2. REVIEW LITERATURE
Plague is a deadly infectious disease caused by a bacterium named *Yersinia pestis*. The ultimate reservoir of this zoonosis remains doubtful, but epidemiological chain links wild rodents, anthropophilic rodents, and their fleas. It is one of the most deadly infectious diseases in the history of mankind, being responsible for huge mortality for more than two millennia. Paleo-microbiological data have indicated that *Yersinia pestis* was indeed responsible for two historical pandemics in the 5-7th century and the 14-18th century as well as the third and current pandemic which started in 1894. Indeed plague is not only a disease of the past but it is regarded as a re-emerging infectious disease. *Yersinia pestis* proved to be a rapidly evolving organism which recently acquired genes coding for antibiotic resistance. *Yersinia pestis* is classified in the group A of bioterrorism agents by the Centers of Disease Control and Prevention. It is P3 level bacterial which need to be manipulated in P3 security laboratory.

*Yersinia pestis* is a Gram-negative bacterium classified in the family *Enterobacteriaceae* of gamma sub-group proteobacteria. Molecular analyses indicated that *Yersinia pestis* emerged 1,500-20,000 years ago from a common ancestor with *Yersinia pseudotuberculosis*, an environmental bacterium causing human disease. *Yersinia pestis* isolates can be grouped into three biovars: *Antiqua* biovar (glycerol fermentation and nitrate reduction), biovar Medievalis (glycerol fermentation and lack of nitrate reduction), and biovar Orientalis (lack of glycerol of fermentation and nitrate reduction). A fourth biovar named Microtus has been recently proposed by Chinese microbiologists. Russian microbiologists proposed an alternative classification comprising six different biovars based of the analysis of 18 phenotypic characteristics, and different pathogenicity for guinea pigs. Molecular analysis of insertion sequence IS 100 in the chromosome, of variable number of tandem repeat (VNTR) and of single nucleotide polymorphisms (SNPs) allowed to classify *Yersinia pestis* within 8 populations which do not perfectly match biovars. Paleomicrobiology data indicate that only Orientalis biovar was responsible for the 3 historical pandemics,
biovars *Antiqua* and Medievalis being geographical variants lacking epidemic potential.

The genome of five *Yersinia pestis* isolates has been sequenced, comprising the four biotypes and found a 4.6Mb genome comprising numerous insertion sequences, intra-genomic recombination, and lateral transfer of virulence factor genes and three plasmids. Plasmids encode some virulence factors. Plague is a zoonosis with *Yersinia pestis* infected rodents in contacts man as source. These include wild rodents (marmot) and anthropophilic rodents (*Rattus rattus*). Other mammal’s species have been demonstrated to be an uncommon source of plague, such as camel in North Africa and domestic cats in the United States. After the ingestion of infected wild rodents, domestic cats are a source for pulmonary plague as well as bubonic plague. Also, patients with pulmonary plague can be a source of infection. Transmission of *Yersinia pestis* to the man is by the bite of rodent ectoparasite, particularly the rat flea *Xenopsylla cheopis*, infected by *Yersinia pestis* at the time of blood meal on the septicemic rodent. Different flea species have been shown to transmit *Yersinia pestis*, although the efficacy of this mode of transmission is low. Also, it has been suggested that direct contacts with infected animals may be a route of transmission. Transmission from domestic cat results from direct inoculation by bite or scratch (bubonic plague) or by inhalation (pulmonary plague). At last, inter-human transmission by inhalation from patient with pulmonary plague is possible. This route of transmission could be used in case of criminal use of *Yersinia pestis* by bioterrorists.

2.1 Geographical Distribution

Plague has been reported in Africa, in Asia and in America, but no case in Australia till date. Plague is now essentially a rural zoonosis, a situation quite different than that observed during historical pandemics. It is a re-emerging infectious disease since the number of notified cases is increasing for 20 years. In 1997, 5,419 cases including 274 deaths were notified by 14 countries. In Africa,
Madagascar notified 50% of all cases, Congo Democratic republic, Malawi, Tanzania and Vietnam in Asia were the further countries which notified the greatest number of cases. Recently a small epidemic of few cases has been confirmed in the Oran area in Algeria in 2003. New epidemic has been notified in 2005 by the Democratic Republic of Congo.

2.2 Types of Plague

**Bubonic plague** is the most common form of the disease. After 2-7 day incubation, onset is brutal with severe infectious syndrome. Fever is of 39-40°C with headaches and shivers. Agitation or prostration can be observed as well as enteric signs and symptoms including nausea and vomiting.

**Septicemic plague** has common signs of Gram negative septicaemia with higher frequency of enteric signs and symptoms. Pulmonary plague is rarely a primitive form, but results from septicemic plague.

**Pulmonary plague** presents as influenza-like disease with fever of 39-40°C followed by pneumonia with hemoptoic sputum. Mortality of untreated plague is high.

2.3 Symptoms and disease progression

**Bubonic plague**
- Incubation period of 2–6 days, when the bacteria is actively replicating
- General malaise
- Fever
- Headache and chills occur suddenly at the end of the incubation period
- Swelling of lymph nodes resulting in buboes

**Septicemic plague**
- Hypotension
- Hepatosplenomegaly
- Delirium
• Seizures in children
• Shock
• General malaise
• Fever
• Symptoms of bubonic or pneumonic plague are not always present

**Pneumonic plague**

• Fever
• Chills
• Coughing
• Chest pain
• Dyspnea
• Lethargy
• Hypotension
• Shock
• Symptoms of bubonic or septicemic plague are not always present

### 2.4 Diagnosis

**Diagnosis:** The diagnosis of plague has to be evoked in a case of swollen, painful lymph node in a febrile patient who has been exposed to rodents. As for pulmonary and septicemic forms which cannot be clinically distinguished from other infections, knowledge of the patient exposure to rodents or cat is essential.

**Clinical specimen collection:** The bubo pus must be punctured into a sterile tube; blood must be collected into an aerobic blood culture system; sputum must be collected into a sterile tube. Serum has to be collected into a tube without anticoagulant. Clinical specimens will be handled into P2 laboratory under a septic cabinet.

**Microscopic examination and culture:** It is possible to determine the biotype (*Antiqua*, *Medievalis*, *Orientalis*) of the isolate by studying glycerol acidification and nitrate reduction. Molecular analysis includes PCR amplification and sequencing of the plasmid-encoded gene *pla* and genotyping using Multi Spacer Typing (MST).
A original method named Multi-Spacer-Genotyping (MST) has been proposed for genotyping *Yersinia pestis*. This new method is based on sequencing several intergenic regions (spacers). These regions have been chosen after bioinformatic comparison of three sequenced genomes, they are shorter than 500 bases pairs, and comprise variable sequences and conserved region in the 5and 3extremities, for the definition of PCR primers. This new method allowed us to cluster isolates according to their biotype and has been applied to a collection of isolates and ancient human specimens collected in individuals suspected of plague.

**Indirect diagnosis:** Serological diagnosis of plague is commercially available as ELISA test detecting antibodies against the F1 capsular antigen. However, the positive predictive value is very low for patient leaving outside an endemic area and the serological diagnosis must be confirmed by Western-blot.

**Hygiene:** Although plague is not a contagious disease by direct contacts, plague patients may be isolated for two days after initiation of antibiotic treatment due to the risk of secondary pulmonary plague. Clothes must be autoclaved in case of plague in patient infected.

**Antibiotic treatment:** All cases of plague must be treated with antibiotic. Parenteral gentamycin or streptomycin is recommended. Doxycycline or fluoroquinolone are second line recommendations.

**Vaccination:** This is one plague vaccine which is commercially available in Australia only. It is Commonwealth Serum Laboratories Limited, Australia, vaccine which is made with the heat-killed, virulent *Yersinia pestis* strain 195-P. Vaccine is administrated in three injections within two months. Secondary effects occur in 10% of vaccinated persons. The vaccine protects against bubonic plague but not against pulmonary plague. Synthetic vaccine including the capsular F1 and V antigens and alhydrogel as adjuvant is under evaluation. Vaccination is recommended for persons with professional risk of exposure (people working in bacteriology laboratories, veterinary).

**Other measures**

For **people living in endemic area**, destroy nest of rodents, limiting rodent access to house, insecticide treatment of domestic dogs and cats, avoiding direct contacts with dead wild rodents, handle sick cats with caution.

As for the **people with recreational activities including hiking, camping, field scientific research on plague**, Avoid manipulation of sick or dead animals, avoid contact with burrow and rodents nests, use repellent containing N, N-diethyl-m-
toluamide (DEET), treat pets with insecticide and handling dead animals with gloves.

For **health care workers and persons with close contacts with a plague patient**, Patient isolation, antibiotic prophylaxis for people with close contacts < 2 metres of patient suspected or confirmed with pulmonary plague.

For **laboratories workers**, clinical specimen must be handled into class 2 laboratory under safety cabinet using gloves and mask and *Yersinia pestis* isolates must be handled into class 3 laboratory.

**Veterinary** must be informed of the risk of plague in the area where cases have been diagnosed. They must wear gloves and glasses. They must avoid inhalation of infected particles when in contacts with sick cats, febrile cats with enlarged lymph node, infra-mandibulare abscess, and pneumonia.

**Plague palemicrobiology** Plague is one of the infectious diseases reported in historical sources for two millennia, for which we have described new techniques for the detection and identification of *Yersinia pestis* DNA in humans.

![Gram-stained Yersinia pestis organisms exhibiting a safety in morphology](image)

**Figure:1 -** Gram-stained *Yersinia pestis* organisms exhibiting a safety in morphology
In 14th century, the Black Death decimated nearly half of the European population—a horror that continues to resonate through the world even today. Dubbed "the great dying," the mere prospect of a return to such times is enough to put a population on edge. Today, some researchers speculate that the world's first pandemic may have actually been a hemorrhagic fever, but the term "plague" continues to cling to another long-standing suspect and current Category A biological weapon: the Yersinia pestis bacterium.

Plague exists in two main strains: bubonic and pneumonic. Bubonic plague typically spreads by bites from infected fleas, but also can be transmitted from person to person through contact with infected bodily fluids. This strain is named for the swollen glands, or buboes, around the groin, armpit and neck. This swelling is accompanied by fever, chills, headache and exhaustion. Symptoms occur within two or three days and typically last between one and six days. Unless treated within the first 24 hours of infection, 70 percent of those infected die. Pneumonic plague is less common and spreads through the air by coughs, sneezes and face-to-face contact. Its symptoms include high fever, cough, bloody mucus and difficulty breathing. Plague victims themselves, both dead and alive; have historically served as effective delivery vehicles for this biological weapon. A 1940 plague epidemic occurred in China following a Japanese attack that involved dropping sacks of infected fleas out of airplanes. Today, experts predict that plague would likely be weaponized in the form of an aerosol, resulting in an outbreak of pneumonic plague. However, low-tech, vermin-based attacks are still possible. Several countries have explored the use of plague as a bioweapon and, as the disease still occurs naturally throughout the world, copies of the bacterium are relatively easy to come by. With appropriate treatment, plague's mortality rate can dip as low as 5 percent.

Figure: 2 - *Yersinia pestis* colonies grown on CIN agar
Plague, or Black Death as it is commonly called, is an infectious disease caused by the bacterial agent *Yersinia pestis*. Popular tradition dictated that the disease derived its ominous name from the black coloration of the swollen and very tender lymph glands that characterize the bubonic form of the infection, or the black coloration of those who died of septic plague. Other experts suggested that the name came from the Latin *atra mors*, which translates into 'dreadful death' or 'black death.'

The first global pandemic of plague was believed to have began in the Middle East in the 6th Century CE. It reached Egypt by 542 CE, brought destruction upon the Eastern Roman Empire under Justinian, and spread across the European Continent. Constantinople suffered approximately 40% fatality, and the destruction ushered in the Dark Ages in Europe. Spontaneous outbreaks continued until the 8th Century CE.

The second pandemic began in China in 1330s. Between the years 1337 and 1346, a series of environmental disasters ranging from floods to locusts to earthquakes struck China. On the heels of these disasters came a plague that slowly spread westward along trade routes. In 1346, the plague arrived in Asia Minor and the suffering precipitated violence between the Tartars and the Genoese merchants who retreated to the Crimean coastal city of Kaffa, the present day Ukrainian city of Feodosia. The Tartars besieged the city and catapulted bodies of plague victims across the walls. Plague spread within the besieged city, more likely because of flea-infested rats rather than the gruesome Tartar projectiles. The Genoese merchants abandoned the city to return to Europe with the deadly bacteria in tow. Historians suspected the plague entered Europe through other trade routes as well. By 1348, the plague swept into Sicily and the Italian peninsula. In the 14th Century, the lack of adequate medical knowledge to address questions regarding the plague's origins and transmission inspired social panic and massive witch hunts. Chronicles recorded that women who survived the epidemic were frequently attacked as witches and 'plague-spreaders.' One legend popular in central Europe and Scandinavia blamed the plague on *Pest Jungfrau*, a maiden who traversed the skies as a blue flame waving her hand or a red handkerchief to spread the deadly disease.

Home remedies against infection ranged from practical suggestions regarding sanitation and disposal of corpses to bathing the victims in rose water and vinegar to drinking stewed mixtures of ground eggshells and marigold flowers. Historical records on the mortality rate vary widely. Most agreed mortality numbered at least 20 million people in Europe and was higher in cities than the countryside.
Best estimates by historians suggest that between 20% and 30% of the population of Europe was destroyed by the plague. The next global epidemic that would cause more deaths was the Spanish influenza of 1918 that killed 50 million in a year. The plague remained in Europe until the Great Plague of London in 1665. The plague started in the overcrowded poor parish of St. Giles-in-the-Field. Authorities quarantined all infected households; a red cross and the words 'Lord have Mercy on Us' were painted on the door to indicate a doomed house. At night, the dead were collected and buried in large communal pits, one at Aldgate and one at Finsbury Fields. In an effort to control the epidemic, the Mayor of London ordered all cats and dogs to be destroyed, but such measures only worsened the plague by allowing rats to thrive without their natural predators. The plague in London caused 15% fatality in the population. Then the plague reached an abrupt conclusion. The Great Fire of London in 1666 that destroyed the city was believed to have destroyed the plague as well. In the late 20th Century, medical historians have questioned the exact nature of Black Death. One argument, spearheaded by British zoologist Graham Twiggs and Edward Thompson of the University of Toronto, suggested that in addition to bubonic plague, anthrax outbreaks augmented the high fatality rates that devastated the Medieval European civilization. Physicians in the 14th Century may have conflated the similar preliminary symptoms of both diseases. Further, plague ravaged 14th Century Iceland, but rats were not introduced to Iceland until the 17th Century. Many historians concede that the two diseases perhaps coexisted to bring about the high fatalities associated with the Black Death.

In 1894, Swiss-French bacteriologist Alexandre Yersin of the Pasteur Institute was credited as the first to isolate the bacteria *Yersinia pestis* that causes the plague. In 1894, Yersin joined the Colonial Health Corps and traveled to Hong Kong where the plague raged. In Hong Kong, Yersin isolated the bacteria, connected the bacteria with bubonic plague, and published his results in French. Yersin named the bacteria *Pasteurella pestis* after his mentor, Louis Pasteur, but by 1970, the bacteria was renamed *Yersinia pestis* after the bacteriologist who discovered the bacteria and linked it to the disease. In 1896, during an outbreak of
plague in Bombay, the Bombay authorities turned to Waldemar Haffkine for a medical miracle. Haffkine raced to find a vaccine for *Yersinia pestis* in his makeshift laboratory at Grant Medical College. By January 1897, a vaccine created with killed plague bacteria was developed. Haffkine tested the vaccine on himself before testing it on volunteers from the Byculla jail. In 1898, EH Hawkin and Paul Louis Simond were credited with discovering the role of rats in transmission of bubonic plague, and in 1900, Simond was further credited with uncovering the role of the flea as well.

The third pandemic of plague began in 1892 in the Yunnan Province of China, and spread around the world killing an estimated 6 million in India alone. In 1899, a ship from Hong Kong arrived in San Francisco with two plague victims onboard. Although initially quarantined on Angel Island, the ship was allowed to dock and in 1900, and a fatal case of bubonic plague was discovered in San Francisco's Chinatown. Local authorities burned all houses that contained plague victims and moved the Chinatown population to camps outside the city. A 1906 earthquake and a 1907 bounty placed on rats finally helped end the epidemic. At the beginning of the 21st Century, the third pandemic of plague continues to appear sporadically on a worldwide scale except in Australia and Antarctica.

In the late 20th Century, bubonic plague continued to appear in central, southwestern, and northern India. In September 1993, an earthquake measuring 6.4 on the Richter scale caused more than 10,000 deaths in the Western Indian state of Maharashtra. In 1994, the monsoons flooded low-lying slum areas in the city of Surat and left in its wake piles of garbage lingering in the narrow streets. According to epidemiologists, the first natural disaster dislocated rodent wildlife from nearby forested areas and brought them in contact with domestic rats. The second natural disaster and the garbage piles left in its aftermath provided the plague-carrying rats with a ready home. In August 1994, in the village of Mamala near Beed, a city in the state of Maharashtra, rats began to die off. The first human cases were reported in the Bir district of Maharashtra. Between 26 August
and 18 October, 693 cases of bubonic or pneumonic plague were discovered, and 56 victims died nationwide.

**Plague as a Biological Weapon:** After the Tartar's transformation of plague victims into bioweapons with catapults in 1346, it was the Japanese during World War II that then developed plague into a biological weapons agent. In Unit 731, the Japanese biological weapons program in Manchuria headed by army officer and physician Shiro Ishii, the flea bomb or Uji-50 was created. The simple design of the bomb was a ceramic container filled with plague-infested fleas and flour. Once the container reached the ground and broke, the fleas would mount the rats, and the rats would spread the disease. The Japanese tested the weapon on Manchurian villages. It was reported that Ishii had devised *Operation Cherry Blossoms at Night*, a plan to send kamikaze bombers loaded with plague to San Diego, California. The operation was scheduled for 22 September 1945. The Atomic bomb was dropped on Hiroshima on 6 August 1945. Beyond bubonic plague spread by plague-carrying fleas, the *Yersina pestis* bacteria would most likely be weaponized as aerosolized, antibiotic resistant pneumonic plague, the most deadly form of the disease. During the 1950s and 1960s, the United States and the Soviet Union biological weapons programs both experimented with aerosolized *Yersina pestis*. In a 1970 report, the World Health Organization (WHO) estimated that 50 kilograms of *Yersina pestis* bacteria sprayed over a city of 5 million inhabitants would result in 150,000 cases of pneumonic plague and 36,000 deaths. Aerosolized pneumonic plague remains one of the most deadly biological weapons agents due to universal susceptibility to the disease, high morbidity and mortality induced by the disease, and quick person-to-person transmission of the pneumonic form of disease.