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**Chapter**

# *Drosophila melanogaster*: A Robust Tool to Study Candidate Drug against Epidemic and Pandemic Diseases

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## Abstract

*Drosophila melanogaster* is a widely used, dynamic model organism to study various pathogenic diseases observed ubiquitously in the human population. Drosophila, at present, is extensively used to conduct preclinical studies besides its counterpart rodents. The epidemic and pandemic diseases are discussed in this review to demonstrate *Drosophila melanogaster* as a key model. Epidemic and pandemic diseases are still claiming more than 5 million lives every year, and these diseases were well studied in flies. Currently there is no cure for the disease like HIV; the bacterial and fungal infections usually seen in HIV/AIDS patients could be demonstrated elaborately in *Drosophila melanogaster*. Diseases like myocardial infractions and cancer causing viral infection are long term effects of ART (anti-retroviral therapy) that could be experimented in flies. Stable Drosophila S2 cell line, Transgenic flies, transfusion of bacteria and fungi could be implemented to study several infectious diseases and for vaccine development. The latest trends in understanding pathogenic diseases and its potential biochemical markers in flies are discussed in this review to utilize the fruit flies as a functional tool and to explore further it in drug development. The advantages and disadvantages of the fly as a model of infection are discussed along with the epidemiology and the cellular pathophysiology

**Keywords:** *Drosophila melanogaster*, HIV, Influenza, cholera, tuberculosis, pneumonia, viral diseases, epidemic and pandemic diseases

## 1. Introduction

### 1.1 Epidemic disease

The term epidemic is derived from Greek word "epi" meaning "upon" and "demos" meaning "people". It refers to a communicable disease which spreads rapidly in a given population within a very short period of time. Any infectious disease existing in a region does not make it epidemic unless it causes faster mortality. A death rate of around 1.6 folds higher than usual death rate (baseline) caused by a

disease in a population within a fixed period could be considered as an epidemic disease. A disease lower than this fold increase, observed in a population could be designated as an outbreak of a disease [1].

Diseases like tuberculosis, hepatitis, yellow fever, chikungunya, ebola virus disease, marburg virus disease, Crimean-Congo haemorrhagic fever, rift valley fever, typhoid fever, Shigellosis, plague, lassa fever, West Nile fever, zika virus disease, meningitis, MERS-CoV, plague, monkeypox, nodding syndrome, nipah virus infection are considered as epidemic diseases as per World Health Organization [2]. Epidemic diseases like plague, small pox and cholera caused unsurpassed deaths in human population till the end of eighteenth century [3].

## 1.2 Pandemic disease

The term pandemic is derived from Greek word "pan" meaning "all" and "demos" meaning "people". It refers to an epidemic disease which spreads among large population possibly across geographic locations or continents within a short time span [4].

Influenza, along with viral pneumonia, HIV and cholera are considered as pandemic disease and caused millions to die beside high rate of hospitalization and life threatening conditions across the globe [2]. The viral diseases like Influenza, cholera and HIV caused maximum deaths in the twenty-first century [3].

## 1.3 Vaccination

Vaccines are available for most of the epidemic and pandemic diseases [5]. Vaccination is the most effective prevention technique to suppress the infection in healthy population [6]. However, poor and conflicted regions of Asia and Africa are deprived of these vaccines [7]. World Health Organization plays a major role in epidemic preparedness in these regions and provides extended healthcare facilities during an epidemic outbreak [8].

## 1.4 Global requirement

Considering, the disease outbreak and its transmission is high in a poor population of developing region [9]. First line and second line antibiotics are the most effective medicines for infected subjects as the vaccines are ineffective after the infection had taken place. First line therapy includes antibiotics are the most commonly prescribed medicines to alleviate the infection process, often not responsive on several types of multi drug resistant infection [10]. Hence it is important to select a cost effective model to screen the first line antibiotics or antivirals.

## 1.5 Fruit fly as a model organism for drug screening

Today, we need to discover more efficacious antibiotics to fight the infectious diseases. *Drosophila melanogaster* could be a useful model organism to study the infection process and to screen an efficient drug. Due to its shorter lifespan and vast genetic similarity towards vertebrates allows conducting the drug screening experiments. It is reviewed here that *Drosophila melanogaster* was already been used to study infections caused by pandemic and epidemic diseases. But how to utilize the fruit flies to study different infectious disease and techniques to screen a potential drug candidate were not well reviewed.

## 2. Epidemiology of infectious diseases

### 2.1 Lower respiratory tract infection epidemiology

As per World Health Organization lower respiratory tract infections are caused mainly by influenza, pneumococcal pneumonia and viral pneumonia are responsible for 3 million deaths [11]. The WHO reported in 2018, 3–5 million cases of Influenza with 290,000–650,000 death cases annually [12]. As per the Global Disease burden (GDB) study report of 2015 there were around 1.5 million deaths in all age groups caused due to pneumococcal pneumonia [13]. SARS (Corona virus) causes viral pneumonia; it is epidemic to more than 30 countries with 8000 reported cases and 774 deaths during the year 2002–2003. MERS is a viral pneumonia causing infections in 688 persons and 282 deaths reported by WHO in 20 countries during 2012 [14].

### 2.2 Influenza

Influenza originates from *Orthomyxoviridae* family it can be differentiated into three types Influenza pandemic caused by Influenza A/B virus, seasonal Influenza and avian influenza (H5N1) [15]. Influenza virus causes upper respiratory tract infection often found to cause lower respiratory tract infection in association with bacterial co-invasion. The seasonal influenza leads to maximum hospitalization resulting fatality in infants during the seasonal outbreak [16].

### 2.3 Pneumonia and viral pneumonia

Pneumonia is caused due to several communicable infections usually known as community acquired pneumonia (CAP), often seen in hospitalized patients. Pneumonia can be caused by bacteria like *Streptococcus pneumoniae, Haemophilus influenzae type b (Hib),* and *Chlamydia pneumoniae, Staphylococcus aureus*, *Klebsiella pneumonia* and *Mycoplasma pneumonia.* Viruses like syncytial virus, adenovirus, rhinovirus, metapneumovirus, Influenza A/B viruses, Coronaviruses, parainfluenza virus including MERS and SARS causes viral pneumonia [17]. The viral pneumonia is the influenza often associated with bacterial infection thereby causing fatality better known as superinfection [18].

### 2.4 Tuberculosis

Tuberculosis is caused by *Mycobacterium tuberculosis*, a gram negative facultative anaerobic bacteria. In 2017 around 10 million people were infected with tuberculosis causing mycobacterium killing 1.6 million peoples across the world [19]. The current estimate of tuberculosis is not significantly different from the 2015 WHO report [20].

### 2.5 HIV

Currently HIV is the most fatal disease observed in human population across the globe. It caused maximum number of deaths around the world in the last 3 decades. As per the latest WHO report of 2019, HIV/AIDS have claimed more than 35 million deaths till date. Currently 36.9 million (31.1–43.9 million) peoples are living with HIV as of 2017 [21]. Although the rate of infection has decreased in the recent years, still HIV remains a global burden on world economy.

**2.6 Diarrhoeal disease**

*2.6.1 Cholera*

The term "cholera" was derived from Sanskrit meaning "stomach disturbance" [22]. Since, early 1800 century cholera outbreak turned out to be pandemic and caused millions to die, altogether six different pandemics took place the seventh started in the year 1961 and is still ongoing [23, 24]. In 2019 WHO report suggests 1.3 million to 4.0 million cases of cholera with an estimated 21,000–143,000 deaths worldwide [25].

**2.7 Hepatitis**

Viral hepatitis is one of the most life threatening disease, it causes death to 1.4 million peoples across the globe reported in 2018 [26]. Globally around 260 million peoples are infected with HBV and 71 million with HCV infections are reported causing 90% of deaths among viral hepatitis patients [27]. The HBV and HCV has the highest prevalence rate in the global population at present, hepatitis viruses like HAV, HAD and HEV are endemic in many countries [26]. Currently there is no vaccine available for HCV till date.

**2.8 Typhoid**

The term Typhoid was coined from the Greek word "typhus" which means "Smoky" was used to relate the delirium symptom often associated with typhoid fever [28]. Typhoid fever is caused by gram-negative bacteria known as *Salmonella enterica serovar typhi*. Around 11–21 million cases of typhoid fever outbreak are reported annually, among that it causes death of 128,000–161,000 individuals worldwide [29].

**2.9 Malaria**

Malaria fever is a severe parasitic disease caused by *Plasmodium falciparum* and *Plasmodium vivax transmits through* female *Anopheles gambiae* mosquitoes. In the year 2017 219 million cases were noted by World Health Organization, this seasonal outbreak of malaria in 87 countries led to 435,000 deaths [30].

**2.10 Viral meningitis, viral encephalitis and hemorrhagic fever viruses**

Viruses like herpes simplex virus HSV, HIV, mumps virus, measles virus and west Nile virus causes meningitis which causes frequent outbreaks in some regions [31]. Japanese encephalitis virus along with genus *Alphavirus* Togaviridae family viruses are arbovirus (arthropod borne virus) like California encephalitis, Chikungunya, dengue, Eastern equine encephalitis, Powassan, St. Louis encephalitis, Sindbis virus, West Nile, Yellow Fever and Zika virus are capable of causing encephalitis in humans [32, 33]. The viruses capable of causing hemorrhagic fever are dengue virus, rift valley virus, yellow virus, Crimean-Congo Hemorrhagic Fever, Lassa virus, Marburg virus and Ebola virus are epidemic diseases [34].

## 3. Drosophila model to study highly infectious diseases

There are at present several bacterial, fungal and viral models of infection which were successfully demonstrated to infect flies and used it to understand drug efficacy. Drosophila model of infectious disease could be very low cost model

to study drug efficacy in-vivo; it could help to save lives by saving time during an epidemic outbreak. Understanding the disease pathogenesis in humans and drawing out a similar model in *Drosophila melanogaster* would suggest the target genes and proteins responsible for the underlying disease [35].

In the recent past several research works has been conducted to understand the immune system of *Drosophila melanogaster*. At present the immune system of *Drosophila* is a well-studied model to study infectious disease [35]. Adult flies have brain, heart, lung (spiracle), liver (fat body), kidney (renal tubule), GI tract (gut/crop), ovary/testis and versatile circulatory system (hemocyte) [36]. Apart from physiological resemblance Drosophila has 75% genetical similarity with human disease genes, due to this fact genetically tractable model could be generated to what extent is discussed here [35].

## 4. Host-pathogen interaction

*Drosophila melanogaster* has a well-built immune system to withstand pathogenic incursion, comprising of cellular, humoral and innate immunity in an effective but in simpler form than humans [37]. However, due to evolutionarily conserved immune pathways found in vertebrates and invertebrates, several components of fly immune system are homologous to humans [38]. The immune activation in flies against pathogens involves processes like recognition, coagulation, melanisation, phagocytosis, apoptosis, regulation of iron metabolism, synthesis of antimicrobial peptides and production of reactive oxygen species [39].

The bacterial and fungal infection leads to the activation of dToll, Imd, Eiger (TNF family homolog) and insulin like receptors (FOXO) in *Drosophila*. The drosophila toll and Imd (immune deficiency) pathways function as innate immunity. Toll receptors in flies play an important part during viral, fungal and bacterial infection. The patterns recognition receptors (PRRs) initiate the signal in fly immune system depending on the type of pathogen upon interaction [40]. Gram positive and gram negative bacterial infection activates peptidoglycan recognition protein SA (PGRP-SA) and Gram-negative binding protein 1 (GNBP1) respectively. PGRP-SA causes proteolytic cleavage of Spatzle upon stimulation of dToll, it mediates downstream signalling of dMyD88, Tube, Pelle, and DIF (dorsalrelated immunity factor) the NF-kB homolog. Imd an intracellular signalling protein located close to the transmembrane PGRP-LE and PGRP-LC proteins, activates Relish protein to trigger autophagy and phagocytosis through ImD regulated genes by rendering cellular immunity against gram negative bacteria [41]. Toll activates the nuclear factor DIF and it promotes humoral immunity in the fat body by producing varieties of anti-microbial peptides AMPs like attacin, cecropin, drosomycin, defensin, metchnikowin, diptericin and drosocin [42].

The fungal pathogen was found to be recognized by GNBP3 along with PGRPSA and GNBP1 it activates the drosophila toll receptors [43]. The Drosophila toll-5 (Tehao) and toll-9 plays major role during fungal infection by inducing Drosomycin gene [44].

During the preliminary stage of viral infection Drosophila toll receptor homolog of human TLR, Imd (TNF-alpha), Domeless (Jak–STAT), and RNAi plays a major role against viral infection these are components of innate immune system [45]. Similar to humans the viral glycoproteins are recognized by toll receptors like toll-4, while toll-7 dependent autophagy observed during viral infection in flies [42, 46]. Jak–STAT and Imd together mediates effective immunity against viral attack in flies [47]. The domeless-hop-stat2 pathway stimulated by upd1/2/3 activates Jak–STAT regulated genes responsible for controlling viral load; it is homologous to mammalian Jak–STAT pathway [48]. The Drosophila P53 and dP38 mediates apoptosis in

flies upon stress response generated due to DNA damage, P53 mediated apoptotic genes are regulated by Jak–STAT-MAPK [49]. The dP38 stimulation in flies triggers Unpraired gene (upd protein) a mammalian IL-6 homolog further activates Jak–STAT-*Turandots* pathway which increases tolerance towards the viral invasion [50]. The intrinsic to cell the Dicer2 a viral sensor protein mediates silencing through RNA-induced silencing complex (RISC) dependent RNAi production which inhibits viral components transcription and vago gene activation finally controls viral growth [51, 52]. The anti-viral RNAi are transported from one cell to another through canonical nano-tubes structures [53]. dERK pathway regulates viral infection of flies gut epithelial infection during orally challenge of arbovirus, Sindbis virus and vesicular stomatitis virus [54]. Despite of dynamic immune response against the viral infection viruses like Nora virus, Sigma virus (DmelSV), Drosophila C virus (DCV), and Drosophila X virus (DXV) can cause fatality in flies [55].

## 5. Markers of infectious diseases

In the recent decades extensive research has been conducted to understand the regulation of immune system in *Drosophila melanogaster*. Using techniques like genome wide screens, *Drosophila* S2 cell line in-vitro models and tissue specific loss of function mutation in transgenic GAL4/UAS fly allows studying selective pathways of immune response [56]. Up-regulation of antimicrobial peptides (AMP) in flies during bacterial and fungal infection was frequently observed, these six AMP genes expression level could be analyzed in flies [42, 57]. ROS level in flies trigger several pathways responsible for tolerance (cell survival) and apoptosis (cell death) could be assayed in virally infected flies [49, 50]. Rescue of diseased transgenic flies upon feeding of desired drug could reveal drug efficacy [56]. Survival of flies would further reveal the effect of drugs during an ongoing pathogenesis [58].

## 6. Behavioral and physiological characterization of infected flies

### 6.1 Negative geotaxis assay

Negative geotaxis assay serves the purpose to manifest ongoing pathogenesis inside the live model. It was demonstrated previously that infected flies display significantly lesser motility than healthy flies when exposed under bright light. It could be considered as an important parameter to explain drug efficacy while screening anti-microbial drugs in flies [59].

### 6.2 Circadian rhythm

Circadian rhythm in flies was studied, the genes timeless or period controls the circadian rhythm of activity-sleep cycle during day-night respectively. It has been observed that infected flies exhibit interrupted circadian control of locomotion thus flies with this deficit shows restlessness at the same time gets lesser sleep than normal flies. The behavioral changes could also be studied in infected flies beside the control/uninfected flies [60].

### 6.3 Wasting

Wasting is commonly seen physiological changes associated with prolonged diseased condition in humans. Wasting is a common symptom in HIV/AIDS,

tuberculosis and cholera patients. Similarly rapid loss in weight could be seen in infected flies prior to its death [61, 62].

## 7. Factors contributing to suitable infection model

It was previously reported that in order to replicate the outcome of future studies it is important to optimize the lethal dosage selection and the route of inoculum [63]. It is suggested that the selection of microbial strain and gender of flies are two important factors which could potentially impact the findings of future research.

### 7.1 Route of inoculum

There are two prime techniques for inducing infection in flies, primarily by feeding the flies with the microbes secondly by pricking micro needles dipped in bacterial liquid (inoculum) into fly's abdomen or thorax [62, 63]. Flies could be pricked in the abdomen with micro-needle dipped in the microbial solution, known amount can be useful in pharmacodynamic as well as pharmacokinetic studies [64].

### 7.2 Flies gender selection

Selecting gender should be considered strictly, few studies do not prefer to report the reason behind choosing the gender male/female type. In a study with *Vibrio cholera* infection narrated that female flies survived approximately 24 h longer than male flies [65].

## 8. In-vivo models for epidemic and pandemic diseases

The existing models using live bacterial infusion, feeding fungal strains and transgenic flies expressing viral proteins. Under immuno-suppressed condition would serve multiple purposes like studying host pathogen interaction and conducting preclinical trials [62, 66].

### 8.1 HIV models

Since human viruses do not usually invade insects the use of *Drosophila melanogaster* as a model organism is critical and currently in less usage [67]. In order to establish an HIV model for drug screening in *Drosophila melanogaster* it is important to understand the structural components of human immunodeficiency virus (HIV). The envelope components are comprised of Gp120 and Gp41 encoded by *env* sequence, pol-Gag RNA material encodes for *Matrix, capcid, nuclear capsid, p6, Protease, reverse transcriptase, and Integrase), Vif, Vpr, Nef, vpu, tat,* and *Rev* [68]. Transfection of Tat (transcription activator), Vpu (helps virion budding), Nef (regulator of structural gene expression) and Rev. (Nuclear export protein) in flies (in-vitro/in-vivo) were previously shown, these transfection models could be useful due to the fact that there is no marketed drug to target these viral proteins [69].

The incapacity of Drosophila S2 cells is only associated with the expression of HIV-1 envelope proteins. It is possible to express gycosylated and cleaved Gp120 in S2 cells but fusion with CD4+ receptors of T-helper cells could not be achieved in the model expression system [70]. In another study the expression of Gp120 in drosophila was carried out in S2 cell line, the antigen Gp-120 did not exhibited T-helper cell mediated humoral immune system activation and IgG antibody generation,

when introduced in mice [71]. Due to this usual challenge in a different study they expressed HIV-1 virus like proteins in Drosophila S2 cells [72].

The nef transgenic flies exhibited JNK mediated apoptosis further nef inhibits NF-kB necessary for Relish gene activation similarly decreased immune response is common in AIDS patients [73]. In a study transfected viral protein Vpu was shown to cause immune suppression in fat body of flies via toll dependent pathway, in wings the Vpu expression caused apoptosis and hindered wing development, in mammals Vpu is known for causing T-cell lymphocyte death in infected patients [74, 75]. Active microbial invasion in nef flies should be further confirmed before targeting with potent anti-nef drug candidate. The *Rev* transfected S2 cells revealed that expression of Rev. protein directed the translocation of viral mRNA sequence into the cytoplasm, blocked by leptomycin B a secondary metabolite of *Streptomyces* species [76]. Leptomycin B remained unapproved in clinical trials due to high toxicity in cancer patients [77]. The ART drugs like zidovudine, lamivudine, stavudine, didanosine and abacavir were introduced in *D. melanogaster* to study genotoxicity profile [78, 79]. In Drosophila oocytes Tat a nuclear shuttling protein, displayed interaction with tubulin causing dorso-ventral axis mislocalization resulting in delayed microtubule polymerization, similarly tubulin dysfunction causes neurological symptoms observed in HIV+ individuals [80]. The transfected viral proteins in live Drosophila could be used to target drug in a thoughtfully designed model.

Cryptococcosis, Candidiasis and Aspergillosis are common types of fungal infections observed as clinical challenge in HIV-positive patients [81]. Under immunosuppressed condition the invasion of fungi in flies causes fatality. In Drosophila fungal infection could be difficult to achieve as the innate immune system mediates anti-fungal peptide production by haemocyte causing decrease of fungal load and increases fly survival rate [82]. Hence Toll mutant flies were generated and used to induce fungal infection.

Fluconazole and voriconazole showed anti-fungal activity against *Candida albicans* and *Aspergilus fumigatus* respectively in flies [64, 83]. Among *Cryptococcus* species only *Cryptococcus neoformans* is capable of killing flies with mutated toll receptors in drosophila, susceptible to infection acquired from *Cryptococcus* species like *Cryptococcus kuetzingii* or *Cryptococcus laurentii* [83]. Although, the toll mutant flies do not demonstrate an HIV model, it is used to induce fungal infection which can serve as a model for fungal infection in Drosophila for drug screening purpose.

In order to study HPV and EBV there are two model systems to study the effect in flies. In the study with HPV co-expression of viral oncoprotein E6 and human UBE3A did not resulted in tumorigenesis requires Ras or Notch pathway in flies, E6-UBE3A requires insulin receptors for cancer to develop [84]. Upon introducing the BZLF1 gene of EBV led to interaction with shaven gene in flies a homolog of pax gene family of humans responsible for B-cell development [85]. Expression of BRLF1 and BZLF1 genes using *GMR-R* model in Drosophila showed BRLF1 caused overproliferation of cells in flies whereas, BZLF1 resulted in interaction with several tumor suppressor genes and both viral genes showed interactions with core tumor suppressor genes like *reaper, p53, Rab5*, and *Tor* [86]. EVB DNA injection in flies caused Imd mediated pathway to increase diptericin production at the same time hemocyte proliferation and remarkable increase in numbers of hemocyte cells [87]. Human cytomegalovirus derived immediate early gene transfection in flies resulted in embryonic lethality similar to humans [88].

## 8.2 Influenza infection models

Influenza virus like most other viruses fails to infect the *Drosophila melanogaster*. To construct a suitable model for drug screening was also an important aspect due

to high mortality rate caused by flu virus. The influenza virus coat protein consists of hemagglutinin (HA) and neuraminidase (NA). Matrix protein 2 (M2) plays a vital role in maintaining the pH level through proton transport enabling viral uncoating. Expression of M2 protein in flies is achieved through insertion of M2 cDNA sequence in upstream activation sequence (UAS) of pCaSpeR3 p-element insertion vector gave rise to UAS-M2 flies. The crossover between UAS-M2 and C135-Gal4 flies resulted in death at the pupal stage. Therefore the larvae were exposed and not the adult flies to anti-influenza drug amantidine which is a M2 antagonist. Amantadine and several other drugs of its class are not capable of acting against the flu virus due to varying viral strain types. Moving further the flu-fly model of UAS-M2 could be used to study potential anti-influenza drug [56].

### 8.3 Pneumococcal pneumonia models

There are several pneumococcal pneumonia infection model studied in drosophila using *Streptococcus pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia.* The biofilm formation is widely observed during *Streptococcus pneumoniae* infection in humans [89]. The nasopharyngeal tract is colonized primarily by these gram positive rods bacteria prior to infecting the lower respiratory tract. Similarly the *Pseudomonas aeruginosa* exhibit the biofilm formation during nasocomial infection in humans, a common culprit causing community acquired pneumonia hospitalized in patients [57, 90]. *Pseudomonas aeruginosa* was shown to infect *Drosophila melanogaster* causing its gut epithelium inflammation [57].

*Staphylococcus aureus* causes osteomyelitis, endocarditis, septicaemia and pneumoniae in humans, it can be selected for mimicking pneumoniae infection in Drosophila [91]. *Staphylococcus aureus* caused rapid death of flies within 48 h due to inoculation of high lethal dosage, extended survival seen upon exposure to antibiotic (tetracycline) [92]. The teichoic acid of peptidoglycan layer in *Staphylococcus aureus* was found to suppress the toll receptors of flies similar to *Streptococcus pneumoniae* toxins autolysin and pneumolysin interacts with toll receptors of macrophages in human [93–95]. *Klebsiella pneumonia* the gram positive bacteria are capable of killing drosophila at higher dose [96]. *Streptococcus pneumoniae* causes the maximum deaths in human causing pneumoniae which could be used as a suitable model for antibiotic screening in *Drosophila melanogaster*.

### 8.4 Tuberculosis models

There are at present two bacterial models for studying mycobacterium infection in flies, induced by *Mycobacterium marinum* and *Mycobacterium abscessus*. *Mycobacterium marinum* is a non spore forming, non motile, gram positive acid-fast bacillus, which is genetically 99.3% similar *to Mycobacterium tuberculosis* [97, 98]. Vacuole acidification is inhibited by *M. marinum* in drosophila phagocytic cells has been previously identified to be similar with tuberculosis pathogenesis in humans [99, 100]. Tigecycline plus linezolid was shown to have extended fly survival during the *Mycobacterium abscessus* infection. Rifampicin a very potential wide range antibiotics effective to inhibit multi drug resistance tuberculosis (MDRTb), it showed antimycobacterial efficacy in Drosophila infected with *Mycobacterium marinum* [101]. Any potential drug candidate capable of anti-mycobacterial activity can be studied in these models.

### 8.5 Cholera models

The bacteria *Vibrio cholerae* is a gram-negative and motile bacterium causes diarrheal disease in human. The pathogenesis of *Vibrio cholera* infection in humans

was previously reported to be symbolized as comparable disease progression in *Drosophila melanogaster*. Ingestion of cholera bacterium results in lethal infection induced by the toxins in the intestinal cells of the flies. The toxins ingestion could not cause equivalent lethal effect on flies was explained previously. The *V. cholera* infection results in inhibition of adenylyl cyclase, Gsα, or the Gardos K+ channel causing death due to oral ingestion in flies. Clotrimazole a Potassium Calcium-Activated Channel Subfamily N Member 4 (KCNN4) inhibitor exposure increased flies susceptibility to *V. cholera* infection [61]. Quorum sensing is the ability to detect and to respond to a specific density of cell population through gene regulation [102]. *Drosophila melanogaster* initiates quorum sensing during vibrio cholera infection by suppressing succinate (substrate of KEBS cycle) uptake in flies intestine, limiting the wasting process [62]. Quorum sensing enables the bacterium to remain sessile in the flies gut and Vibrio polysaccharide (VPS) gene expression was shown to have increased during *V. cholera* infection of flies [103].

## 9. Importance of in-vitro model infection in Drosophila

The Drosophila S2 cells were first discovered by I. Schneider in 1972 [104]. S2 cells are derived from primary cell culture of late phase embryo of *Drosophila melanogaster*. S2 cells are macrophage like cells potentially grows in serum free medium as non-adherent suspension. S2 cells can express variety of heterologous proteins, upto 12 proteins could be co-expressed at a time in highly controlled manner, doubling at a rate equivalent to any cell lines derived from human cancerous cell line [105]. These cells do not form coherent clusters with no noisy gene expression profiles by maintaining uniformity during expression and chromosomal aneuploidy gets compensated during expression self adjusted to one gene copy number per cell unlike cancerous lineage [106]. These viable and potent cellular characteristics of S2 cell allows to be chosen for vaccine development, large scale enzyme as well as hormones production similar to Chinese hamster ovary CHO cell lines [104, 107]. The post translational glycosylation process is often not achievable in S2 cells making it disadvantageous [105]. The viral infections models are slow in inducing fatality in immuno-suppressed mutant flies, 50% death occurs after around 18–30 days post infection in live model [44, 108]. Therefore, S2 cell line model could requite certain challenges usually observed during in-vivo infection models.

## 10. In-vitro model of epidemic and pandemic infectious disease

Using drosophila S2 cell model a study showed that intercellular *Mycobacterium smegmatis* growth inside the host phagosomes is restricted by Rab7, CG8743, and the ESCRT factors [109]. *Cryptococcus neoformans* a fungi responsible for meningoencephalitis infection, S2 cells infected by this fungus up-regulates autophagy initiating proteins like Atg2a, Atg5 and Atg9a beside lysosomal markers like LAMP-1 and cathepsin D [110]. The hepatitis B virus surface antigen (HBsAg) coded by S gene was transfected in S2 cell line gave rise to no variation in expressed protein suggesting S2 cells useful for expression system [111]. The *Plasmodium falciparum* reticulocyte-binding protein homolog 5 (PfRH5) was expressed in S2 cells of Drosophila to produce non-glycosylated variants capable of binding to its receptor in rabbits resulted in IgG production against PfRH5 protein [112]. Highly potential vaccine VAR2CSA against malaria was successfully produced in S2 cells of *Drosophila melanogaster* [113].

## 11. Viral meningitis, encephalitis and hemorrhagic fever S2 cell line model

Herpes simplex virus was studied in Drosophila S2 cells where transfection of two viral proteins PILRα and gB responsible for binding to mammalian cells were expressed found to be poorly glycosylated [114]. The RNAi pathway was indulged by host cells to inhibit the Dengue virus (Flavivirus family) infection, by knocking down Argonaute (Ago1/2) and Dicer (Dcr1/2) showed sustained viral infection, currently clinical trial is underway NCT00936429 [115, 116]. Japanese encephalitis virus envelope glycoprotein E transfected in Drosophila S2 cells resulted in stable protein expression, this glycoprotein exposure in mice led antibody production against it [117]. Infection of Sindbis virus in live flies led activation of Notch, Jak–STAT and ImD pathway to intervene viral invasion [54]. Notch pathway mediated assimilation of ankyrin, plap, syx13, unc-13, csp, rab1 and rab8 during Sindbis virus infection in S2 cells [115]. The human antibody MR191 specific against Marburg virus was fused with recombinant RAVV GP ectodomain produced in S2 cell line [118]. The Zika virus structural envelope (E) protein were efficiently produced and secreted from transfected Drosophila S2 cell line model [119]. Flies produces RNAi against west Nile virus infection as a result of innate immune response similarly it was seen in S2 cell line, S2 cell lines were used for WNV infection, currently vaccine development NCT01477580 and NCT00707642 is underway [116, 120]. In a study mice were injected with glycoprotein GP of Ebola virus expressed in Drosophila S2 cell line found to produce antibodies against the infused antigen [121] (**Table 1**).

| Epidemic/ Pandemic Disease | Microbes | Vaccine/Drugs screened or derived out of fly model (in-vitro/ in-vivo) | References |
|---|---|---|---|
| HIV/AIDS | Human Immuno virus | Leptomycin B (In-Vitro) Unapproved under clinical trials Zidovudine, lamivudine, stavudine, didanosine, Abacavir | [76, 77] [78, 79] |
| Flu | Influenza A | Amantidine | [56] |
| Cholera | *Vibrio cholera* | Clotrimazole | [61] |
| Pneumonia | *Streptococcus pneumoniae* | Tetracycline | [92] |
| Tuberculosis | *Mycobacterium tuberculosis* | Rifampicin, Tigecycline + Linezolid | [100, 101] |
| SARS | SARS Corona virus | — | |
| MERS | MERS corona virus | — | |
| Measles | Rubeola virus | — | |
| Typhoid | Salmonella typhi | — | |
| Hepatitis | Hepatitis A and B | HBsAg expressed in S2 cell line | [111] |
| Small pox | Variola virus | — | |
| Malaria | *Plasmodium falciparum* | VAR2CSA/PfRH5 viral protein expressed in S2 cell line | [112, 113] |
| Zika Fever | Zika virus | Structural envelope (E) protein expressed in S2 cells | [119] |
| Dengue Fever | Dengue Virus | DEN1-80E expressed in S2 cells | [115, 116] |
| Encephalitis | Japanese encephalitis virus | JEV E protein expression in S2 cell line | [117] |

| Epidemic/<br>Pandemic Disease | Microbes | Vaccine/Drugs screened or<br>derived out of fly model (in-vitro/<br>in-vivo) | References |
|---|---|---|---|
| Haemorrhagic fever | Ebola virus | glycoprotein GP expressed in S2<br>cell line | [121] |
| Haemorrhagic fever | Marburg Virus | MR191 expressed in S2 cell line | [118] |
| Plague | Yersinia pestis | — | |
| Yellow fever | Yellow fever virus | — | |
| West Nile Fever | West Nile Virus | WN-80E expressed in S2 cell line | [116, 120] |

**Table 1.**
*List of Drugs/vaccines screened or developed against Infectious diseases in* Drosophila melanogaster *as a model organism.*

## 12. Disadvantages of Drosophila model for drug screening

*Drosophila melanogaster* being ectothermic organism unlike humans are endothermic homeotherms maintains physiological temperature constantly at 37°C, making it difficult to infect flies with bacteria like *Mycobacterium tuberculosis* grows strictly at 37°C [36, 122]. Several pathogenic viruses capable of infecting humans cannot naturally infect *Drosophila melanogaster* [55, 67]. The fungal dose response in flies is difficult to measure in oral infection model therefore this model is limited to study only the anti-fungal drug efficacy [64]. The presence of symbiotic microbes like Wolbachia a gram negative bacteria associate mostly with drosophila gut, improves the fly immunity against viral infection [123]. Superinfection like viral pneumonia cannot be studied at present to undertake preclinical trial using fly as a model.

## 13. Future perspective

Irrespective of multiple disadvantages flies could be used for studying drug efficacy. Multi-drug resistance tuberculosis infection could be studied in flies. The ART medication impairs human heart by causing prolonged QT, prolonged arrhythmic condition leads to myocardial infraction, *Drosophila* could be a suitable model to study the effect of anti retroviral therapy on fly heart [124–126]. Shockingly infection induced in flies by vesicular stomatitis virus in toll-7 depleted flies where 50% flies displayed death after 18 days, suggesting HIV infection could also kill toll7 mutant flies, as toll mutant flies displayed fungal invasion, this yet to be confirmed [44]. Alternative to this only viral DNA had been shown in a recent study to evoke immune activation in Drosophila by injecting it in thoracic region [87]. Kaposi sarcoma associated Herpesvirus (KSHV) needs a model which is yet to be studied in flies, however the KSHV viral gene latent nuclear antigen (LANA) interacts with RING3 of human which is homologous to drosophila female sterile homeotic (fsh) has already been identified [127]. Drosophila wound healing an important concern while inducing bacterial infection currently it is well understood and was found regulated by EGFR/ERK pathway essential for tissue repair [128]. The RNAi screens against Dengue or Influenza virus infection in cell culture could not identify Jak–STAT, ImD and toll dependent gene activation suggesting possible alternative pathway associated in infection modulation and no stimulation of inflammatory cytokine activation [40]. The food borne *Salmonella typhimurium* causes stomach flu (*gastroenteritis*) is well studied in Drosophila, but not epidemic pathogen *Salmonella typhi*.

## 14. Conclusions

Currently the existing models of infection in drosophila are capable of causing infection using viruses, bacteria (gram negative and gram positive) and fungi. These models are of great use since the efficacy of a drug capable of modifying diseased condition could be studied in detail in live *Drosophila* or in-vitro S2 cell. In this detailed review on epidemiology of infectious disease, it could be predicted that infection alone is a threat to overall population imposing death to more than 5 million individuals. Diseases like influenza, HIV, pneumonia, tuberculosis and cholera could be studied in flies. Currently there are 20 diseases which caused epidemic worldwide [2], 13 of the pathogens were studied in *Drosophila melanogaster* and diseases caused by yellow fever virus, Nipah virus, MERS, Hepatitis C virus, Salmonella typhi, Crimean congo hemorrhagic fever virus, chikungunya virus, monkeypox virus, Nipah virus and shigellosis bacteria are yet to be studied in-vitro/in-vivo. These diseases are of pandemic and epidemic criteria it causes huge number of deaths globally. Controlling the epidemic and pandemic diseases should be the main focus of the healthcare sector in the next decade.

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## Conflict of interest

The author has no conflict of interest.

## Notes/Thanks/Other declarations

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