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Plague: A zoonotic disease, wiping out many civilizations

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Abstract

Plague is affecting human being for centuries. It is a zoonotic infection caused by gram negative bacteria Yersinia pestis. Yersinia pestis has different virulence determinants which aid in its proliferation including several proteins such as plasminogen activator which breaks blood clots and allows bacteria to reach distant sites and F1 capsular antigen forms a gel like capsule for protection against immune system. Culturing bacteria, staining procedures such as gram stain, Giemsa stains and other techniques including PCR and fluorescent antibody staining are used. Suspected individuals should start the course of antimicrobial drugs including streptomycin and tetracyclines until the diagnostic tests confirm plague, as it can be fatal for the patients. No FDA approved vaccines are available due to their ineffective property of short-term protection and adverse reactions. Prevention can be followed by observing hygienic conditions such as isolation of individuals with pneumonic plague, flea repellents and reduced interaction with diseased animal. Despite Pakistan is located in plague infested geographical zone but no cases have been reported yet. Vaccines with long term protection and less or no side effects should be produced so that any future outbreaks can be prevented.

Key words; Plague; Yersinia pestis; rodent hosts; Flea; borne; transmission; PCR; anti-microbial drugs.

1. Introduction

It is an acute zoonotic infection caused by a virulent gram-negative bacteria Yersinia pestis. It is an infectious disease of animals including rodents and their fleas. Human beings are the accidental hosts and are infected as a result of being bitten by an infected flea, direct contact with disease reservoir animals and their products or inhaling air droplets suspended in the air as a result of sneezing or coughing of an infected person. It is considered to be one of the most dreadful diseases. Some historians believe that Plague had major

contribution to the development of modern civilization. Until now three major pandemics have been observed in human populations including The Black Death, which wiped out the 75% of the European population, causing the decline of Roman Empire. It still continues to be a life-threatening disease unless detected and treated at early stages [1][2].

2. Causative Agent; Structure and Genetics

The disease-causing agent of Plague is Yersinia pestis. It is one the most virulent bacteria known. It is a pleomorphic, non-motile, coccobacillus gram- negative bacteria of family Enterobacteriaceae. **(Table.1)** It shows bipolar staining i.e. resembles a safety pin in appearance. Only one serotype of Y. pestis has been identified. It can be divided into three biovars: Antiqua, Mediaevalis, and Orientalis [3].

Kingdom	**Eubacteria**
Phylum	Proteobacteria
Class	Gamma proteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	Yersinia
Specie	Pestis

Table.1. Taxonomy of Yersinia pestis.

The genome of the Y. pestis consists of many genes that encode many factors and plasmids which enhance its pathogenicity. Low-calcium response plasmid, or pYV or pCD encodes type III secretion system (TTSS), yersinial outer proteins (Yops) and V antigen. Another plasmid PST/pPCP1 encodes the plasminogen activator (Pla) and the bacteriocin pesticin. Plasmid called pFra/pMT1 that encodes a capsular protein called fraction 1(F1) antigen and the murine toxin. Chromosomal genes that are important for its pathogenicity include pigmentation locus (pgm) that promote iron uptake and hemin storage gene (hms) encodes yersiniabactin (Ybt) that promote iron transportation in bacteria [4].

3. Vectors

Fleas are the primary vectors of plague. More than thousands of flea species had been identified but among them few have been demonstrated as competent vectors. Globally the most important vector specie is the oriental rat flea (Xenopsylla cheopis) which lives on rats of genus Rattus. This flea specie was responsible for the major world's flea- borne plague epidemic. Other vectors including louse and ticks also have been shown experimentally to transmit plague [5].

4. Hosts

Some of the organisms are susceptible to the infection while others are susceptible to the plague. [1] Rodents including animals like Prairie dogs, rats, mice; and lagomorphs such as rabbits and Pikas are the important host species for Yersinia pestis. Y. pestis must reach level as much as 10 to 100 million per millilitre of blood, for biting fleas to become infected [6]. It is transmitted from these hosts to other organisms through flea bites. These organisms develop high levels of bacteraemia in order to infect the fleas. Both the rodent and lagomorphs show high susceptibility to the disease [1].

Insectivores such as shrews show high susceptibility to infection but are moderately resistant to disease [7]. Carnivores are highly susceptible to infection but some including the domestic dogs develop antibodies on exposure and are moderately susceptible to the disease while some are highly susceptible to plague such as domestic cats and mountain lions [1].

5. Modes of Transmission in Human Beings

The plague bacteria can be transmitted to human beings in following ways:

5.1 Flea borne Transmission of Y. pestis:

This transmission can be divided into following phases from the exposure of the flea to the infection to transmission to other mammals.

I. **Day 0:** The flea becomes infected with the bacteria. It becomes infected by ingesting Y. pestis when they feed on a host mammal [8][9].

II. **Day 1-4:** Early phase transmission involves the transmission of the bacteria from the flea to the host as soon as possible with the initiation of the infection. Some flea species are able to transmit the bacteria within the three hours of the infection whereas in some species it can continue up to 4 days [10] [11]. The ability of transmitting bacteria depends upon the location of the bacteria in its digestive tract. Initially when the flea feeds, the bacteria remain within the esophagus and can be transmitted easily to other hosts upon feeding but as the time passes the bacteria begins to move further into its gut, hence decreasing the possibility of the transmission. Esophageal infections are more common than hindgut infections [12].Some species of the fleas are poor transmitters and eliminate Yersinia pestis in their faeces. In some fleas Yersinia pestis multiplies within their gut and form huge clumps which cannot be passed out due to huge size, thus preventing their elimination through faeces [1].

III. **Day 5 and Later:** In some flea species Yersinia pestis forms biofilms in the proventriculus by adhering to the spines lining it. The biofilm formation occurs between the duration of 3 to 9 days when the flea feeds on infected host. The bacteria may then extend from proventriculus to the esophagus. The biofilm prevents the ingested blood from entering the stomach [13][14]. Due to starvation when the flea repeatedly tries to feed, the blood sucked from the host is regurgitated and interacts with the host blood. In this way the flea dies due to starvation within 5 days and the mammal host becomes infected. Yersinia pestis travels from the site of flea bite to lymph nodes where it proliferates, enters blood stream and spreads to other organs. During this the bacterial concentration in blood is increased, if a flea feeds on this mammal it would become infected and can spread the infection to other hosts [11].

5.2 Direct contact with infected animals:

Yersinia pestis can be directly transmitted to humans hosts after interaction with infected animal tissues through punctured or injured skin such as during skinning of an animal [1].

5.3 Ingesting infected animals:

Ingestion of an infected animal's meat, especially raw meat, can also cause plague [1].

5.4 Airborne transmission:

Inhaling droplets infected with Yersinia pestis, is one of the ways by which it can be transmitted to human host [1].

6. Ecology of Plague

The ecology of the disease depends upon the life cycle of Yersinia pestis. The life cycle of Yersinia pestis is reliant on its transmission between rodent hosts and their fleas. Its transmission consists of two cycles;

6.1 Sylvatic cycle

In this cycle the transmission of Yersinia pestis takes place between wild rodents and their fleas. These wild rodents can act as a source of infection for other animals including the humans. Transmission to other animals majorly occurs by flea bites [1].

6.2 Domestic cycle

This cycle takes place between the domestic rodents and their fleas. As these rodents live with the human beings, their interaction with them may act as a source of plague for humans. Transmission can occur in number of ways [1].

7. Types of plague in Humans

Depending upon the severity of the infection and infected organs, plague can be classified into following types. Primarily there are three main forms of plague observed in humans:

7.1 Bubonic Plague

It is one of the most common form of the disease and responsible for the past major plague pandemics. It is derived from Gk.buobon, groin. It is named so because the lymph nodes near to the infected site after contact become swollen, these are termed as buboes and mostly appear firstly in the groin region, but they can also occur in other lymph nodes of the body, depending upon the site of exposure. Within 2 to 6 days of exposure the patients start developing symptoms such as headache, chills, fever, lymphadenitis, malaise and fatigue. Early treatment with antibiotic reduces the disease progression, thus the mortality rate. In later stages the disease progresses into septicemic plague [15].

7.2 Septicemic Plague

It is less common form of plague as compared to other forms. When the bacteria directly enter the bloodstream it initially develops bacteremia, followed by sepsis. During this stage the levels of bacteria are high enough and can be helpful in diagnosis of the disease. The symptoms include fever, chills, malaise and gastrointestinal infection. If the patient is not treated with antibiotics, it causes other severe conditions such as gangrene and necrosis. When bacteria enter the bloodstream, it releases some endotoxins which causes disseminated intravascular coagulation, in which clots are formed throughout the body, resulting into a condition known as ischaemic necrosis. The tissues die due to lack of blood and cause gangrene. In DIC the body loses its capability of clotting due to shortage of clotting factors, the body bleeds internally and causes rashes. Mortality rate with septicemic plague is less as compared to bubonic plague.

There are two types of septicemic plague:

I. Primary septicemic plague results when the bacteria enter the blood stream and during this stage the lymph nodes are not swollen.

II. In extremities it progresses into secondary septicemic plague. It spreads throughout the body and infects the lungs [1].

7.3 Pneumonic Plague

If primary septic emic plague is not treated it progresses into secondary septicemic plague. In secondary phase the bacteria enter the lung via the blood and causes pneumonic plague. After exposure, within 1 to 3 days the patient suffers with flu like fever which progresses into pneumonia. Mostly it is caused by inhalation of infectious droplets. Mortality rates with this form of plague are very high because the patient suffers severe respiratory conditions such as respiratory shock or failure [1].

Other forms of plague include:

7.4 Pestis Minor

It is a form of bubonic plague and occurs rarely. The symptoms include, headache, fever, lymphadenitis and fatigue. The patients recover within one week without any treatment [16].

7.5 Meningeal Plague

It can occur as a result of secondary bubonic plague when the lymph nodes in the neck and armpit region

become infected [16]. The patient suffers with acute meningitis along with fever and stiff neck after 9 to 17 days of illness [17]. The bacteria can also spread to central nervous system. It can be treated with antibiotics such as Chloramphenicol [1].

7.6 Pharyngeal Plague

It is caused due to the inhalation of infected particles or eating infected meat. Cervical buboes develop due to settlement of bacteria in tonsils and pharynx. Symptoms include sore throat, weakness and fever [1].

7.8 Cutaneous Plague

Several skin problems occur during plague and this condition is referred to as cutaneous plague. Skin lesions, ulcers, bruises and gangrene occur on the skin due to bacteremia [1].

8. Pathogenesis

Yersinia pestis is a highly virulent pathogen because of its astonishing abilities gained by its virulence determinants, which makes it able to evade host defenses successfully and continue its proliferation, thus resulting into severe lethal infection. It has several virulence factors that work at different stages in order to make it survive the host defense mechanisms. These determinants are encoded by different plasmids present in bacteria. The pathogenesis starts with the interaction of the bacteria with host defense molecules:

1. The host defense molecules are capable of differentiating between self-molecules and pathogen molecules called as pathogen-associated molecular patterns [PAMPs] via germ line-encoded pattern recognition receptors (PRRs). These PRRs then activates the innate immunity pathway and induces immune response by production of different cytokines, inflammatory response, macrophages and neutrophils [18-20].
2. After flea bite, the pathogen invades the host skin barrier and is exposed to phagocytes such as neutrophils and macrophages. Neutrophils are capable of killing these bacteria but macrophages only engulf them. These macrophages act as a niche for the bacteria and facilitate bacterial growth. Bacteria infect these macrophages after recognition with specific surface-associated molecules and inside them develop a number of protective determinants that prevent their further engulfment and exposure to other immune defence molecules [21]. Bacteria releases plasminogen activator which dissolves the blood clots at the site of flea bite [22][23]. Another protein F1 capsular antigen forms a gel like capsule around bacteria making it resistance against all other defence molecules [24]. The bacteria within the macrophages then are translocate to the lymph nodes by the lymphatic system. The bacteria are now resistant, they disrupt these macrophages and are released into the extracellular environment. In gram negative bacteria a system of protein known as Type three secretion system is present which promotes bacterial growth in extracellular environment. When bacteria are replicating within the macrophages, the expression of T3SS proteins is increased and it forms needle like protein complex structures on bacterial surface [25]. The bacteria after being released, when interact with other defence molecules release effector proteins from these needles which penetrate host cell membrane and in turn suppresses immune response. This protein is released by T3SS is Yersinia outer proteins (Yops). These proteins directly reduce the production of all other immune cells reaching the inflammatory site. These Yops also induce apoptosis of these macrophages by migrating into the nucleus and interfering with signalling pathways that regulate functions like gene expression and cell proliferation [22]. It is that stage of plague when buboes develop and is referred to as bubonic plague.
3. It reaches secondary lymphoid organs such as spleen and liver where it continuously proliferates and kills other macrophages. It produces another protein V antigen and injects it into host cells that prevent the formation of other proteins of host that may act as signalling molecules for initiation of inflammatory responses. The visceral organs are destroyed and it then enters the blood stream causing septicaemia. When in blood there are some other virulence determinant which promotes its proliferation and prevents from other immune responses in blood. These determinants include:

Yersiniabactin siderophore system (Ybt) enables the Yersinia pestis to acquire iron for the host blood. Lipopolysaccharides (LPS) in its cell wall makes it resistant towards other proteins found in host blood and prevents its destruction. Endotoxin is also its structural component which is released upon bacterial cell lysis. When it is released, sign and symptoms begin to appear followed by septic shock [6] [26]. All these virulence determinants promote bacterial proliferation and disease progression. The host immune system is attenuated, if not treated in early stages the patient ultimately dies.

9. Medical Diagnosis:

There are different diagnostic tests used for detecting Yersinia pestis and confirmation of the illness in an individual. On the basis of the test reports, the individuals are then classified as suspect, presumptive or confirmed in accordance with the diagnostic criteria guidelines [1].

Primarily the diagnosis is performed on the basis of appearance of clinical symptoms and individual's exposure. Tissue and body fluid samples such blood, sputum, lymph node aspirates, and nasopharyngeal swabs can be used for visualizing bacterial by staining or culturing. Staining techniques including Wayson or Giemsa stains and Gram's stain can also be used to analyse the smears of the samples. Fluorescent antibody testing (anti-F1 antibody) is another diagnostic method for identification of unique antigens of bacteria by targeting them fluorescently labelled antibodies. These antigens are usually absent in normal mammalian cells [1]. A proper diagnosis of plague can be made when Y. pestis colonies grow in growth media inoculated with tissue or body fluid sample from the patient. Colonies of Y. pestis grow slowly at least in 2 days to become visible. They are opaque with asymmetrical edges, somehow like a hammered-metal [26]. Polymerase chain reaction is another process used for the diagnosis of Yersinia pestis. Two different genes are present in its plasmids which produces proteins. These proteins aid in pathogenicity. Plasminogen activator (pla) is a protease which breaks blood clots and the F1 capsule antigen (caf1) forms capsule, both of these factors help in causing and spreading infection in host. PCR targets these genes [27-31].

10. Treatments:

Plague is an acute zoonotic disease and its causative agent is Yersinia pestis. It is a bacterium and can be treated with antimicrobial therapy. Individuals suspected or confirmed after diagnosis should be started with antimicrobials as soon as possible because as the disease progresses, it becomes severe and can lead to fatality [32]. Following antibacterial drugs approved by FDA can be used for treating Plague;

10.1 Streptomycin

It is a bactericide and most affective drug against Yersinia pestis. It is a protein synthesis inhibitor and kills the bacteria by binding to its small ribosomal subunit 16s of small 30s ribosomal unit. The translation pathway of the bacteria is disrupted and it ultimately dies [33]. It can be given intravenously or by intramuscular injections [34-37].

10.2 Doxycycline

It belongs to the tetracycline class of drugs. It is a bacteriostatic drug and also inhibits the growth of bacteria by interrupting the protein synthesis pathway [38]. It can be given either orally or intravenously [39].

10.3 Ciprofloxacin

It is a Fluoroquinolone [40-41]. It is a bactericide and kills bacteria by inhibiting the functioning of Gyrase enzyme, which causes the unwinding of the bacterial DNA during replication [42-43]. It is given orally or intravenously [44-45].

11. Prevention

The first mass vaccination campaigns against plague were carried out simultaneously in 1934 by Georges Girard and Jean-Marie Robic (strain EV) in Madagascar and by L Otten (strain Tjiwidej) in Java with live attenuated plague bacilli [46]. but their short-term protection and adverse effects lead to their decreased

utilization. Currently there are no vaccines approved by FDA for prevention from plague. Hence, some ordinary precautionary measures can be observed in order to protect individuals from plague;

a. Flea Repellents should be used against protection from fleas.
b. Patients suffering with pneumonic plague should be kept isolated until the infection cease to exist.
c. Standardized procedures should be followed in laboratory while experimenting or other procedures such as skinning.
d. Debris in nearby places and outside the house should be cleaned to prevent infected rodents from infesting the bacteria [1].

12. Conclusion

Plague is historically one the deadliest diseases that wiped out major civilizations of the world. With the advent of antimicrobial therapy and diagnostic procedures it as now curable and millions of souls had been saved from this deadly disease. It can be diagnosed at early stages and treated with proper medications as no vaccines are available currently for utilization. Awareness about the disease and proper treatment can save lives and prevent any major outbreak in the future. Other than that, ordinary precautionary measures can also prevent the spread of plague bacteria by their reservoirs.

13. Recommendations

Plague is an acute bacterial disease that can be transmitted to other host animals via flea bites, infectious droplet particles or direct contact with infected animals. It can be treated with antimicrobial drugs or also can be prevented by observing precautionary measures such as flea repellents, standardized procedures in laboratory and protective measures, cleaning the environment around the houses to avoid availability of food and shelter to plague reservoirs and avoiding interaction with dead or infected animals.

Although in past vaccines have been developed to prevent infection in animals including human beings and it also proved to be affective against plague. The morbidity and mortality rates were decrease after utilizing vaccines but it had two problems which lead to its decreased usage. Firstly, these vaccines provided short term protection and secondly after dosage it started severe adverse reactions in individuals causing complications in health.

While developing vaccines for plague these two conditions should be kept in view. Vaccines having long term protection and low side effects should be manufactured in order to prevent any plague outbreaks in future.

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