Chapter 2

Review of Literature

2.1 INFECTIOUS DISEASES

IDs are a group of diseases caused by distinctive pathogens such as bacterium, virus, fungus or protozoan. IDs occur when the pathogens make their entry into the host cell and then, the pathogens initiates its replication process. The duration taken by the pathogen to cause the disease is completely depends on the virulence of the causative agent (Mehnaz, 2009). Even though, there are much advancement in diagnostic techniques and treatment procedure, still IDs continue to be serious threat to human beings as many of the people especially children in the developing countries do not have access to these advance methods (Gwatkin et al., 1999). The diseases caused by infectious agents are usually falls into five phases namely 1) incubation period 2) prodromal phase 3) clinical phase 4) decline phase and 5) recovery phase. The incubation period is the duration between the infection of the agent and the appearance of signs and symptoms in the host where as in the prodromal phase, the patient shows temperate symptoms. In the clinical phase, the actual signs and symptoms of the disease is found in the infected patients and in the last two phases, the infection of the patient gets lower and recovered from the disease (Murray et al., 2012).

IDs can be broadly classified into three groups based on the time taken by the pathogen to show the symptoms and lasting of those symptoms

- Acute
- Chronic
- Latent

It may be localized or systemic infection. Localized infection is referred to cause the infection in a particular site whereas the systemic infection is spreading of the infection from localized site to blood stream or other tissues (Goldmann, 1992; Saiman et al., 2001)

2.2 *Streptococcus pneumoniae*

2.2.1 GENERAL CHARACTERISTICS

The *S. pneumoniae* is a GP encapsulated (Verma and Khanna, 2012) coccus, alpha-hemolytic and belongs to a member of lactic acid bacteria. In 1881, a French scientist named Louis Pasteur was the first to segregate and develop *S. pneumoniae*. The organism is also termed as *Pneumococcus* or Diplococcus pneumoniae. *S. pneumoniae* is a human pathogen (Tettelin et al., 2001) and it is often associated with a extensive range of community-acquired diseases which covers meningitis, pneumococcal infections of upper respiratory tract that results in pneumonia and other infections like sepsis, pericarditis, peritonitis, acute sinusitis, cellulitis, conjunctivitis, septic arthritis, osteomyelitis, endocarditis, and otitis media (Allegrucci and Sauer, 2007). Collectively, these diseases are responsible for millions of deaths worldwide annually and both children and adults are the affected individuals.

2.2.2 GENETICS

The genome of *S. pneumoniae* consists of a single circular chromosome and the length ranges from 2,240 to 2,270 kilo base pair (bp) with approximately 39.7% of GC content. The genome has large number of insertion sequences which cover almost 5% of the genome (Tettelin et al., 2001) but most of these sequences were non-functional because of insertions, deletions or point mutations. The physical mapping studies on *S. pneumoniae* reveals that the genome of *S. pneumoniae* has at least six rRNA operons and three structural RNAs (a tRNA like/mRNA like (tm) RNA, signal recognition particle RNA and a ribonuclease P RNA) (Gasc et al., 1991). The genome has large amount of ATP-dependent transporters and it also has sodium ion/proton exchanger and sodium ion driven transporters. Around 30% of transporters in this organism were thought to be sugar transporters (Paulsen et al., 2000). The extracellular enzymes present in *S. pneumoniae* metabolize hexosamies and polysaccharides. This result in supplying of nitrogen and carbon to *S. pneumoniae* that plays a vital role in the synthesis of capsule and factors involving in virulent activity (Tettelin et al., 2001). Approximately 90 varied serotypes of *S. pneumoniae* are

determined on the basis of variation in the content of polysaccharide capsule (Verma and Khanna, 2012).

2.2.3 CULTURAL CHARACTERISTICS

S. pneumoniae is one of the fastidious bacteria and the optimal growth of the organism is at 35-37°C with approximately 5% CO_2. The organism is commonly cultured on blood agar but it also has the ability to grow on chocolate agar plate (CAP). The colonies on blood agar seems to be tiny, moist (may be mucoidal sometimes) and grey in color. It also forms a zone of α-hemolysis in green color. This α-hemolytic characteristics play an important role in differentiation of *S. pneumoniae* from various other species but not possible to differentiate from viridans Streptococci. It can be differentiated from viridans Streptococci only after 24-48 hours of culturing as the colonies will get smoothed and the middle part of the colonies will become depressed which is not happening in the viridans Streptococci (World Health Organization, 2011).

The colonies of *S. pneumoniae* are opaque on the transparent surface of agar as the organism undergoes the phase variation. Three phase variants have been determined on the basis of colony morphology (Cundell et al., 1995; Weiser et al., 1996; Overweg et al., 2000). The transparent variants are capable of colonizing the nasopharynx expeditiously and adhere the human endothelial cells and type II lung cells in culture (Cundell et al., 1995) where as the opaque variants are insufficient to colonize (Weiser et al., 1994). The organism has its own destructive mechanism to catabolise the following pentitols through pentose phosphate pathway (Tettelin et al., 2001).

- Cellobiose
- Fructose
- Fucose
- Galactose
- Galactitol
- Glucose
- Glycerol
- Lactose
- Mannitol
- Mannose
- Raffinose
- Sucrose
- Trehalose
- Maltosaccharides

2.2.4 VIRULENCE FACTORS

The polysaccharide capsule, toxin pneumolysin, different surface-located and metal binding proteins and neuraminidases are the most vital virulent factors produced by *S. pneumoniae* (Kadioglu et al., 2008; Mitchell and Mitchell, 2010). The polysaccharide capsule has anti-phagocytic characteristics and thus, it is considered as a major virulent determinant. The capsule also minimizes the entrapment in mucus and at least 90 varied capsule types have been determined (Brown et al., 1983; Hoskins et al., 2001; Abeyta et al., 2003; Hyams et al., 2010). The encapsulated strains of *S. pneumoniae* are 10*5 times more virulent than the strains which do not have capsule.

There are large numbers of surface-associated proteins present in *S. pneumoniae.* Among them, pneumococcal surface protein A (PspA) and C (PspC) are considered to be as the best marked choline-binding proteins. PspA act as a central factor in immune escape of *S. pneumoniae* by interrupting the complement activation and deposition which is mediated through the classical and alternative pathways. PspA has the ability to bind with lactoferrin. PspC joins with human IgA and polymeric immunoglobulin receptor which provokes transcytosis and adhesion (Li et al., 2007; Ricci et al., 2013). The other virulent factor toxin pneumolysin of *S. pneumoniae* elicits the production of inflammatory cytokines like tumor necrosis factor α and interleukin-1 β. It also has the capacity to restrain the whipping of cilia present on human respiratory epithelial cells and to interrupt the monolayers of cultured epithelial cells in upper respiratory tract and alveoli. Thereby, it diminishes the bactericidal activity and migration of neutrophils and damages the blood-brain-barrier (AlonsoDeVelasco et al., 1995; Rogers et al., 2003). The neuraminidases of *S. pneumoniae* are the enzyme involves in the cleavage of sialic acid from glycolipids and gangliosides of host and then it attaches the epithelial cells (Krivan et al., 1988).

The murein hydrolase is an N-acetylmuramoyl-L-alanine amidase and it is referred to as autolysin. It is required to bind with choline which is present on teichoic acid for their normal functioning (Briese and Hakenbeck, 1985). In addition, another important choline binding protein of *S. pneumoniae* is CbpA which helps in bacterial adherence (Rosenow et al., 1997; Gosink et al., 2000; Jedrzejas, 2001).

2.2.5 PATHOGENESIS

S. pneumoniae is normally carried by many healthy individuals in nasopharynx with an incidence of about 40% and 15% in infants (2-15 years) and adults (≥ 16 years) respectively (Barichello et al., 2012). *S. pneumoniae* can be transferred from one person to other person through coughing and sneezing (AlonsoDeVelasco et al., 1995; Bogaert et al., 2004). The organism is responsible for causing varied infections like pneumonia, bacteremia, meningitis and otitis media. The organism infects people of all ages and the children below <2 years and elderly adults are at the higher risk towards this infection (Burman et al., 1985; Pallares et al., 2003). It should intakes essential nutrients to survive and replicate in the host during various forms of infections (Honsa et al., 2013). The most important characteristics of *S. pneumoniae* are to cause the pneumococcal disease with the nasopharyngeal colonization by the homologous strain of *S. pneumoniae* (Gray et al., 1980; Faden et al., 1997). The pathogenic route for *S. pneumoniae* is shown in Fig. 2.1.

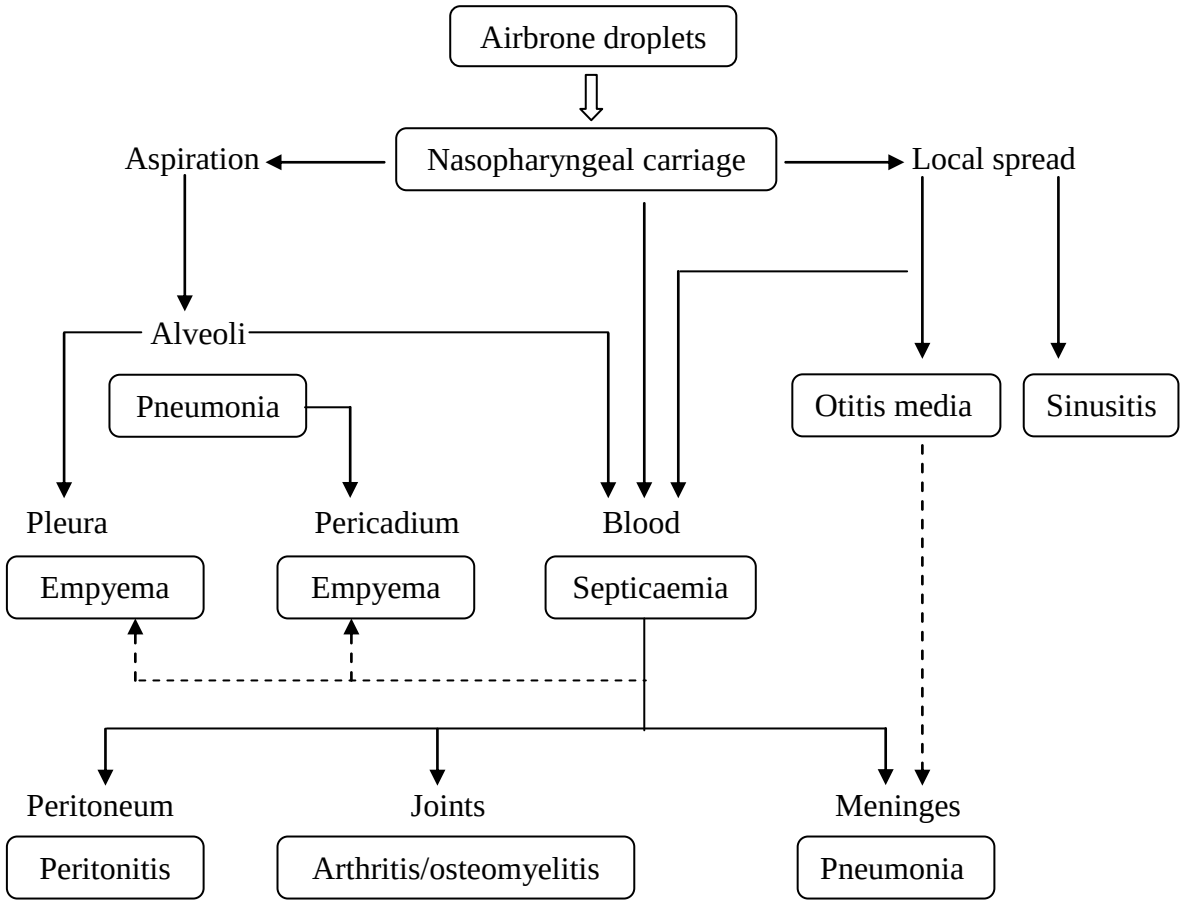

Fig. 2.1: Pathogenic route for *S. pneumoniae* infection

2.2.6 LABORATORY DIAGNOSIS

S. pneumoniae can be determined by performing Optochin test, Gram stain and Catalase tests. The serological tests can be done for the identification of their serotype if these tests indicate that the isolate is *S. pneumoniae*. The identification and characterization of *S. pneumoniae* is shown in Fig. 2.2 (World Health Organization, 2011)

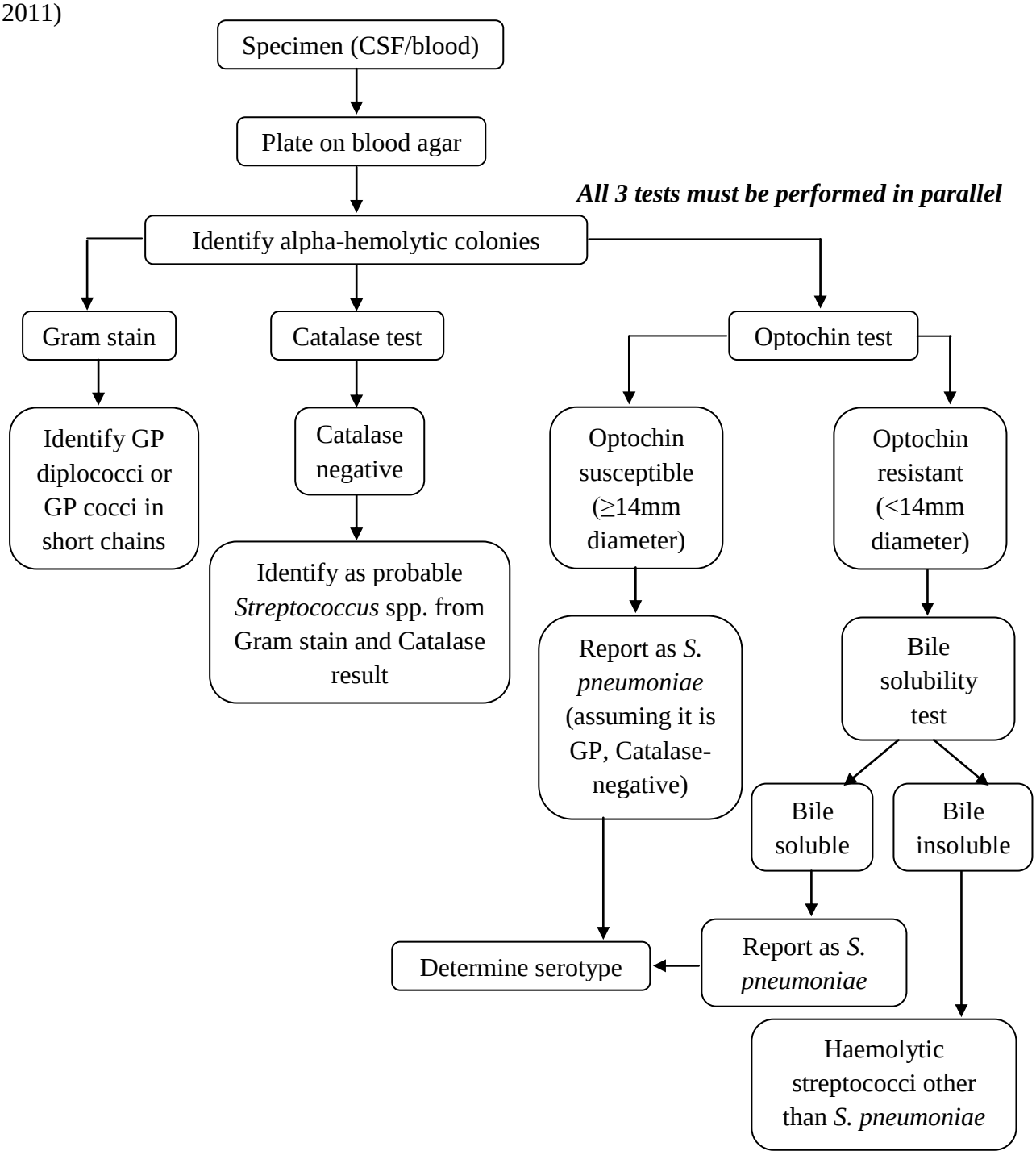

Fig. 2.2: Work flow for characterization and determination of a *S. pneumoniae* isolate

2.3 *Haemophilus influenzae*

2.3.1 HISTORY

Richard Pfeiffer, a German bacteriologist was the first to identify a bacillus present in sputum of patients suffering from influenza during the period 1889-92 and in 1892; he confirmed that this bacillus is the etiological agent for influenza. After a year, he isolated the organism on the media containing blood. He had an ambiguity on importance of organism which is developed during 1918-19 pandemic when the bacillus could not be found in early victims, but Pfeiffer's claims would be reflected in the nomenclature chosen in 1920, Haemophilus (blood-lover) influenzae (Hirschmann and Everett, 1979).

H. influenzae is a fastidious GN bacillus and coccobacillus, non-motile, pleomorphic and non encapsulated (Oliveira and Pijoan, 2004). The organism is habitually found in upper respiratory tract of healthy humans (Laurenzi et al., 1961). *H. influenzae* causes various systemic life-threatening infections and in addition, it commonly causes serious diseases that affect upper and lower respiratory tract (Peltola, 2000). *H. influenzae* respiratory tract infections include pneumonia in children and adults, acute otitis media, acute maxillary sinusitis, epiglottitis and acute exacerbations of bronchitis in patients with underlying chronic obstructive pulmonary disease (Kilian et al., 1972; Chapin and Doern, 1983). Thus, the organism is considered as an important community-acquired pathogen and cause significant morbidity and mortality throughout the world (Ramirez and Anzueto, 2011).

2.3.2 GENETICS

The genome of *H. influenzae* is of 1.83 Mega bp long and it is the first living organism to have its complete genome sequenced. In this genome, there are 1703 coding sequences (CDS) are present and among these, 58% of CDS are allotted to one of 102 biological categories (Fleischmann et al., 1995; Strouts et al., 2012). The rest of the regions are novel proteins and they are not matched with other proteins in the databases. And also, they do not exhibit any significant similarity with other genes. An updated investigation on genome and encoded proteins identified that a general

function can be assigned for 83% of *H. influenzae* proteins (Tatusov et al., 1996). The DNA of *H. influenzae* is composed of 37 mol% of G+C content and hence, the restriction enzymes having C and G alone can not cut the DNA repeatedly than the enzymes with sites having all four bases (Roy and Smith, 1973; Butler and Moxon, 1990).

2.3.3 CULTURAL CHARACTERISTICS

The optimal growth of *H. influenzae* is at 35-37°C with approximately 5% of CO_2 or in a candle-jar. The organism needs hemin, an X factor and nicotinamide-adenine-dinucleotide (NAD), a V factor for their growth. CAP is a standard medium which is used to grow *H. influenzae* and this medium has horse blood which is heat lysed. The horse blood is a good resource for hemin and NAD factors and sometimes, sheep blood is also used to grow *H. influenzae*. The heating process while preparing chocolate agar releases NAD and also inactivates all the growth inhibitors. The hemin is obtained from both hemolyzed and non-hemolyzed blood cells. This leads to development of colonies of *H. influenzae* CAP. In some cases, the NAD factor is available commercially or it can be made in laboratory and then they are incorporated into chocolate agar as media supplements. The colonies of *H. influenzae* appear as big, circular, convex, smooth and opaque on CAP. The color of the colonies may be ranges from colorless to grey. The capsulated *H. influenzae* strains are more mucoidal than the non-capsulated *H. influenzae* strains. The colonies of non-capsulated strains are tiny and compact grey color. There is no evident for hemolysis or discoloration of colonies on CAP. The colonies of this organism have pungent indol odour and hence, the cultured plates should not be opened (World Health Organization, 2011).

Many studies have been reported that 38% of *H. influenzae* genes are not capable to grow on nutrient rich medium and most of the putative essential genes of *H. influenzae* are identified as nonessential in some other organisms (Akerley et al., 2002; Mohd-Zain et al., 2004). The organism has the ability to identify essential pathways which have different mechanism in other organisms that has more genome content. *H. influenzae* also has some factors that will be destructive when essential gene is reduced (Karlin et al., 1996; Glass et al., 2006).

2.3.4 VIRULENCE FACTORS

The possession of type b capsule has been appearing to be a major virulent factor in *H. influenzae*. The type b strains of *H. influenzae* have more virulent activity than type a strains that are more virulent than any other capsular strains of *H.* influenzae. This was again confirmed during the animal studies where the isogenic strains of *H. influenzae* are transforming with capsule related DNA (Zwahlen et al., 1989; St Geme and Cutter, 1996; Gilsdorf et al., 2004). The capsule in *H. influenzae* protects them against complement-mediated lysis and phagocytosis. Thus, it helps in colonisation of bacterium and considered as an important virulence factor for invasive disease (Moxon and Kroll, 1990; Tunkel and Scheld, 1993).

Another most vital virulent factor present in *H. influenzae* is endotoxin or its sub element lipooligosaccharide (LOS) (Makela, 1988). The LOS of *H. influenzae* is similar to the lipopolysaccharide (LPS) of enteric GN bacteria (Gibson et al., 1993). It has O-antigen units which is not present in classic enterobacterial LPS and in this way, it differs from LPS of classic enterobacteriaceae family. The endotoxin is found in outer membrane of *H. influenzae* and however, it is released in biologically active state by *H. influenzae* when the organism is growing or being responded to any antibiotics. (Mandrell et al., 1992; Gu et al., 1995; Gilsdorf, 1998). When the endotoxin interacts with polymorphonuclear neutrophils, macrophages and other cell types, it releases different vasoactive amines and some other mediators of inflammation (Valvano, 1992; Moxon et al., 1994; DeMaria et al., 1997).

The pili of *H. influenzae* are peritrichous (tail like structures all over surface) and polymeric in nature which projects from the outer membrane of *H. influenzae* (Stull et al., 1984; Gilsdorf et al., 1997) and it attaches to sialic acid that has lactosylceramide structures on epithelial cell surfaces (Van Alphen et al., 1991). 5 genes namely hifA, hifB, hifC, hifD and hiFE are required for the production of pili and all these five genes are found in hifE clusters (Watson et al., 1994; Gilsdorf et al., 1997). The phase variation changes the expression of pili in *H. influenzae* and this process takes the help of slipped strand impairing (Gilsdorf et al., 1997). This shows that *H. influenzae* has the ability to change and adapt the environment quickly.

2.3.5 PATHOGENESIS

The following are the infections caused by *H. influenzae*

- Meningitis
- Epiglottitis
- Pneumonia
- Otitis media and other infections

The asymptomatic colonization of nasopharynx is the initial process in causing invasive and respiratory *H. influenzae* infections. This process will occur in both capsulated as well as non-capsulated *H. influenzae* strains (Van Eldere et al., 2014). The droplets from *H. influenzae* infected patients are mainly responsible for the transmission of disease. Initially, the organism makes a contact with respiratory mucus soon after the inhalation of *H. influenzae* and attaches to respiratory epithelial cells with the help of different adhesions (Lipsitch and Moxon, 1997; Foxwell et al., 1998; Virkola et al., 2000). Some of the important adhesions of *H. influenzae* include pili, fimbriae, penetration and adherence proteins, lipo-oligosaccharides, *Haemophilus* surface fibrils (Hsf) and opacity-coupled protein. Furthermore, NTHi has epithelial cell adhesions which are large proteins with more molecular weight and these adhesions are alternate form of Hsf of Hib (Rodriguez et al., 2003; Ecevit at al., 2004). The Hib strain causes pneumonia which is normally segmental or lobar. The clinical characteristics and radiological appearances of pneumonia caused by Hib strain are same to that of pneumonia caused by *S. Pneumonia*. In chronic bronchitis, the bronchi do not have the ability to sterilise them self and hence, they easily are accountable for colonisation by non-capsulated *H. influenzae* (Turk, 1984).

2.3.6 LABORATORY DIAGNOSIS

Kovac's oxidase test is used to recognize *H. influenzae* and it is essential to find out the presence of NAD and hemin factors. When these tests turned out to be positive for *H. influenzae*, serotyping tests can be carried out. The identification and characterization of *H. influenzae* is shown in Fig. 2.3 (World Health Organization, 2011).

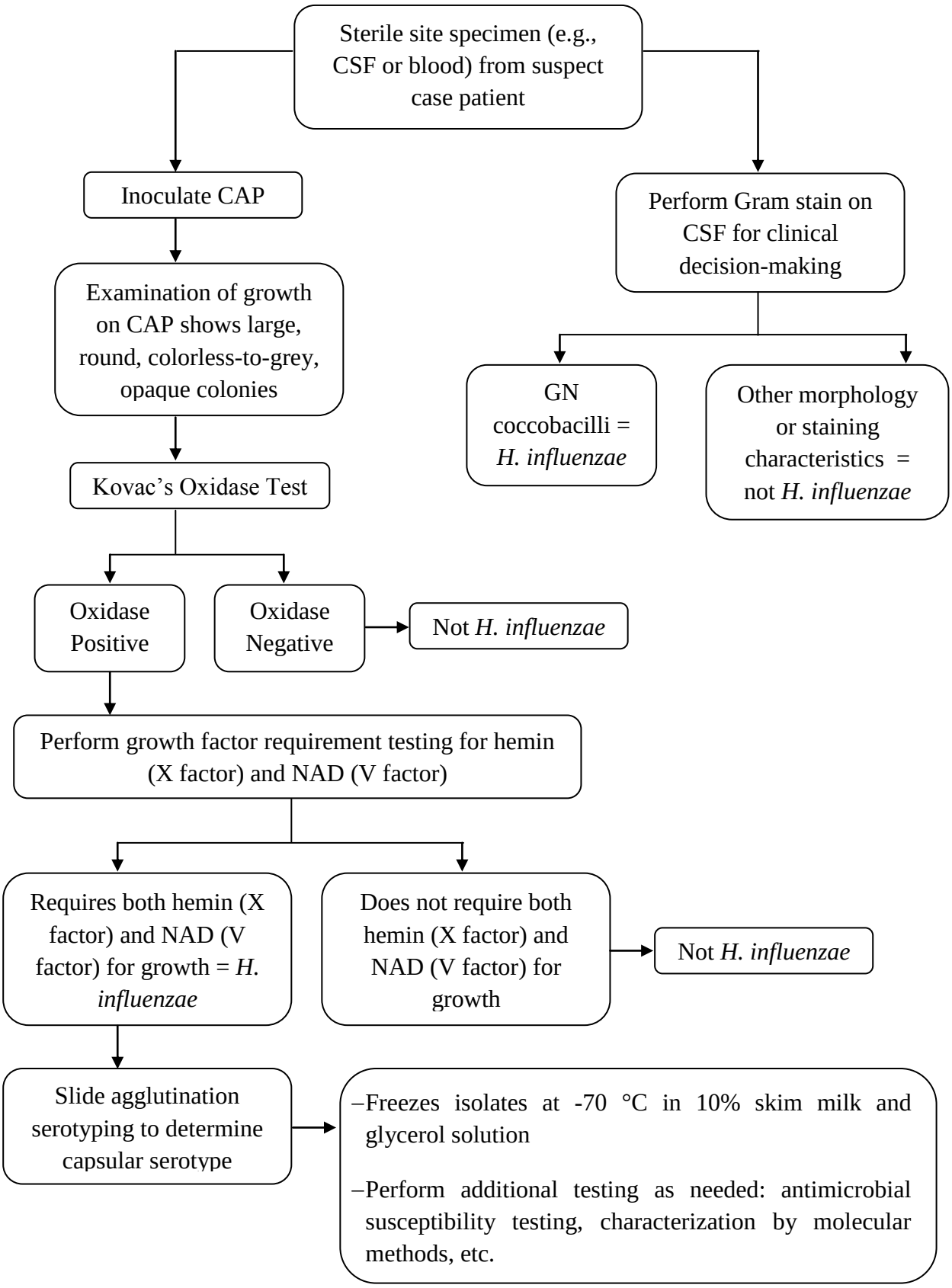

Fig. 2.3: Work flow for identification and characterization of a *H. influenzae* isolate

2.4 MELIOIDOSIS

2.4.1 HISTORY

Melioidosis is a tropical disease, caused by a soil bacterium *B. pseudomallei* and it affects the people who live in Southeast Asia and northern Australia (Valade et al., 2009; Chalmers et al., 2014). Foremost, *B. pseudomallei* were determined by Alfred Whimore, a British pathologist from a young man who was dying with pneumonia disease at a hospital in Burma. Hence, the bacterium is also called as Whitemore bacillus (Smith et al., 1987). *B. pseudomallei* is an environmental GN bacillus, motile and facultative intracellular bacterium (Kespichayawattana et al., 2000; Cheng and Currie, 2005; Burtnick et al., 2008). The name melioidosis was obtained from two Greek words; "melis", which means distemper of asses and "eidos", which means resemblance (Raja et al., 2005). In 1921, Stanton and Fletcher created the term melioidosis (Boruah et al., 2013). The disease is considered as an important reason for causing fatal septicaemia in endemic tropical regions (Cheng and Currie, 2005). Melioidosis is portrayed by the pneumonia disease normally and some times, it is characterised by various organ abscesses in infected patients (Campos et al., 2013). The death rate associated with this deadly disease is up to 40%. Previously, melioidosis clinically depicts like a glanders disease which is also a bacterial disease caused by *Burkholderia mallei*. (Jabbar and Currie, 2013).

Earlier, *B. pseudomallei* was categorized under *Pseudomonas* species because the biochemical, cultural and morphological properties of the organism were very much alike with the properties of *Pseudomonas* species. (Loveleena et al., 2004; Alice et al., 2006). Later on, 7 species in *Pseudomonas* were grouped into the new genus named *Burkholderia*. This grouping was carried out on the basis of phenotypic properties, 16S ribosomal RNA sequences, DNA-DNA homology values and finally based on cellular lipid and fatty acid composition (Yabuuchi et al., 1992; U'Ren et al., 2007). *B. pseudomallei* is incorporated in CDC list as a category B agent because the organism has the ability to infect and cause the disease in individuals even through the inhalation of this organism (Holden et al., 2004; Liguori et al., 2011; Wiersinga et al., 2012; Yeager et al., 2012).

2.4.2 GENOME PLASTICITY

There are two chromosomes present in the genome of *B. pseudomallei* (Ong et al., 2004). The chromosome 1 is of 4.07 Mega bp long with 3,460 CDS whereas the chromosome 2 is of 3.17 Mega bp with 2,395 CDS. The chromosome 1 is responsible for core functioning connected with cell growth and metabolism and the chromosome 2 accomplishes different accessory functions related to adaption of the organism and their survival in various niches (Stevens et al., 2002). The chromosome 1 and 2 do not exhibits greater likeness apart from rRNA bundles. *B. pseudomallei* has 26 pseudogenes (genes that do not expressed) and 42 insertion sequence factors. One of the most outstanding characteristics of *B. pseudomallei* is that it has 16 genomic islands which make up 6.1% of *B. pseudomallei* genome (Holden et al., 2004). The proteins of *B. pseudomallei* have extensive number of Orthologous matched with *Ralstonia solanacearum* when compared with that of other pseudomonads (Salanoubat et al., 2002).

2.4.3 VIRULENCE FACTORS

B. pseudomallei is flexible in nature so that it has the ability to live in different adverse conditions like very high or low temperature, lack of nutrients, acidity or alkali pH and in any antiseptic or disinfectant solution. The organism generates following enzymes for their adaptation in host cell.

- Catalase
- Lecithinase
- Lipase
- Peroxidase
- Protease
- Superoxide dismutase

It also produces hemolysins, siderophore and a cytotoxic exolipid. Normally, *B. pseudomallei* exhibit resistance against positive charged peptidases and complement lysosomal defensins (White, 2003). The major virulent determinant of *B. pseudomallei* is polysaccharide capsule (Steinmetz et al., 1995; Reckseidler-Zenteno

et al., 2005). This capsule forms microcolonies in a secured condition and there is a significant alteration in phenotype of *B. pseudomallei*. Thereby, it leads to resistant to variety of antibiotics (Vorachit et al., 1993; Sawasdidoln et al., 2010).

B. pseudomallei changes the outer membrane of infected cells and make its surface to merge with external surface of nearby cells. This results in the formation of multi nucleated giant cell and causes increase in cell to cell dissemination in infected hosts (Kespichayawattana et al., 2000; St John et al., 2014).

2.4.4 PATHOGENESIS

Melioidosis commonly infect the people those who have routine work in soil and water environment. Melioidosis causing organism, *B. pseudomallei* can affect the individuals by entering through the cut in already existing lesions or by drinking of organism polluted water or by iatrogenic inoculation or by infection spreads from laboratory handling *B. pseudomallei*. Amongst, the most widely recognized procedure of infection is inoculation (Loveleena et al., 2004; Cheng and Currie, 2005).

The clinical features of melioidosis typically show extensive clinical series from benign localized infection to quickly deadly septicemia (Chantratita et al., 2012). *B. pseudomallei* has the ability to stay inactive in host for many days. When the situation gets positive for the organism to increase their numbers, the organism breaks out and then become active (Ashdown and Guard, 1984; Naigowit et al., 1992; Van Phung et al., 1993). In most of the endemic areas, a noteworthy extent of people who are in good condition has antibodies against *B. pseudomallei* and it may be the outcome of subclinical infection (Barnes et al., 2004; Rolim et al., 2011).

Many studies have confirmed that *B. pseudomallei* can live and replicated inside the efficient phagocytes such as monocytes or macrophages and cell lines of neutrophils (Pruksachartvuthi et al., 1990; Jones et al., 1996; Mulye et al., 2014; Williams et al., 2014). *B. pseudomallei* avoids the fusion between phagosome and lysosome and thereby, it demolishes the membrane of phagosome immediately after 15 minutes of ingestion (Harley et al., 1998). The association between *B. pseudomallei* and macrophages are very well understood through ultra structural

studies. These studies reveal that the individuals suffering with melioidosis has reduced the initial fusion of phagolysosome and thereby, it increases the quantity of intracellular bacteria. These results were compared with healthy controls and they do not exhibit such a reduction in the phagolysosome fusion (Cheng and Currie, 2005; Chieng et al., 2012; Weiss and Schaible, 2015).

Tumor Necrosis Factor (TNF)-α is principally developed by macrophages and sometimes, by B or T cells and fibroblasts. It is known as a powerful proinflammatory cytokine. The highest intensity of TNF-α is related with death rate. TNF-α has the ability to decrease the infection and when the TNF-α is neutralized, it rises the sensitivity towards melioidosis (Santanirand et al., 1997). Interferon-γ is considered to play an effective role in controlling the disease. Natural-killer cells and activated CD8+ cells are responsible for developing interferon-γ during the nonspecific immune response against *B. pseudomallei* (Lertmemongkolchai et al., 2001; Haque et al., 2006; Koo and Gan, 2006; Breitbach et al., 2009). Many studies reported that melioidosis infect the individuals who lack T-cell activities and the individuals with good T-cell functioning do not develop the disease (Barnes et al., 2004; Ye et al., 2008).

2.4.5 CLINICAL DESCRIPTION

B. pseudomallei takes a lot of time to initiate the clinical features after its entry to the host and it is very difficult to characterize the duration (Currie et al., 2010; Morris et al., 2015; Welkos et al., 2015). Studies have been proposed that *B. pseudomallei* takes 9 days of time on average to exhibit the symptoms of melioidosis and usually it varies from 1-21 days. This condition was observed in 25% of melioidosis infected patients who were being exposed to soil environment during the floods (Currie et al., 2000; Nelson et al., 2011).

The clinical features of melioidosis are septicaemia, pneumonia, acute localized or chronic infections. *B. pseudomallei* can be transferred from one person to another person by skin contacting each other, swallowing or breathing of organism contaminated resource (Suputtamongkol et al., 1994).

The most adverse form of melioidosis is an acute bacteremic infection and it is often associated with high degree of death rate. Usually, the disease represent as instinctive abscesses, or septic joint inflammation or fever with temperature > 38.3°C which cannot be diagnosed even after a week (Jesudason et al., 2003). Histopathologically, melioidosis is portrayed by swelling of different organs and fascia accumulated with pus and sepsis. Cutaneous abcesses results from bacteremic spread to skin of infected individuals (Mickail et al., 2012). Sometimes, the cutaneous abcesses happens without the presence of systemic infection (Teo et al., 2006). The signs of cutaneous melioidosis are serious tiny blisters with pus, systemic vasculitis and broad ecthyme like lesions. The severity of cutaneous melioidosis can be indicated by low intensity of albumin or lymphocytes and high intensity of white blood cells (Gibney et al., 2008). The major characteristics of neurological melioidosis are weakness in single limb, damaging cerebellum and partial paralysis of limb (Currie et al., 2000). The symptoms of pulmonary melioidosis are high fever and pulmonary distress with swelling and the patient may die if the disease is not treated (Jansen et al., 2005).

2.4.6 LABORATORY DIAGNOSIS

The medicinal practitioner can believe that the disease as melioidosis when the patient has fever with a record of navigation from endemic areas where the disease is more common or made a contact with *B. pseudomallei* or the occurrence of risk factors like kidney disease, liver disease and diabetes mellitus (Limmathurotsakul and Peacock, 2011).

The standard method for examination of melioidosis is *B. pseudomallei* determination by culture procedure from samples like blood, sputum (combination of saliva and mucus) or other clinical based specimen (Pongsunk et al., 1999; Novak et al., 2006; Nhem et al., 2014). There are many problems arises during the culturing method like, the method needs more days of incubation and this leads to postponement of antibiotics treatment to melioidosis infected patients (Brook et al., 1997; Limmathurotsakul et al., 2010; Goodyear et al., 2013). It is also not easy to determine *B. pseudomallei* from the contaminated culture and the organism shows resistant activity to various antibiotics needed in empirical therapy (O'Brien et al.,

2004; Wuthiekanun et al., 2011). Hence, nucleic acid or antigen or antibody detection tests can be carried out to fasten the diagnosis and these tests afford results more quickly whereas the standard culturing method take long period to provide the results.

The sensitivity and specificity of direct immunofluorescent technique used to diagnose melioidosis was 66% and 99.4% and it gives the result in 2 hours. Whereas, the sensitivity of rapid immunofluorescent method is as same as direct technique but the specificity was 99.5% and produce the results in 10 min (Walsh et al., 1994; Wuthiekanun et al., 2005). The indirect immunofluorescent method is used for antibody detection in the diagnosis of melioidosis and it is a faster technique providing the results more quickly with 92% of negative predictive value for IgM antibody detection test and 100% of positive predictive value for combined IgM and IgG antibody detection test (Mathai et al., 2003).

The most commonly used technique for diagnosing melioidosis is indirect haemagglutination assay and the method fails to exhibit sensitivity (Ashdown, 1987; Harris et al., 2009; Harris et al., 2011). This failure leads to the improvement of other serological tests such as enzyme linked immunosorbent assay and immuno polymerase chain reaction which are helpful in the recognition of *B. pseudomallei* antibodies (Cooper et al., 2013). The immunochromatographic technique which provides the results rapidly is also used to spot the melioidosis among the patients who were migrated from prevalent regions of melioidosis (O'Brien et al., 2004).

Hence, the early determination of melioidosis can be done with the help of both culturing techniques and any serological antibody or antigen tests. So that there will not be any postponement of antibiotic treatment.

2.4.7 EPIDEMIOLOGY

In 1949, melioidosis was foremost reported in Northern Queensland of Australia from a flare-up in sheep (Dance, 1991). From 1989 to 1999, the yearly frequency of melioidosis occurrence was 16.5/1, 00,000 in peak of Northern Territory (Currie et al., 2000). After 10 years, the frequency has been raised up to 41.7/1, 00,000 because of heavy rainfall annually (Currie et al., 2004). Melioidosis is

considered as promising disease in Western Province and it is rare in Port Moresby area (Currie, 1993).

Melioidosis is also commonly found in Southeast Asian countries (Dance, 1991) and it is also found to be endemic in China and Korea (Howe et al., 1971). Even though the disease is endemic in tropical countries, some cases occur in temperate and subtropical areas occasionally. In India, melioidosis was first recorded in 1991 and 92 patients were diagnosed as melioidosis as of 2009. These patients were from different parts of India (Raghavan et al., 1991; Bharadwaj et al., 1994; Cherian et al., 1995; John et al., 1996; Saravu et al., 2010)

2.4.8 TREATMENT

Antimicrobial resistance is now become a global issue (Sekhri, 2013). *B. pseudomallei* show resistance towards penicillin and its derivatives, 1st and 2nd generation cephalosporins and it is susceptible to some 3rd generation cephalosporins (particularly ceftazidime, but sometimes, cefotaxime and ceftriaxone) (Dance et al., 1989; Leelarasamee and Bovornkitti, 1989), carabapenem and fluroquinolones (Ashdown, 1988; Dance et al., 1989), amoxicillin-clavulanate and co-trimaxazole (a mixture of trimethoprime-sulfamethoxale (TMP_SMX)) (Chaowagul, 2000).

Antibiotic therapy should be based on

- Anti-microbial susceptibility test on the isolate from patients infected with melioidosis.

2.5 EBOLA VIRUS DISEASE

2.5.1 BACKGROUND

Filoviruses belong to a family known as *Filoviridae* and these viruses consist of a RNA genome only which is of single stranded. The *Filoviridae* family comprises of two members and they are usually called as *Ebolavirus* and *Marburgvirus* derived from a same ancestor (Suzuki and Gojobori, 1997; Belyi et al., 2013; Carroll et al., 2013). The major characteristic of Filoviridae is that the viruses in this family are

enveloped viruses. Thereby, it permits the viruses to target a particular cell type and make their entry into the cell (Beer et al., 1999). The genus *Ebolavirus* covers of five different species namely

- *Zaire ebolavirus* (EBOV)
- *Sudan ebolavirus* (SUDV)
- *Reston ebolavirus* (RESTV)
- *Bundibugyo ebolavirus* (BDBV) and
- *Tai Forest ebolavirus* (TAFV)

The length of *Ebolavirus* genome is approximately 19,000 bases and it has been aligned the genes continuously nucleotides long that is converted into 7 structural proteins and 1 non-structural protein (Feldmann et al., 1993; Feng et al., 2007). There exists some differences of about 30-40% between the genomes of each *Ebolavirus* species and this differences is mainly because variation in their evolution record. Among 5 species of *Ebolavirus*, EBOV is interrelated with SUDV and show variations in virulent capacity, serological and biochemical properties. Both these strains were never been seclude from non-human beings which are not affected by these two strains experimentally (Jahrling et al., 1990; Okware et al., 2002). In 1989, RESTV was initially secluded from cynomolgus monkeys (*Macaca fascicularis*) in Reston area where people were restricted for their entry in that region (Miranda and Miranda, 2011). These infected monkeys were originally from Philippines and the individuals handling these monkeys were also affected by RESTV. They produced antibodies against RESTV without developing any symptoms for EVD. RESTV is observed to be more pathogenic to monkeys than human beings (Le Guenno et al., 1995; Gray et al., 2014).

EBOV are accountable for causing large outbreaks of haemorrhagic fever or EVD and they are often correlated with high death rate. This characteristic is one of the promising qualities of this pathogen. EBOV replicates quickly which will not permit the human beings to induce the innate immunity. There is no prophylaxis or anti-viral therapy available for EVD (Sullivan et al., 2000; Friedrich et al., 2012; Falasca et al., 2015).

2.5.2 VIRULENT PROTEINS & RESERVOIRS

The filoviruses include minimum 7 structural proteins and each protein is a product of translation of an mRNA molecule which has a length of poly adenine residues in 3' tail. Among these proteins, glycoprotein (gp) is responsible for the *Ebolavirus* replication (Lee and Saphire, 2009). GP is exists in 2 forms,

- Structural protein, integrates into virion of *Ebolavirus*
- Secreted gp, performs different function.

gp is involved in the development of trimers and it favours the attachment of trimers with endothelium. gp is considered as a mouse retroviral vector which helps the gene for the transcription (Yang et al., 1998). The pseudotyping research on gp states that there was reduction in titer of *Ebolavirus* and rises the level of expression of gp. This results in modification of morphological structure of *Ebolavirus* and this modification is not observed in gp of murine leukemia virus. These observations show that gp of *Ebolavirus* may specifically involve in toxic effects to living cells (Yang et al., 2000). gp is the product of gene expression of fourth gene in those 7 structural proteins. It has N and O-linked carbohydrates which makes it to have more molecular mass than those obtained from deduced protein sequences. It plays a major role in attaching with receptor molecule and combines with cellular membrane that allows the *Ebolavirus* entry into the cell. These activities are due to the presence of single transmembrane gp which leads to the formation of spikes on the surface of *Ebolavirus* (Leroy et al., 2004).

2.5.3 EPIDEMIOLOGY

EBOV, SUDV and BDBV are the three *Ebolaviruses* that have brought huge episodes of haemorrhagic fever in sub-Saharan Africa (Hartman et al., 2010; Feldmann and Geisbert, 2011; Kalra et al., 2014). The disease is quickly spread to Uganda in East Africa, Gabon in west coast of Africa, Sudan in North-east Africa and the Democratic Republic of Congo (Chippaux, 2014). In the Philippines country, the dissemination of *Reston ebolavirus* causes infection in placental mammals but it does not affect humans (Miranda et al., 1999). Other species, TAFV was first identified Tai

Forest in Ivory Coast from an individual who had a contact with chimpanzee which was suffering from EVD (Formenty et al., 1999). Despite the fact that this incident shows the occurrence of TAFV in West Africa, this subregion was not thought to be a region where EBOV was endemic. EBOV is predominantly accumulated in fruit bats of *Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquate*. These fruit bats are mostly found in West Africa.

In 2014, in the month of March, medical clinics and general wellbeing administrations alerted the Guinea's Ministry of Health. After this alertness, 8 individuals were admitted in hospital because of EVD. Among them, 3 were expired and further, deaths were declared amongst the family members of the affected individuals. The large number of deaths was noted in Macenta which even includes the staff members working in those hospitals.

The people of 5 countries in West Africa such as Sierra Leone, Guinea, Senegal, Liberia and Nigeria were infected by EVD. The number of confirmed and likely EVD cases was found to be 4507 as on 14th September, 2014 and deaths were declared for 2296 EVD infected patients (WHO Ebola Response Team, 2014). The present epidemiology of EVD explains that the death rate is much higher than all those earlier epidemics which were combined together. The real numbers of deaths by EVD is undoubtedly high.

2.5.4 PATHOGENESIS

Ebolavirus outbreaks happen suddenly from an unknown resource which is followed by transmission from one individual to another individual. *Ebolavirus* has tendency to cause haemorrhagic fever and large number of deaths have observed in numerous individual outbreaks (Bowen et al., 1977; Sullivan et al., 2003), but the factors involved in causing EVD is poorly understood. The last phase of disease is portrayed by severe bleeding, improper supply of blood to important organs and fever with major participation of reticuloendothelial and mononuclear phagocytic cell arrangement. Many studies suggested that immune system of the patients may play an important role in the *Ebolavirus* pathogenesis and it is also observed that the intensity

of inflammatory cytokine has been elevated more in serious cases than in infected patients (Baize et al., 1999; Villinger et al., 1999).

The well-known major symptoms in assured cases of EVD were severe fever, diarrhoea and frequent vomiting (Baize et al., 2014; Kratz et al., 2015). The structural studies on monkeys infected with RESTV and EBOV confirms that monocytes or macrophages and fibroblast are favoured sites of *Ebolavirus* replication in initial phase (Geisbert et al., 1992) and other types of cells will be associated during the advanced condition of the disease. In culture method also, monocytes or macrophages show sensitivity towards EVD and leads to huge making of virus and lysis of viral cell (Feldmann et al., 1996). In spite of the fact that the reports on non-human primates did not recognize endothelial cells as a region of enormous viral replication, many *in vitro* studies showed that those cells are appropriate target for the replication of virus (Schnittler et al., 1993). *Ebolavirus* infection may results in lysis of cell and damaging of endothelial cells. These results are acting as major steps in the pathogenesis of EVD.

In vitro studies proved that cultures of supernatants of *Ebolavirus*-infected monocytes or macrophage have the ability to expand the endothelial permeability through paracellular mechanism (Feldmann et al., 1996). While analyzing the mediators present in supernatants of those cells showed that there is a rise in the levels of secreted TNF-α. This analysis support the theory of vascular instability activated by mediator and thereby, boost the permeability which is an important process in developing shock syndrome and it is observed in most serious cases (Schnittler and Feldmann, 1999). As a result of shock, the bleeding decreases the blood flow. During tissue and organ culture demonstration, it was noted that the bleeding in EVD is because of damage in endothelial cells which is caused by viral replication (directly) and cytokine-mediated processes (indirectly) and loss of integrity of endothelium (Fisher-Hoch et al., 1985; Geisbert et al., 1992; Schnittler et al., 1993; Feldmann et al., 1996; Schnittler and Feldmann, 1999; Baize et al., 2014; Kratz et al., 2015). The viral replication also provokes proinflammatory endothelial cells and then activates the coagulation process.

2.5.5 LABORATORY DIAGNOSIS

It is not possible to determine *Ebolavirus* immediately as the signs of EVD is fever which is not only particular to *Ebolavirus* infection but it is also commonly observed in patients with general infections namely, typhoid, malaria and other non-feverish diseases (Kratz et al., 2015). In any case, if an individual has manifestations of EVD and has a record of get in touch with blood or any fluids of an EVD infected patients or animals, the individual should be separated from the general public and it has to inform to medical practitioner or professionals. Then, the investigations should be carried out from the samples of affected individuals and further, tested to verify infection. The sample used for identifying *Ebolavirus* is blood which can be spotted at noticeable point only after 3 days of EVD signs (Towner et al., 2004; Saijo et al., 2006). The tests which are used in diagnosis of EVD are shown in Fig. 2.4.

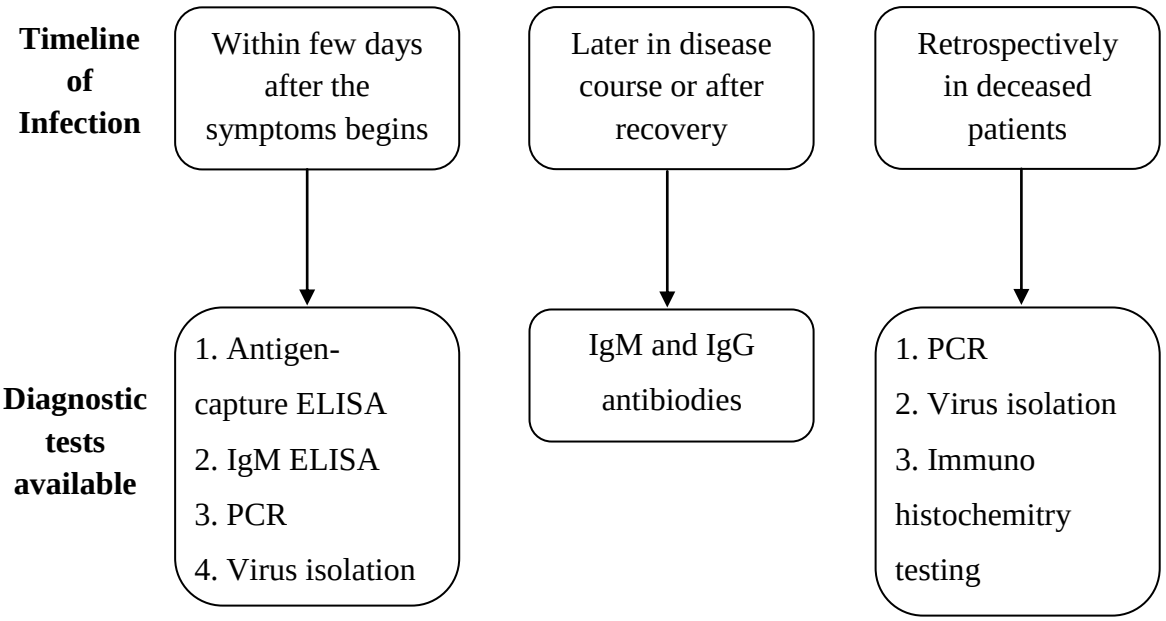

Fig. 2.4: Laboratory test used in diagnosis of EVD

2.6 Infectious diseases databases

The following are the databases describing about their organism causing the specific IDs

2.6.1 EUROPEAN HEPATITIS C VIRUS DATABASE (euHCVdb)

The hepatitis C virus (HCV) genome exhibits an outstanding sequence inconsistency which leads to the categorization of at least 6 major genotypes, several subtypes and innumerable of quasispecies within a given host. The euHCVdb allows the users to examine the structural and genetic differences present in all the available HCV sequences. This analysis provides an idea about the virology and pathogenesis of HCV and also helps in the drug designing and vaccine development. The euHCVdb merges the computer-annotated HCV sequences with the protein three-dimensional (3-D) models and it is linked to the large number of sequences and structure analysis tools (Combet et al., 2007). The database can be reachable through the http://euhcvdb.ibcp.fr.

2.6.2 EchoBASE

EchoBase is a database which curates latest experimental and bioinformatic information on the genes and gene products of the model bacterium *Escherichia coli* K-12 strain MG1655. The goal of the database is to assemble the information's from extensive range of sources to afford evidence on the functions of around 1500 gene products that have no definite cellular function. The proteomics studies, microarray data, protein–protein interaction data, structural data and bioinformatics studies can be performed in the EchoBase (Misra et al., 2005). The database can be accessible through the http://www.ecoli-york.org.

2.6.3 TubercuList

The TubercuList is a relation database that provides the genome-derived information about H37Rv, the paradigm strain of *Mycobacterium tuberculosis*. It is a knowledge base that aims to combine the genomic and proteomic features, drug and transcriptome data, mutant and operon annotation, bibliography, structural views and

comparative genomics, in a structured manner required for the rational development of new diagnostic, therapeutic and prophylactic measures against tuberculosis (Lew et al., 2011). TubercuList is available through the URL: http://tuberculist.epfl.ch/

2.6.4 *Pseudomonas* GENOME DATABASE

Pseudomonas is a genus of bacteria which is familiar for its metabolic ability and the capacity to live in a broad range of environments as free-living soil microorganisms or as severe opportunistic pathogens. The Pseudomonas community annotation project was initially created to meet the requirement of providing a conventional, peer-reviewed annotation of the *Pseudomonas aeruginosa* PAO1 genome sequence with the help of internet-based approach along with community assisted genome annotation. This led to the development of the Pseudomonas genome database (http://www.pseudomonas.com), which at first was specific for *P. aeruginosa*. Later, when the number of *Pseudomonas* genomic sequences increased, the project community realised the significance of comparative genome analysis. The comparative genomic analysis for genus *Pseudomonas* helps in the analysis of phenotypic differences between closely related isolates and strains (Winsor et al., 2011).