to survive for prolonged periods in the environment contributing to the transmission during outbreaks (Jawad \textit{et al.}, 1998). This organism is also being capable of surviving for weeks under dry conditions in hospital equipments suggesting that it could serve as a secondary reservoir for the infection (Catalano \textit{et al.}, 1999).

2.8. \textbf{Food borne infections}

All the food borne infections are associated with poor hygienic practices where the oral - faecal route is maintained with the food providing the vital link between hosts. Factors contributing to foodborne illness include improper cleaning of raw foods, cross contamination with microbes, inadequate heating and insufficient cooking of foods (Centers for Disease Control and Prevention, 2004a). Insects such as houseflies or cockroaches feeding on faecal waste also have a role in transferring microbes to food either mechanically through their body parts or through faecal pellets. Fomites too play a role in the maintenance of the faecal-oral route of transmission. Characteristics of food borne illness vary from pathogen to pathogen. Complications due to illness are often found in the elderly, young children and immune suppressed (Kendall \textit{et al.}, 2006; Koehler \textit{et al.}, 2006). The predominant bacterial flora involved in food spoilage or toxin production are as follows: \textit{Salmonella} spp., \textit{Shigella} spp., \textit{S. aureus}, \textit{C. jejuni}, \textit{Enterococcus} spp., \textit{E. coli}, \textit{L. monocytogenes}, \textit{Y. enterocolytica}, \textit{Aeromonas aerophila}, \textit{V. cholerae} and \textit{V. parahaemolyticus}. 
Salmonellosis is generally a self-limiting gastroenteritis mostly caused by non-typhoidal *Salmonella* such as *Salmonella typhimurium, Salmonella enteritidis, Salmonella cholerasuis, Salmonella hader, Salmonella virchow, Salmonella dublin* etc. with a tendency to produce severe illness in immunocompromised individuals (Sakai and Chalermchaikit, 1996; Hohmann, 2001). Despite the strong link between food of animal origin and human salmonellosis, it has been noticed that infection may also be acquired by cross contamination through contact as well as by ingestion of vegetables and fruits (Neto *et al.*, 2010). There have been evidences to suggest that as low as 10-100 cells of *Salmonella* may initiate an infection under certain conditions and with some foods (D’Aoust, 1994). Septicaemia occurs as a complication of gastroenteritis which can be fatal in immunocompromised hosts. The emergence of MDR *Salmonella* has become a concern as infection with MDR *Salmonella typhimurium* DT 104 result in greater morbidity and mortality when compared to non-resistant *Salmonella* (Helms *et al.*, 2002).

*Campylobacter* is the second most common identified organism in foodborne disease and the species associated with human illness is *Campylobacter jejuni.* It is zoonotic in origin with reservoirs like rodents, wild birds, sheep, cow and poultry. Contaminated shellfish and vegetables may also serve as vehicles of these bacteria (Altekruse *et al.*, 1999). Most *Campylobacter* spp. are associated with diarrhoea and extra-intestinal infections including bacteraemia, urinary tract infections, meningitis and Guillain-Barre Syndrome.

*Cl. Perfringens* is one of the most common cause of human gastrointestinal illness in U. S and Europe and is the third most commonly reported food borne disease (Lynch *et al.*, 2006). The enterotoxin is
responsible for the clinical manifestation associated with its food poisoning. It is a self limiting disease in normal adults though it may lead to death in immunocompromised (Lynch et al., 2006). Botulism caused by *Clostridium botulinum* if not treated can lead to death.

Shigellosis is caused by *Shigella* spp., viz. *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei* (Kuo et al., 2008). Among *Shigella* species, *S. flexneri* prevails in developing countries while *S. sonnei* in industrialized zones (Pazhani et al., 2005; Savadkoohi and Kacho, 2007). *Shigella* has been recognised as a waterborne pathogen associated contaminated water sources (Fleming et al., 2000). Foodborne outbreaks of *Shigella* are also common especially with foods that are unhygienically prepared or exposed to a limited heat treatment or are delivered raw to the consumer (Wu et al., 2000). The infective dose for *Shigella* may be as low as 10 cells of *S. dysenteriae* to 500 cells of *S. sonnei* (Kothary and Babu, 2001). Very young, very old or the immunocompromised persons may be more susceptible to infection. Typical symptoms of infection include bloody diarrhoea, abdominal pain, fever and malaise.

*Escherichia coli* constitutes part of the normal flora of humans and other warm blooded animals (Holt et al., 1994) and is often considered as an indicator of faecal contamination. However this perspective has been changed with the recognition of the bacterium as the cause food borne illness. Diarrhoeagenic *E.coli* can be categorized into specific groups viz. enteropathogenic *E.coli* (EPEC), enteroinvasive *E.coli* (EIEC), enteroaggregative *E.coli* (EAEC), enterohaemorrhagic *E.coli* (EHEC) and enterotoxigenic *E.coli* (ETEC) based on their virulence properties, mechanism of pathogenicity, clinical syndromes and distinct O:H serotypes. *E.coli* O157:H7 is a shiga toxin producing *E. coli* and ingestion of 10-100
organisms is enough to produce infection. Human illness follows consumption of food or water contaminated with cow’s faeces causing bloody diarrhoea and painful abdominal cramps. Rarely the infection terminates in haemolytic uremic syndrome (HUS) with manifestations such as temporary anaemia, profuse bleeding and kidney failure (Breum and Boel, 2010; Newell et al., 2010; Wang et al., 2011). E. coli 0104: H4 is a mutant of E. coli 0157: H7. Food such as cucumbers, lettuce, tomatoes and bean sprouts has been mainly implicated in the latest outbreak of food borne E. coli 0104: H4.

Certain strains of S. aureus produce staphylococcal enterotoxins (SEs) and are associated with food poisoning. It is an important pathogen due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance (Loir et al., 2003). Different types of food which have been linked with staphylococcal food poisoning include milk, cream, butter, ham, cheeses, sausages, canned meat, salads, cooked meals and sandwiches. S. intermedius is the only other Staphylococcus that has been clearly involved in staphylococcal food poisoning outbreaks (Khambaty et al., 1994). The symptoms of Staphylococcus food poisoning are abdominal cramps, nausea, vomiting often followed by diarrhoea.

Although B. cereus is the major pathogen of the genus Bacillus, other Bacillus species viz. B. licheniformis, B. Subtilis, B. Pumilus, B. brevis are also implicated in food poisoning. It can cause two distinct types of illness depending upon the type of toxin produced; an emetic illness, characterized by nausea and vomiting with an incubation period of 1-6 hr and a diarrhoeal type, with an incubation period of 4-16 hr associated with a wide range of food.
Chapter 2

*V. parahaemolyticus* is a halophilic bacterium that inhabits warm estuarine waters worldwide (Daniels *et al.*, 2000). It causes acute gastroenteritis and food poisoning in humans who consume raw or improperly cooked seafood (Hlady and Klontz, 1996; Pan *et al.*, 1997). Thermostable direct haemolysin (encoded by *tdh*) is the virulence factor responsible for illness (Nishibuchi *et al.*, 1992). When ingested, *V. parahaemolyticus* causes watery diarrhoea often with abdominal cramping, nausea, vomiting, fever and chills. Severe disease is rare and occurs more commonly in persons with weakened immune systems.

*Aeromonas hydrophila* is present in fresh water as well as brakish water. Infection acquired by man is often through open wounds or by ingestion of food or water with sufficient numbers of organisms. *A. hydrophila* often causes gastroenteritis in healthy individuals and septicemia in immunocompromised. Gastroenteritis associated with *A. hydrophila* may be presented as cholera-like illness or as dysentery type.

*Yersinia* is a facultative anaerobic bacterium with the ability to grow in refrigerated condition. Infection with *Y. enterocolytica*, the yersiniosis, leads to acute intestinal infection with mesenteric lymphadenitis and illitis. There have been reports of pandemic due to *Y. enterocolitica* strains of serogroups O3 and O9 in Europe, Japan in 1970s and in North America by the end of 1980s. These strains appear to have a reservoir in pigs and are transmitted to humans by consumption of or contact with raw pork or pork products (Bamaiyi, 2011).

Listeriosis is a severe invasive food borne infection in human beings caused by *L. monocytogenes*. Though healthy people rarely contract listeriosis, the illness can be serious for the elderly, newborns, pregnant
women and those with weakened immune systems. However, as the organism is not detected by routine stool culture, *L. monocytogenes* as the cause of gastroenteritis and fever associated with listeriosis is rarely diagnosed (Dalton *et al.*, 1997). Early spontaneous abortion or miscarriage accompanying as a sequelae of listeriosis may also be under diagnosed.

### 2.9. The genus *Listeria*

#### 2.9.1. History

Although as early as 1891 Hayem in France and in 1893 Henle in Germany observed Gram positive rods in tissue sections from patients who died of the disease that was almost certainly *Listeria* infection, Hilphers (1911) appears to have been the first to record the organism now known as *Listeria monocytogenes*. In 1926, Murray isolated the bacterium in pure culture during a spontaneous epidemic disease of rabbits and was named *Bacterium monocytogenes* because of monocytosis in natural and artificially infected monogastric animals (Murray *et al.*, 1926). The generic name *Listerella* was chosen for this bacterium in honor of Lord Lister, the well known pioneer in the field of microbiology. In 1940, Pirie suggested the name *Listeria* and was adopted in the sixth edition Bergey’s Manual of Determinative Bacteriology and approved the judicial commission on bacteriological nomenclature and taxonomy. Gill is credited with the first isolation of *Listeria monocytogenes* from farm animals. The first confirmed report of *Listeria* infection in man was made by Nyfeldt in 1929.

#### 2.9.2. Classification

Taxonomically, the genus *Listeria* comprises of six species: *L. monocytogenes, L. ivanovii, L. welshimeri, L. seeligeri* and *L. grayi* (Vázquez-Boland *et al.*, 2001). The two definitive pathogens of the genus *Listeria* are *L. ivanovii*, principally an animal pathogen and
L. monocytogenes, an intracellular food-borne pathogen infecting both man and animals (Low and Donachie, 1997).

2.9.3. Reservoir, host range and transmission

Listeria species are isolated from a variety of environmental sources including decaying vegetation, soil, water, effluents, a large variety of foods and from the faeces of man and animals (Vázquez-Boland et al., 2001). This association is believed to contribute to the inclusion of many animals including ruminants, birds, insects, fish, crustacean and humans in an ecological cycle in which the organism is consumed during feeding on contaminated soils and vegetation, carried to new sites and excreted and subsequently dispersing into new environments (Nightingale et al., 2004). The organism has been reported in the mesenteric lymph nodes of healthy cattle, pigs and sheep. Domestic poultry are also found to be a source of the organism. The distribution of L. monocytogenes is so widespread that it is often considered an environmental organism with an opportunistic pathogen status rather than a primary parasite of humans or animals. The organism has been emerged as a food borne pathogen associated with processed food such as coleslaw, soft cheese and pasteurized milk (Schlech et al., 1983; Fleming et al., 1985; Farber et al., 1987). McLauchlin and Nichols, (1994) demonstrated a direct relationship between poor hygiene and the presence of Listeria spp. in sea food and ready to eat food.

2.9.4. Morphology

The Listeria genus comprises of motile, regular, non-sporing, non capsulated, Gram positive coco-bacillus shaped bacteria (Seeliger and Jones, 1986). Its size ranges from 0.4–0.5 µm wide and 0.5–2.0 µm long. In smears it occurs singly or in short chains arranged in a V or Y shape.
2.9.5. Cultural characters

Tryptose peptone is an excellent medium for cultivation and preservation of the bacterium with an additional advantage of being clear and colourless. The colonies of *Listeria* have a finely textured surface with a distinctive blue colour (Gray, 1957). After 24 hr of incubation at 37°C the colonies appear round, translucent, slightly raised with an entire margin and are blue - grey by normal illumination with a size range from 0.3-1.5 mm in diameter. In blood containing medium a narrow zone of haemolysis around the colonies is characteristic of *Listeria monocytogenes*. A wide zone of beta haemolysis may be noticed with *Listeria ivanovii* where as haemolysis produced by *Listeria seeligeri* is weak.

2.9.6. Biochemical differentiation of *Listeria* species

*Listeria* species are catalase positive, oxidase and indole negative with no ability to hydrolyse urea or starch. Gelatine and coagulated serum are not liquefied by any species. *Listeria* species utilize dextrose, esculin and maltose and some species ferment mannitol, rhamnose and xylose with production of acid (Gawade *et al*., 2010). *L. grayi* ferments mannitol with acid production. *L. monocytogenes*, *L. ivanovii* and *L. seeligeri* produce haemolysis on sheep blood agar. Of these three species, *L. monocytogenes* ferments rhamnose but fails to ferment xylose. In CAMP test *L. seeligeri* shows enhanced haemolysis at the *S. aureus* streak whereas *L. ivanovii* shows enhanced hemolysis at the *R. equi* streak. Of the non-hemolytic species, *L. innocua* may provide the same rhamnose-xylose reactions as *L. monocytogenes* but it is negative for the CAMP test. *L. innocua* gives variable results of rhamnose fermentation. A *L. welshimeri* isolate that is rhamnose-negative may be confused with a weakly haemolytic *L. seeligeri*
isolate unless resolved by the CAMP test. \textit{L. monocytogenes} shows methyl red, Voges–Proskauer test positive after 24 hr incubation at 28 ± 1°C.

\textbf{Table 1}

\textbf{Biochemical differentiation of \textit{Listeria} species}

<table>
<thead>
<tr>
<th>\textit{Listeria} spp</th>
<th>(\beta)-haemolysis</th>
<th>D-Xylose</th>
<th>L-Rhamnose</th>
<th>(\alpha)-Methyl D-mannoside</th>
<th>Nitratreduction</th>
<th>D-Mannitol</th>
<th>CAMP with \textit{S. aureus}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{L. monocytogenes}</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>\textit{L. innocua}</td>
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</tr>
<tr>
<td>\textit{L. ivanovii}</td>
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<tr>
<td>\textit{L. seeligeri}</td>
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<td>\textit{L. welshimeri}</td>
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<td>\textit{L. grayi}</td>
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V - Variable

2.9.7. \textbf{Foodborne listeriosis}

Listeriosis is a severe invasive foodborne infection in human beings caused by \textit{L. monocytogenes}. Though healthy people rarely contract listeriosis, the illness can be serious for the elderly, newborns, pregnant women and those with weakened immune systems. However, Bula \textit{et al.}, (1995) reported listeriosis in healthy adults without any predisposing factors. Symptoms may include fever, fatigue, nausea, vomiting and diarrhoea. It may often leads to sepsis or meningitis or both and in seldom cases cause brain stem encephalitis (Antal \textit{et al.}, 2005). Other clinical illness includes pneumonia, endocarditis, septic arthritis, and abscess (Schett \textit{et al.}, 2005). Listeriosis in pregnant women may show flu-like symptoms; complications can result in miscarriage, stillbirth, or septicaemia or meningitis in the newborn.
2.9.8. Different stages of \textit{L. monocytogenes} cellular infectious process

**Figure 1**

Cellular infectious process - \textit{L. monocytogenes} (Portnoy \textit{et al}., 2002)

In order for infection, a high dose of approximately $10^6$ cells/gram is required. The cycle begins with the adhesion of intestinal epithelium and paysers patches following passage through the stomach (Vazquez-Boland \textit{et al}., 2001; Sleater \textit{et al}., 2009). \textit{Listeria} can induce their own internalization in macrophage or non-phagocytic cells such as epithelial cells, fibroblast cells, hepatocytes, endothelial cells, various types of nerve cells and dendritic cells where they undergo intra cellular cycle (Gaillard \textit{et al}., 1987; Kuhn \textit{et al}., 1988; Dramsi \textit{et al}., 1995; Guzman \textit{et al}., 1995; Drevets \textit{et al}., 1995; Dramsi \textit{et al}., 1998). \textit{L. monocytogenes} invades non-phagocytic cells by a zipper - like mechanism in which the host cells engulf the bacterium and cell membrane surrounds the bacterial cells forming a phagocytic vacuole (Gaillard \textit{et al}., 1987). After about 30 min., the phagosome membrane is lysed by the bacteria, escapes into the cytoplasm and proliferates. This process is mediated by listeriolysin - O in combination with
phosphatidylinositol (PI- PLC) and phosphatidylcholine (PC- PLC) phospholipase. Intracytoplasmic bacteria are then surrounded by fibrillar material composed of actin filaments which later form an actin tail at one pole of the bacterium. It propels the bacterium in the cytoplasm finally reaching the cells periphery. In contact with the cell membrane a finger like protrusion is formed with the bacterium at the tip. These pseudopodates penetrate the uninfected neighbouring cells where it is engulfed by phagocytosis forming a secondary phagosome. The bacteria are covered with two membranes, the donor cell forming the inner and the newly infected cell forming the outer membrane. Bacteria rapidly dissolve these membranes and initiate another round of intercellar proliferation in the cytoplasm.

2.10. Virulence factors of Listeria

The virulent factors of *Listeria monocytogenes* are encoded on a 9-kb pathogenicity island known as LIPI 1 (*Listeria Pathogenicity Island 1*) (Portnoy *et al.*, 1992). Different proteins involved in adhesion and invasion of these bacteria are internalins A and internalin B. Factors involved in intracellular life cycle include listeriolysin O (LLO), phospholipase A (Plc A), phospholipase B (Plc B), hexose phosphate translocase (Hpt) and metalloprotease. The virulent genes Prs and Idh genes are housekeeping genes encoding phosphoribosyl pyrophosphate synthetase and lactate dehydrogenase respectively (Gouin *et al.*, 1994).

Internalins A and B (InlA, InlB) are the surface proteins of *L. monocytogenes* encoded by the *inlAB* operon mediating the invasion of non-phagocytic cells including the epithelial cells that line the human stomach cells (Lingnau *et al.*, 1995). The receptors of Inl A are E cadherin expressed on epithelial cells of the skin, liver, and the gastrointestinal tract (Manguad *et al.*,
Inl A plays an important role in crossing the intestinal barrier, in binding and internalization into intestinal epithelial cells. Internalin B mediates internalization into a wider variety of cell types such as hepatocytes, fibroblasts, epithelioid cells, lymphocytes, neutrophils and endothelial cells and the entry is mediated by Met receptor tyrosine kinase (Shen et al., 2000). Another internalin InlC, encoded by the gene inlC, is important for full virulence in mice but its role in human pathogenesis remains unclear (Lecuit et al., 1997; Portnoy et al., 2002).

The cholesterol-dependent pore forming listeriolysin O (LLO) encoded by the gene hly is the virulence factor primarily responsible for escape of L. monocytogenes from the host vacuole (Birmingham et al., 2008). LLO disrupts the phagocytic vacuole through generation of small pores in the phagosome membrane causing a passive flux of ions and macromolecules (Shaughnessy et al., 2006). As a consequence in a short time vacuole is lysed and the bacterium is released into the cytosol. Mengaud et al., (1988) have reported the presence of the listeriolysin gene only in L. monocytogenes. However, Leimeister-Wachter and Chakraborty, (1989) noticed highly homologous listeriolysins in L. ivanovii, and in L. seeligeri.

Two different phospholipase C have been demonstrated in pathogenic Listeria spp. viz. phosphatidylinositol phospholipase (PI –PLC) and phosphatidylecholine phospholipase (PC – PLC) encoded by Plc A and Plc B respectively (Goldfine et al., 1993). PI-PLC activity is a reliable marker for permitting discrimination between pathogenic and nonpathogenic Listeria species (Serve et al., 1991). In particular, detection of PI-PLC activity clearly separates the nonpathogenic but haemolytic L. seeligeri from the pathogenic and haemolytic species L. monocytogenes and L. ivanovii. Though Plc A has only a minor role in virulence, it acts synergistically with Plc B in conjunction with
LLO for the optimal level of escape from the phagosome (Marquis et al., 1995; Gedde et al., 2000). PlcB has the ability to react with several enzymes but one of its main roles is to assist in the disruption of the phagosome and lysing the vacuole as part of the invasion process.

Act A is a surface based protein of *Listeria monocytogenes* encoded by *actA* needed for host cell actin polymerization based bacterial movement and for spread of the bacteria into adjacent cells (Kocks et al., 1992; Cameron et al., 1999). The metalloprotease (Mpl) of *Listeria monocytogenes* is a thermolysin-like protease which is involved in the maturation of PlcB contributing to the intracellular survival of this food borne bacterial pathogen (Bitar et al., 2008). Positive regulatory factor A (PrfA) dependent expression of hexose phosphate translocase (Hpt) facilitates the use of glucose 1 phosphate by the bacterium. The Hpt encoded by the gene *hpt* is important for rapid growth in the host cytosol and assists in the intracellular multiplication of the *L. monocytogenes* (Goldfine and Knob, 1992; Scortti et al., 2007). Bile salt hydrolase deconjugates conjugated bile salts and facilitates the survival of *Listeria* cells from the bile salt toxicity in the intestinal wall (Dussurget et al., 2002). FbpA is a surface protein of *L. monocytogenes* which binds to fibronectin and mediates HEp-2 cells adhesion. It also mediates protein level of LLO and InlB and behaves as an escort protein for two important virulence factors (Dramsi et al., 2004). Sortases are transpeptidases involved in cell wall anchoring of surface proteins and contributing to virulence. Auto is a surface protein encoded by aut gene and is expressed independently of the virulence gene regulator PrfA (Cabanès et al., 2004). These proteins are especially involved in invasion of eukaryotic cells especially in early and late stages of the infection process. P60 is a virulence factor which act in concert of with internalin to achieve optimal uptake into nonprofessional phagocytes and macrophages (Hess et al., 1995).
2.11. Biofilm formation in *Listeria*

The term biofilm describes the sessile and colonised form of microbial life characterized by adhesion of microorganisms to biotic or abiotic surfaces by producing a three-dimensional matrix of extracellular polymeric substances (EPS) (Donlan, 2002; Marques *et al*., 2007). Recent reports suggest that the biofilm matrix is mostly composed of extracellular proteins, exopolysaccharides and extracellular DNA (Langille *et al*., 2000; Mattos-Guaraldi *et al*., 2000). Biofilm represents a survival strategy where microorganisms exist in a dynamic equilibrium which aggregate, mature and detach to disseminate to new surfaces subsequently initiating infection or contamination. Reports of *Vibrio*, *Staphylococcus*, *Psuedomonas*, *Klebsiella*, *Enterococcus* and *E.coli* using the strategy of biofilm formation to persist in diverse environmental niches as well as opportunistically infecting a human host have been documented (Watnick and Kolter, 1999; Yanagihara *et al*., 2000; Fux *et al*., 2004; Mohamed and Huang, 2007; Salo *et al*., 2009; Stahlhut *et al*., 2012). The ability of *L. monocytogenes* forming biofilms on food-processing surfaces potentially leading to food product contamination has been recorded by many investigators (Borucki *et al*., 2003; Carpentier and Chassaing, 2004). In addition to the intrinsic properties of *L. monocytogenes* forming biofilm, certain extrinsic factors such as temperature, pH, salt, sugar and the presence of other bacteria have been observed to be influencing biofilm formation (Møretrø and Langsrud, 2004). Biofilms are generally considered to be more resistant to antimicrobial agents and disinfectants than their planktonic counter parts. Various studies on *L. monocytogenes* biofilms noticed this concept agreeable by observing more resistance to various disinfectants compared with planktonic cells (Folsom and Frank, 2006; Berrang *et al*., 2008).
Chapter 2

2.12. Biological and physicochemical parameters influencing the growth of *Listeria*

Bile is a bactericidal agent associated with the digestive system. The bile tolerance of bacteria is likely to be important for its survival and colonization in the intestinal tract. Several studies elucidated the bactericidal role of bile on bacteria (Kheadr, *et al.*., 2007; Paterson *et al.*, 2009). *L. monocytogenes* is capable of colonizing the human gallbladder as well as the intestinal tract pointing its ability to survive in the presence of bile (Begley *et al.*, 2005a). The *bilE* and *bsh* genes have been shown to play a role in providing bile resistance in *L. monocytogenes* (Begley *et al.*, 2005b). Begley *et al.*, (2009) noticed the changes in cell morphology of *L. monocytogenes* on exposure to bile resulting in enhanced biofilm formation of this bacterial species and proposed their enhanced survival and colonization in the intestinal tract as well as in the gallbladder.

Lysozyme is a potent antimicrobial peptide causing bacteriolysis. Lysozyme has been suggested to have roles as a preservative and its effectiveness on *L. monocytogenes* has been reported (Hughes and Johnson, 1987). Smith *et al.*, (1991) reported the increased resistance of *Listeria monocytogenes* to lytic action of lysozyme when grown at 37°C than the cells grown at 19°C, 12°C or 5°C.

The ability of *Listeria* spp. to grow a wide range of temperature (1–45°C) as reported by Chavant *et al.*, (2002) endow this bacteria with the capacity to survive food processing and food storage conditions. In spite of its ability to grow in a wide range of temperature, optimal growth of the organism occurs between 30–37°C (Petran and Zottola, 1989). A higher temperature of 60°C, however, is found to be detrimental to bacteria making pasteurization a suitable food processing technique to eliminate this bacterium from dairy.
products (Seeliger and Jonesy, 1986). Like many other bacteria, *Listeria species* too grow optimally at pH close to neutrality but growth has been reported at pH values ranging from 4.5 to 9.2 ((Parish and Higgins, 1989; Petran and Zottola, 1989). NaCl is one of the most commonly employed agents for food conservation allowing considerable increase in storage time by reducing water activity and the electrochemical potential across the cell membrane. *Listeria* spp. have been found to tolerate extremely high levels of salt and it has been reported that *L. monocytogenes* survived in commercial cheese brine (23.8% NaCl, pH 4.9) stored for 259 days at 4°C (Larson et al., 1999). The survival of *L. monocytogenes* for more than 18 weeks in 25.5% sodium chloride has been reported by Shahamat et al., (1980). Juneja and Eblen, (1999) observed the protective effect of salt in *L. monocytogenes* against the lethal effect of thermal treatment.

### 2.13. Resistance to heavy metals in *Listeria species*

Heavy metals in high concentrations as found in many environments can be toxic to bacteria. Heavy metal efflux systems and the presence of metal binding proteins are the genetic mechanisms in bacteria where by intracellular homeostasis of essential metal ions is maintained and resistance against toxic metals is acquired (Olafson et al., 1988; Nies and Silver, 1995; Silver and Phung, 1996). Plasmid mediated resistance to the heavy metal cadmium in *L. monocytogenes* has been observed by Lebrun et al., (1994). *cadA* operon is a part of the efflux system and has been reported to provide cadmium resistance as well as to benzalkonium chloride in many bacteria including *Listeria monocytogenes* (Lebrun et al., 1994; Mullapudi et al., 2010). The bacteria with plasmid borne clustered resistance genes may subsequently be transferred on to other bacteria through horizontal gene transfer enabling them a better chance of survival. Katharios -Lanwermeyer et al., (2012) suggested the reservoir status of
nonpathogenic *Listeria* spp. for disinfectant and heavy metal resistance genes for other *Listeria* including the pathogenic *L. monocytogenes*.

### 2.14. Influence of disinfectants

Disinfection is a process of removal or inactivation of pathogenic microorganisms by chemical or physical means. The disinfectants must be effective, safe and easy to use and easily rinsed off from surfaces leaving no toxic residues that could affect the health or the quality of the final products. The common disinfectants are as follows:

Although several alcohols have been shown to be effective against microorganisms, ethyl alcohol, isopropyl alcohol and *n*-propanol are the most widely used. Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria including mycobacteria, viruses, and fungi. However, they are widely used for both hard-surface disinfection and skin antisepsis. It is generally believed that they act by causing membrane damage and rapid denaturation of proteins with subsequent interference with the metabolism and cell lysis.

Glutaraldehyde is an important dialdehyde that has found usage as a disinfectant and sterilant in particular for low-temperature disinfection and sterilization of endoscopes and surgical equipment and as a fixative in electron microscopy. It has a broad spectrum of activity against bacteria and their spores, fungi, and viruses. The possible reactive sites of this disinfectant are the unprotonated amines in the outer layers of bacterial cells (Bruck, 1991). Formaldehyde is a monoaldehyde generally used as a disinfectant and sterilant in liquid or in combination with low temperature steam. Formaldehyde is bactericidal, sporicidal and virucidal, but it works more slowly than glutaraldehyde (Power, 1995). Formaldehyde is an extremely reactive chemical
(Russell and Hopwood, 1976) that interacts with protein, DNA and RNA in vitro. It has considered also sporicidal by virtue of its ability to penetrate into the interior of the bacterial spore.

Triclorocarbanilide (TCC) is the most extensively studied anilide and is used mostly in consumer soaps and deodorants. TCC is active against Gram positive bacteria but less so active against Gram negative and fungi. The anilides are thought to act by adsorbing to and destroying the semipermeable character of the cytoplasmic membrane leading to cell death.

Chlorhexidine is the most important biguanide and is used as either the acetate or gluconate salt (McDonnell and Russell, 1999). It is probably the most widely used biocide in antiseptic products particularly in oral and hand washing products. It is also used as a disinfectant and preservative. It has a broad spectrum of activity with bacteriostatic and bactericidal property against Gram-positive and Gram-negative microorganisms with sporostatic activity. Despite the advantages of chlorhexidine, its activity is pH dependent and its effect is greatly reduced in the presence of organic matter (Russell and Day, 1993). Lethality of biguanides is attributed to the damage to the cytoplasmic membrane causing leakage of intracellular components. A secondary target of biguanides is the enzyme adenosine triphosphatase (Maillard, 2002). The antiviral activity of chlorhexidine is variable.

Chlorine and iodine based compounds are the most significant microbicidal halogens used in the clinic and have been traditionally used for both antiseptic and disinfectant purposes. Chlorine compounds can be considered to be of three types: chlorine gas, hypochlorites and chlorine releasing agents (McDonnell and Russell, 1999; Knight and Cooke, 2002). Chlorine gas can be used for the disinfection of water but sodium hypochlorite
and sodium dichloroisocyanurate are the agents of choice. Chlorine compounds are bactericidal and sporicidal. Their activity is related to their solubility, amount of available chlorine present and pH of the solution. Their activity is impaired in the presence of organic matter. Sodium hypochlorite solutions are extensively used for hard surface disinfection and can be used for disinfecting spillages of blood containing human immunodeficiency virus or HBV. Hypochlorites are powerful oxidants and can induce lysis in Gram-negative bacteria by affecting the cell wall (Maillard, 2002). Sodium dichloroisocyanurate (NaDCC) is a stable source of chlorine and has the advantages of providing a higher concentration of available chlorine and is less susceptible to inactivation by organic matter.

Although less reactive than chlorine, iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal and sporidical (Gottardi, 1991). Iodine is able to penetrate the cell wall of microorganisms quickly and the lethal effect is due to the disruption of protein and nucleic acid structure. Less is known about the antiviral action of iodine, but it is likely that iodine attacks the surface proteins of enveloped viruses. Iodophores contain elemental iodine complexed with chemicals such as surfactants for solubility and phosphoric acid for stability. Iodophors are bactericidal, mycobactericidal and virudical.

Silver and its compounds have long been used as antimicrobial agents. The most important silver compound currently in use is silver sulfadiazine (AgSD) although silver metal, silver acetate, silver nitrate, and silver protein have antimicrobial properties. In recent years, silver compounds have been used to prevent the infection of burns and some eye infections. In addition to its effects on enzymes, Ag+ produces marked inhibition of bacterial growth. It inhibits cell division and damages the cell envelope and the contents of
P. aeruginosa (Richards et al., 1991). Ag⁺ ion also interacts with nucleic acids preferentially with the bases in DNA (Yakabe et al., 1980).

Peracetic acid (PAA) shows broad spectrum of activity even in the presence of heterogeneous organic matter. The absence of persistent toxic or mutagenic residuals or by-products, no quenching requirement, small dependence on pH, short contact time and effectiveness on primary and secondary effluents are the other advantages of this disinfectant (Klaas et al., 2002; Kitis, 2004). Furthermore, PAA has been found to be effective against biofilm bacteria especially if the biofilm contains food residues (Chmielewski and Frank, 2003). PAA probably denatures proteins and enzymes and increases cell wall permeability by disrupting sulfhydryl (-SH) and disulfide (S-S) bonds. The main disadvantages associated with peracetic acid disinfection are the increase of organic content in the effluent due to acetic acid and its pungent odour. Another drawback of the use of peracetic acid is its high cost (Kitis, 2004).

Hydrogen peroxide (H₂O₂) is widely used for disinfection, sterilization and antiseptics. H₂O₂ demonstrates broad-spectrum efficacy against viruses, bacteria, yeasts and bacterial spores (Block, 1991). In general, greater activity is seen against Gram-positive than Gram-negative bacteria. Higher concentrations of H₂O₂ (10 to 30%) and longer contact times are required for sporicidal activity (Russel, 1991).

Phenolic-type antimicrobial agents have long been used for their antiseptic, disinfectant or preservative properties depending on the compound. Phenol induces progressive leakage of intracellular constituents including the release of K⁺ (Lambert and Hammond, 1973). Coagulation of cytoplasmic constituents at higher phenol concentrations causing irreversible cellular damage.
has been described. The phenolics possess antifungal and antiviral properties. Their antifungal action probably involves damage to the plasma membrane resulting in the leakage of intracellular constituents.

QACs are cationic surfactant sanitizers, hard surface cleansers and deodorizers effective against molds, yeast (Carsberg, 1996), Gram positive and Gram negative bacteria except *Pseudomonas* spp. (Langsrud *et al*., 2003). They are more active at a neutral to a slightly alkaline pH but lose their activity at a pH of less than 3.5. These compounds are easily inactivated by inorganic matter, detergent, soaps and hard water. However, they are non corrosive, non irritating and their activity is unaffected by organic load. QACs are membrane-active agents with a target site predominantly on the cytoplasmic membrane in bacteria or the plasma membrane in yeasts. The cationic agents react with phospholipid components in the cytoplasmic membrane thereby producing membrane distortion and protoplast lysis under osmotic stress (Cabral, 1991). QACs are sporostatic and mycobacteriostatic.

Ethylene oxide and propylene oxide are the two most important disinfectants of oxides group. The antimicrobial activity of oxides has been attributed to alkylation of sulfhydryl, amino, carboxyl, phenolic and hydroxyl groups in spores or vegetative cells. The antimicrobial properties of oxides are dependent on factors such as concentration, time of exposure, temperature and water vapour (Block, 1991). Ethylene oxide gas has the disadvantages of being mutagenic and explosive.

Ozone is a powerful oxidizing agent with bactericidal, sporicidal and virucidal activity. It has also been reported that ozone is effective against fungi and protozoa (Block, 1991). Bacterial cell surfaces are the primary target of ozone. Double bonds of unsaturated lipids within the cell membrane are the
primary site of attack. Ozone also reacts with amino acids and modifies purine and pyrimidine bases (Maillard, 2002).

2.15. Factors influencing disinfection

The factors influencing disinfection efficiency are disinfectant concentration, contact time, temperature and pH. Other factors that influence microbial sensitivity to disinfection include attachment to surfaces, encapsulation, aggregation and low-nutrient growth. Antimicrobial activity of a disinfectant varies greatly between different types of microorganisms and might also differ between the strains of the same species. Among vegetative bacteria, *Mycobacteria* are probably the most resistant followed by Gram negative bacteria. The composition and structure of the cell and outer walls of these organisms can account for these phenomena (Maillard, 2002). It has been observed that resistance to various disinfectants to different bacterial species occurring in the environment is much greater than that of the same species grown under laboratory conditions (Carson et al., 1972; Harakeh and Butler, 1985; Kuchta et al., 1985).

2.16. Resistance to disinfectants in bacteria

Effective cleaning and disinfection procedures in the food processing surfaces as well as hospital environment are imperative in preventing the contamination of food products or acquiring infection. The frequent use of commonly used biocides such as phenolics and cationic compounds in their sub inhibitory concentrations has raised concerns about their efficacy and the possible emergence of microbial resistance (Thomas et al., 2000; Chuanchuen et al., 2001; Levy, 2001; Daschner and Schuster, 2004). The impermeability of the cell wall to biocides (Denyer and Maillard, 2002; Cloete, 2003), the efflux pumps enabling the exclusion of intracellular toxic compounds (Levy, 2002;
McKeegan *et al.*, 2003) degradation of the biocide or induction of the cellular stress response can be the inherent or acquired mechanisms of disinfectant resistance commonly encountered.

Propensity of certain *L. monocytogenes* strains towards efficient biofilm formation can provide competitive survival advantage such as enhanced disinfectants resistance. The decreased efficiency of disinfectant on biofilm cells in comparison with their planktonic counterparts has been noticed in earlier studies. The hindered penetration of disinfectants due to the extra polymeric substances (EPS) acting as a permeability barrier, diminished metabolism and quiescence have been hypothesized as major reasons of disinfectant resistance in biofilm (Costerton *et al.*, 1999; Gilbert *et al.*, 2002; Pan *et al.*, 2006).

### 2.17. Influence of antimicrobial agents on bacteria

Antimicrobial agents represent a main therapeutic tool to control and treat a variety of bacterial infectious diseases which may be classified as either bactericidal or bacteriostatic (Hancock, 2005). Bactericidal antimicrobials kill bacteria directly while bacteriostatics prevent them from dividing. However, in practice, both of these are capable of ending a bacterial infection.

Mechanisms of action of antimicrobials include interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of a metabolic pathway and disruption of bacterial membrane structure.
Table 2
Mechanisms of action of antimicrobial agents (Tenover, 2006).

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Antimicrobial(s)</th>
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<tbody>
<tr>
<td>1-Interference with cell wall synthesis</td>
<td>β-lactams: penicillins, cephalosporins,</td>
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<tr>
<td></td>
<td>carbapenems, monobactams.</td>
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<tr>
<td></td>
<td>Glycopeptides: vancomycin, teicoplanin</td>
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<tr>
<td>2 - Protein synthesis inhibition</td>
<td></td>
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<tr>
<td>• Bind to 50S ribosomal subunit</td>
<td>Macrolides, chloramphenicol, clindamycin.</td>
</tr>
<tr>
<td>• Bind to 30S ribosomal subunit</td>
<td>Aminoglycosides, tetracyclines.</td>
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<tr>
<td>3- Interference with nucleic acid</td>
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<tr>
<td>synthesis</td>
<td>Quinolones.</td>
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<tr>
<td>• Inhibit DNA synthesis</td>
<td></td>
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<tr>
<td>• Inhibit RNA synthesis</td>
<td>Rifampin</td>
</tr>
<tr>
<td>4- Inhibition of metabolic pathway</td>
<td>Sulfonamides, folic acid analogues</td>
</tr>
<tr>
<td>5-Disruption of bacterial membrane</td>
<td>Polymyxins, daptomycin.</td>
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<tr>
<td>structure</td>
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</table>

2.17.1. Penicillins

Penicillin group of antibiotic are proved to be potent broad spectrum antibiotics especially when combined with beta lactamase inhibitors. However, several recent studies reported the resistance of penicillin and other beta-lactam antibiotics in the bacterial isolates obtained from cockroaches. Prado et al., (2006) noticed resistance to oxacillin in 61% of coagulase negative Staphylococci isolated from cockroaches. Resistance to methicillin as reported by Gliniewicz et al., (2003) in coagulase negative Staphylococcus obtained from cockroaches suggests the possibility of this insect as one of the sources of methicillin resistant strains in hospital environment. According to
Ahmad et al., (2011), *Enterococci* are the predominant bacterial species in the gut of insects such as house flies and cockroaches and are the reservoir of genes to a wide range of antibiotics including beta-lactams. Though penicillin or ampicillin alone or in combination with an aminoglycoside are the drugs of choice for listeriosis according to Charpentier and Courvalin (1999), there are several reports describing failure with these regimens in this bacterial species (Prazak et al., 2002). Resistance to penicillins may be determined by the organism’s production of penicillin destroying beta-lactamase enzymes which open the beta-lactam ring of penicillins and abolish their antimicrobial activity. Some beta lactamases are plasmid mediated while others are chromosomally mediated.

### 2.17.2. Cephalosporins

Bactericidal effect of this group of antibiotics is through the inhibition of peptidoglycan which is needed for the cell wall synthesis. The first generation of cephalosporins is primarily effective against Gram positive bacteria but second and third generations were later introduced more effective against Gram negative bacteria. Fourth generation cephalosorins are broad spectrum antibiotics with activity against both Gram positive and Gram negative bacteria. *K. pneumoniae* cockroach isolates resistant to ceftazidium was reported by Elgderi et al., (2006). Tilahun et al., (2012) observed high resistance to cephalosporins particularly to cefotaxime and ceftriaxone in all the bacterial isolates from cockroaches. Ennaji et al., (2008) reported 100% of resistance in *L. monocytogenes* to second and third generation cephalosporins. According to MacGowan et al., (1992); Skogberg et al., (1992), cephalosporins are not preferred as the first line treatment against *L. monocytogenes* as its activity against this bacterial species is not efficient.
2.17.3. Tetracycline

Tetracyclines exhibit activity against a wide range of microorganisms and are used extensively in the prophylaxis as well as therapy of human and animal infections. It is also used in animal feed as growth promoters. They inhibit protein synthesis by blocking the attachment of charged aminoacyl–tRNA. The first tetracycline-resistant bacterium *S. dysenteriae* was isolated in 1953. Tetracycline is now frequent in pathogenic, opportunistic and commensal bacteria limiting the use of these antibiotics in treatment. Tachbele *et al.*, (2006) reported tetracycline resistance in the food borne pathogens such as *Shigella*, *Salmonella* and *B. cereus* isolated from cockroaches. The *klebsiella* isolates from this insect resisting tetracycline have been reported by Fotedar *et al.*, (1991). Though over years *Listeria* spp. remained as a sensitive population to antimicrobials, Walsh *et al.*, (2001) noticed tetracycline resistance in *L. innocua* isolates from food. *L. monocytogenes* resisting tetracycline have also been documented by Doucet- Populare *et al.*, (1991); Bertrand *et al.*, (2005). Most of the tetracycline resistant tet genes in bacteria are linked with mobile plasmids or transposons. Rarely pathogens may also acquire resistance by mutation.

2.17.4. Chloramphenicol

Chloramphenicol is a most common broad spectrum antibiotic effective against a variety of Gram negative and Gram positive bacteria including most anaerobic organisms. It binds to the 50S subunit of the ribosome and interferes with the binding of new aminoacids to the nascent peptide chain. Chloramphenicol resistance of enterobacteriaceae members inhabiting in the gut of cockroach has been recorded by Prado *et al.*, (2008). Fotedar *et al.*, (1991); Elgderi *et al.*, (2006) noticed the resistance in isolates
of Klebsiella and Streptococcus from this insect respectively. Though chloramphenicol had been one of the common antimicrobials used against listeriosis, many studies conducted later observed its inefficiency on L. monocytogenes (Scheer and Hirschman, 1982; Richards et al., 1992). Microorganisms resistant to chloramphenicol produce the enzyme chloramphenicol acetyl transferase, which destroys the drug’s activity. The production of this enzyme is usually under the control of a plasmid.

2.17.5. Carbapenams

They have a chemical structure enabling to withstand beta lactamases. They have the broadest antibacterial spectrum of the beta lactam antibiotics. Though active against Gram positive and Gram negative, its effect on intracellular bacteria is restricted. According to Prado et al., (2008) the resistance to imipenam on Enterobacter spp. obtained from cockroaches is less in comparison with other antimicrobials tested. Bouzada et al., (2010) observing imipenam as an effective drug against all the Gram negative bacilli isolated from hospital environment. The efficiency of imipenam on Listeria species was also recorded by Schwaiger et al., (2010).

2.17.6. Quinolones

Quinolones are often used to treat intracellular microbes owing to its ability to penetrate the cell wall. The antimicrobial activity of the first generation quinolones such as nalidixic acid and cinoxacin were highly effective against aerobic Gram negative bacteria but not against aerobic Gram positive bacteria or anaerobic bacteria (Andriole, 2005). The second generation quinolones including norfloxacin, ciprofloxacin, ofloxacin, levofloxacin, lomefloxacin and pefloxacin have antimicrobial activity against both aerobic Gram positive and Gram negative bacteria but they still lacked
activity against anaerobic bacteria. The addition of fluorine at the C-6 position resulted in the innovation of the fluoroquinolones. The third generation fluoroquinolones including grepafloxacin, gatifloxacin, sparfloxacin, and temafloxacin had greater activity against Gram positive bacteria as well as anaerobic bacteria. The fourth generation fluoroquinolones includes trovafloxacin, clinafloxa cin, sitafloxacin, moxifloxacin and gemifloxacin. Fluoroquinolones act by direct inhibition of bacterial DNA synthesis. It inhibits DNA gyrase and topoisomerase IV which have essential roles in DNA replication ultimately resulting in damage to bacterial DNA and bacterial cell death (Drlica and Zhao, 1997).

Resistance to quinolones occurs by altering target mechanism or permeation mechanism through mutation in chromosomal genes. However, the emergence of plasmid-mediated and thus transferable fluoroquinolone resistance has also been reported. The association of fluoroquinolone resistance and ESBL-production in enterobacteriacea is of major concern limiting its use in the treatment of community as well as healthcare acquired urinary tract and intra-abdominal infections as well as traveller’s diarrhoea. According to Dalhoff, (2012) resistance to fluoroquinolone is on a rise among Gram-positive and Gram negative pathogens causing healthcare-associated respiratory tract infections. The reports of resistance to this group in bacterial isolates from cockroach are, however, few. Elgderi et al., (2006); Prado et al., (2008) noticed 100% sensitivity of the enterobacteriacea members isolated from cockroach towards norfloxacin and ciprofloxacin. The intrinsic resistance of *L. monocytogenes* to nalidixic acid was noticed by various studies. Ennaji et al., (2008); Sakaridis et al., (2011) observed 100% resistance to nalidixic acid in *L. monocytogenes*. 
2.17.7. Macrolids

Macrolids have a lactam ring to which deoxy sugars are attached. They are mainly bacteriostatic but in high concentration can be bactericidal. These drugs may interfere with the formation of initiation complexes for peptide chain synthesis or aminoacyl translocation reactions inhibiting protein synthesis. Ortega - Loayza et al., (2010) on their study focusing on MRSA isolates obtained from a cutaneous infection in a paediatric department noticed high level resistance to erythromycin. Resistance of *Staphylococcus* species isolated from domestic cockroaches has been reported by Mpuchane et al., (2006). Ahmed et al., (2011) noticed high frequency of erythromycin resistance in enterococci isolated from house flies and cockroaches. Plasmid mediated transfer of erythromycin in *Listeria* spp. has been demonstrated by Roberts et al., (1996); Charpentier and Courvalin, (1999).

2.17.8. Oxazolidinones

Oxazolidinones exhibit a unique mechanism of protein synthesis inhibition with bacteriostatic activity against many important human pathogens including methicillin-resistant *S. aureus*, vancomycin-resistant enterococci and penicillin and cephalosporin resistant *Streptococcus pneumoniae* (Diekema and Jones, 2000). In consistent with the above observations, Gould et al., (2010) observed the susceptibility of clinical strains of methicillin resistant staphylococci to linezolid. However, Bouzada et al., (2010) observed linezolid resistance in enterococci isolated from hospital environment.

2.17.9. Glycopeptide

Vancomycin and teicoplanin are the members of this group having clinical use. Vancomycin act by inhibiting cell wall synthesis in Gram-
positive bacteria. Due to the different mechanism by which Gram-negative bacteria form their cell walls and the various factors related to entering the outer membrane of Gram negative organisms, vancomycin is not effective on Gram negative bacteria except some non-gonococcal species of Neisseria. However, vancomycin resistance has been reported in Enterococcus and have spread with unanticipated rapidity and are now frequently encountered by hospitals (Jones et al., 1995). Tachbele et al., (2006) noticed S. aureus obtained from P. americana as showing high level resistance to vancomycin. Prado et al., (2008) observed susceptibility in coagulase negative Staphylococcal isolates to this antibiotic.

2.17.10. Aminoglycosides

This group of antibiotics act by binding to 30S sub unit of ribosome and blocking initiation of protein synthesis. They also make mRNA misread and inhibit protein synthesis. Chromosomal resistance of microbes to aminoglycosides principally depends on the lack of a specific protein receptor on the 30S subunit of the ribosome. Plasmid mediated aminoglycoside resistance is by the production of adenylating, phosphorylating or acetylating enzymes that destroy the drugs. A third type of resistance consists of an outer membrane change that reduces active transport of the aminoglycoside into the cell so that the drug cannot reach the ribosome and this is often plasmid mediated. Aminoglycosides are used to treat infections with Gram negative bacteria viz. Acinetobacter spp., P. aeruginosa, Enterobacter spp.
### 2.18. Mechanisms of antimicrobial resistance

**Table 3.**

**Mechanisms of antimicrobial resistance in bacteria**

*McDermott et al., 2003*

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Modification of the antimicrobial agent</td>
<td>Aminoglycosides, chloramphenicol and β-lactams</td>
</tr>
<tr>
<td>2. Alteration or protection of the target site</td>
<td>Aminoglycosides, β-lactams, macrolides, quinolones, rifampicin, trimethoprim, and tetracycline.</td>
</tr>
<tr>
<td>3. Decreased antibiotic accumulation</td>
<td>Many antibiotics (quinolones).</td>
</tr>
<tr>
<td>• Decreased uptake</td>
<td>Tetracycline, macrolides, quinolones and chloramphenicol</td>
</tr>
<tr>
<td>• Increased efflux</td>
<td></td>
</tr>
<tr>
<td>4. Alteration of the metabolic pathway</td>
<td>Sulfonamides, trimethoprim.</td>
</tr>
</tbody>
</table>

Acquiring resistance in bacteria is concern where resistant population spread under selective pressure of use of that agent. Several mechanisms of acquiring antibiotic resistance have been described. The organism may acquire genes encoding enzymes that destroy the antimicrobial agent before producing an effect. The bacteria may acquire efflux pumps that extrude the antimicrobial agent from the cell before it can exert its effect on the target. Genes may also be acquired to produce altered bacterial cell wall which no longer contains the binding site of the antimicrobial agent.

Acquisition of genetic information in bacteria may occur through different methods of horizontal gene transfer viz. transformation, conjugation or transduction. Transformation is a process by which bacteria take up free DNA directly from their environment and in transduction bacterial DNA is moved from one bacterium to another through a bacteriophage. Conjugation is a process by which a living bacterial cell transfers genetic material through
cell-to-cell contact. A number of different DNA elements are involved in the development of resistance in bacteria such as plasmids, transposons, genomic islands, phage, integrons and gene cassettes (Normark and Normark, 2002). Plasmids are extra-chromosomal DNA molecules that replicate independently of the chromosome; often carry genes that confer a selective advantage to the bacterium harbouring them particularly genes conferring antibiotic resistance. Other functions carried by plasmids are synthesis of antibiotics, synthesis of toxins and proteins for bacterial pathogenesis, synthesis of enzymes for the utilization of unusual carbon sources and resistance to heavy metals. Plasmids that carry resistance genes are called R plasmids. These plasmids were first discovered from *Shigella flexneri* strains in the 1950s. Since then, they have been increasingly being reported in both Gram-positive and Gram-negative bacterial pathogens and commensal organisms. A single plasmid may simultaneously mediate resistance to multiple antimicrobials or to be shared among different bacterial genera (Harbottle *et al.*, 2006). In addition to carrying resistance genes, plasmids can serve as vehicles for other genetic elements important in antimicrobial resistance such as transposons and integrons. The bacteria with multiple resistance may become a cause of concern particularly in hospitals and other health care institutions.