# Animal Models to Understand Host–Pathogen Interactions

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**Abstract**

Infectious diseases are the outcome of molecular cross-communication between host and its pathogens. During the molecular cross talks, host–pathogen proteomics, genomics, and immunological responses are highly influenced. Host would respond to their pathogen through several mechanisms for the clearance of pathogens. It is always necessary to identify the underlying molecular mechanisms of pathogenicity. In general, host–pathogen cross talks are complex and dynamic in nature that exploits most of the host cell functions. Immune responses

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are initiated by host cell as a response to the pathogen attack. It was found to be very difficult, exclusive, and ethically inappropriate to evaluate the notorious pathogen interactions that cause adverse effects on human health at the organism level. Hence, the need for experimental animal models to understand host–pathogen interactions always emerges. Incorporation of a host animal model not only allows the identification of host–pathogen interactions but also lights into the phenotypic impacts and molecular mechanisms of pathogenicity. In ancient times, better understanding of virulence determinants and antimicrobial therapy has been hindered by the restrictions of adequate experimental models and necessary tools to measure the severity of infections. Laboratory hosts that have been employed as an alternative for mammal infection models are *Caenorhabditis elegans*, amoeba, *Drosophila melanogaster*, and *Danio rerio*. These models are used as infection models owing to their shorter generation times, flexibility, and affordability to study forward and reverse genetic analysis. Even though humans are excellent model to study human pathogens, their use in studies is limited due to the safety, ethical, and expense concerns. Among other primates, monkey, baboons, and chimpanzees are idyllic and mimic most of the infectious diseases. But rodents such as mice, rats, rabbits, hamsters, and guinea pigs are widely proposed model hosts due the limited application of other primate models. Here, we review the available animal models to study host–pathogen interactions with a focus to decipher pathogenicity mechanisms.

**Keywords**
Infectious disease · Host–pathogen cross talks · Host animal models · Pathogenicity

## 20.1 Introduction

Microorganisms were thought to be the primary invaders that involved in the host–pathogen interactions resulting in disease. Slowly, new insights into the host–pathogen interactions suggested that this does not always cause a disease. This has introduced new terms to enlighten the states in which host and pathogen do not result in a disease (Casadevall and Pirofski 2000). Host–pathogen interactions are also a type of interspecies interactions that result in infections occasionally but need to be figured out at the molecular level as early as possible. Generally, a pathogen protein attach to the receptors of human proteins and manipulates host biological processes. Deciphering the host–pathogen interactions is having an utmost role in developing suitable treatment methods. It has been reviewed that infections rose by HIV, plague, Ebola, cholera, other bacterial, and viral pathogens drag the circumstances more worse owing to the high mortality rate in each year. Infectious diseases not only affect human health but it has adverse effect on the economic status of country (Kösesoy et al. 2019). Host–pathogen interactions are followed by a cascade of cell signaling events. The early events involve the recognition of

pathogen-associated molecular patterns (PAMPs) and the conserved microbial components by host cell pattern recognition receptors (PRRs). This binding is known to determine the rate of failure or success of an immune response. A complex cascade of cellular signaling events takes place followed by the PAMP–PPR interactions. Complex cell signaling events includes an early host response, pathogen clearance, activation of kinase pathways, production of effectors, activation of transcription factors, and modulation of innate and adaptive immune responses and finally leads to a pro-inflammatory or anti-inflammatory response (Bahia et al. 2018).

Emergence of coevolutionary dynamics between the host and pathogen is the most critical and well-studied interspecific interaction. Coevolution has been studied well among widespread ecosystems and found popularly in diverse set of host–parasitic interactions. Host–pathogen coevolution has a special role in shaping the diversity and population structure of hosts and pathogens. Coevolution of host and their pathogen helps in understanding the structure of communities, maintenance of sexual recombination, direction of species invasions, and population dynamics. The flow of coevolution is strongly influenced by the spatial structure of their populations and can occur in relatively short time. All cases of host–pathogen interactions harbor a genetic basis to infection. Size and genetic makeup of the pathogen and the density of susceptible host genotypes in the earlier generations will be the function of the frequency. Also, the chances of host becoming infected is a function of the frequency of pathogen genotypes and past genotypes of both populations. In general, each population can act as a dynamic target for others and hence these dynamics of one partner over the other helps to maintain the polymorphism. There is a pathogen specificity with an enhanced infection on a given host system but decreased infection at the community level due to the polymorphism (Morgan and Koskella 2017). Molecular cross talks between pathogen and host result in infectious diseases. There are several mechanisms underlying the pathogenic rewiring of host cells. Host–pathogen protein–protein interactions also mediate these molecular cross talks. Protein–protein interactions and protein complexes encompass the principal functional modules of the cell. Pathogenic hijacking or rewiring of host proteome involves the intervention at the signaling pathways and cellular functions to determine the strength of the virulent intervention. Phenotypic impact of a pathogen is directly related to its capacity to rewire the host interactome. This describes the impacts of each virulent protein that are linked to their number of interactions with the host proteins. Hence, mapping the host–pathogen protein interactions may offer valuable understandings of biological functions of virulent proteins that are critical to the progression and spread of pathogens. It also provides insights on the molecular basis of pathogenicity and possibly single out the pharmacological intrusion targets (Nicod et al. 2017).

All organisms sense and reply to their external stimuli through the production of second messengers (cyclic nucleotides). A universal second messenger, cyclic diadenosine monophosphate is synthesized by diverse life forms (mammals, fungi, protozoa, and bacteria). cAMP regulates virulence gene expression in host cells owing to their influence on the transcription factors that are dependent on environmental control of secondary messenger production (McDonough and Rodriguez

2012). It is known as the bacterial signaling nucleotide produced by several human pathogens. C-di-AMP has central role in catabolic repression and virulence determinants' expression. Mostly, an infected host cell recognizes the synthesized c-di-AMP and triggers an innate immune response to prevent the colonization and transmission of pathogens and ultimately to clear the pathogens. It has been reported that long-standing interaction of host and pathogens results in the coevolution of both and controls the activation of innate immunity by the signaling molecule. These second messengers will be produced in the host cell in such way that it modulates the host response to intensify the infection by circumventing immune recognition (Devaux et al. 2018).

Most of the knowledge of host–pathogen interactions and their pathogenic mechanisms have risen from the use of various model systems including cell lines and animal models. Model systems are preferred in host–pathogen studies to confirm their pathogenic role causing a disease and also evaluate their immune responses. Cell lines are defined as indispensable powerful tools for learning the molecular and cellular mechanisms of pathogenesis. Recently, animal models are employed to evaluate the pathogenicity of pathogens in host cells, immune responses and to analyze the efficacy of a vaccine. Altogether, both cell lines and animal models are an integral part in the study of host–pathogen interactions and one must be acquainted with the knowledge of these models and their applications (Bhunia 2018). There are several excellent reviews and trailblazing contributions available in this research domain to enhance our understanding in the host–pathogen interactions and to provide new insights for deciphering the interactions through animal models. This chapter summarizes the fascinating reviews addressing various facets of host–pathogen interactions studies in animal models.

## 20.2 Importance of Animal Models

It is not recommended to use humans to evaluate host pathogens and pathogenicity owing to their ethical concerns and safety. From the research point of view, human models are ideal for studying host–pathogen interactions. Some cases of nonfatal diseases human volunteers have been incorporated and studied. To overcome the boundaries of using humans, animal models are frequently used and applied as a substitute for these studies. Most regularly used model hosts are *Caenorhabditis elegans* (nematode), *Dictyostelium discoideum* (amoeba), *Drosophila melanogaster* (fruit fly), *Danio rerio* (Zebra fish), *Cavia porcellus* (guinea pigs), *Mus musculus* (mouse), *Rattus* (rat), *Cricetinae* (hamster), and *Oryctolagus cuniculus* (rabbit). Among them, the nematode, fruit fly, amoeba, and zebra fish have major role in host–pathogen studies as these models exhibit shorter generation times and also due to their amenability and affordability to forward and reverse genetic studies. Owing to the ethical and expense-related concerns, widespread use of some nonhuman primates is limited, which is ideal to mimic many diseases (Lemaitre and Ausubel 2008). Compared to small mammals, morphological and genetic similarities between humans and primates provided an instinctive feeling that they may deliver

more reliable and trustworthy data. Now, clinical studies and data would provide more relevant initial research orientations if it is encouraged (Druilhe et al. 2002).

Immunity of host and their susceptible profile toward infectious agents are defined based on multiple factors such as immune experience, environment, and generics of host and its pathogen. Studying the host–pathogen interactions directly on human models are quite challenging and complex. Hence, host genetics in causing diseases are more depend on rodent model systems (Noll et al. 2019). Most commonly used rodents include mice, rabbits, rats, guinea pigs, and hamsters. However, pathogenic studies using mouse models occupy the central part of the research flow with a successful interpretation of host–pathogen interactions. There are several advantages of using animal models in biomedical research over other model systems which includes: (1) animal models are used in such studies from past many years, (2) features of animal models are well documented for the correct application in different studies, (3) studies on these models provide information regarding current paradigm, and (4) they are readily available from the local suppliers (Druilhe et al. 2002). Among all, vertebrate and invertebrate models are having specific advantages when compared to both. Invertebrate models provide greater advantages over the other one due to their economy of size and ethical concerns. Vertebrate models are vital for the cellular and molecular analysis of host–pathogen interactions (Naglik et al. 2008) (Fig. 20.1).

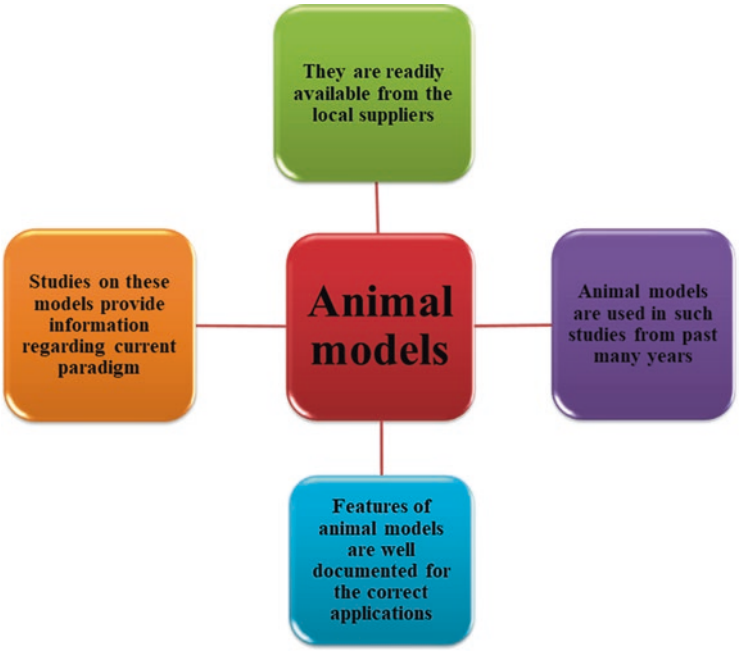


**Fig. 20.1** Advantages of animal models used in different studies of host–pathogen interactions

Animal models also provide prospects to engineer and study the host–microbiota interactions with a level of experimental controls which is not possible with human models. Both the vertebrate and invertebrate models provide enough information regarding the microbial molecular patterns and host recognition receptors. These models are useful for studying the tractable genetics that are essential for enabling symbiosis by both the host and the pathogens. Model systems are extensively used in microbiome studies for revealing the host physiology, skeletal biology, and lipid metabolism. Ever increasing number of studies conducted in host–microbiome research area will prove the associations recognized between the human microbiota and disease (Kostic et al. 2013). Current scenario of biomedical research dealing with host–pathogen studies is dominated by the mouse, fruit fly, and nematode models. Researchers think that these models can be used to summarize the physiology and diseases in different species through manipulating some genes, which would actually make them as perfect models of human biology. However, still there are some limitations of using animal models, which have to be eliminated by the introduction of new or other unconventional model organisms. Most of mice models involved in different studies are young ones but most of diseases evaluated by different researchers are associated with old people such as cancer and neurological disorder. Recently, large scale collaborative research project results showed that genomic responses to acute inflammatory responses are greatly comparable to humans but are not portrayed by corresponding mouse models. New approaches have to be developed to gain more knowledge about the prevention and physiology of diseases. It has been found that animal model research focusing more often on laboratory species may weaken the chances of scientific progress in the forthcoming years (Conti et al. 2014) (Table 20.1).

## 20.3 The Nematode *Caenorhabditis elegans*

*C. elegans* is a soil inhabitant microscopic nematode having a length of 1 mm. Compatibility of *C. elegans* in laboratory work as models is influenced by their shorter generation time of 3 days and also their capacity to produce 300 progeny by a single animal. They naturally grow on agar plates containing *Escherichia coli* OP50, which is a uracil auxotroph facilitating controlled growth. These animals can be accommodated in laboratory conditions and grow quickly in large numbers.

**Table 20.1** Different invertebrate and vertebrate animal models used in host–pathogen studies

| Animal models | |
|---|---|
| Invertebrate models | Vertebrate models |
| *Caenorhabditis elegans* (nematode) | *Mus musculus* (mouse) |
| *Dictyostelium discoideum* (amoeba) | *Cavia porcellus* (guinea pig) |
| *Drosophila melanogaster* (fruit fly) | *Rattus* (rat) |
| *Galleria mellonella* (Greater wax moth) | *Danio rerio* (zebra fish) |
| *Acanthamoeba castellanii* (amoeba) | *Oryctolagus cuniculus* (rabbit) |

Owing to their small size, around 10–20 infected worms can fit to the single well of a 384-well multititer plate. This nematode has become one of the preferred models for cell biologists and geneticists due to their ease of housing in laboratory conditions and simple body organization and hermaphroditic lifestyle. Infection studies using *C. elegans* can be profited from a host influenced circumstances in genetically characterized organisms (Lorenz et al. 2016). Another amazing benefit of using nematode is that adult worms are purely post-mitotic with the exception to germline. Adult worms are developed through the transition between four larval stages. At the end of transition from third to fourth larval stage only changes occur at the growth level of worms, not in their number of somatic cells. Many complexities raised while working with multicellular model systems whereas all cells turn over quickly are eliminated in the case of *C. elegans* model system. Number and identity of each cell vary from one worm to another worm is another advantage of using this model system (Marsh and May 2012).

*C. elegans* have been used as screening platforms for anti-infective molecules from long days. Nematode is an emerging powerful model system to study host–pathogen interactions and can be evaluated for multiple human pathogens for anti-infective development. The development of anti-infective agents is based on the fact that virulence determinants of pathogens causing disease in humans are also involved in killing nematode. *C. elegans* also mounts immune defense produced by the specific pathogen which involves the conserved innate immunity regulators. Pioneer work in *C. elegans* as animal models for screening of anti-infective agents is demonstrated by Frederick Ausubel (Peterson and Pukkila-Worley 2018). This well-known studied animal model for host–pathogen interactions is susceptible to *Enterococcus faecalis*, *Serratia marcescens*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* by causing infection through their intestine. Many of the above-mentioned Gram-negative and Gram-positive bacteria cause infections in humans using the similar virulence factors (James et al. 2018).

There are some conserved immune signaling pathways in *C. elegans* which are always a center of attraction for studies. Immunity studies mainly focus on three approaches: (1) forward genetic screening for nematode mutants showing altered pathogen susceptibility, (2) application of reverse genetic approaches for evaluating conserved genes, and (3) assaying the gene expression that induced by pathogens or by regulation of signaling pathways (Kim 2008). Immune systems of worms are found to be comparably simple and evolutionarily predate those of higher organisms. Specifically, this organism lacks adaptive immunity with devoid of mobile immune cells. However, they carry three pairs of cells (coelomocytes) for detoxification processes that are not involved in any of the immune functions. They use only their innate immunity to mount a response toward pathogens for their removal and to resist them. Innate immune system regulates the signaling pathways of worms upon finding a pathogen at their transcriptional level and organized by several signaling cascades. Major pathways known to date are DAF-16 and DAF-2, p38/PMK-1, DBL-1, and ERK/MPK-1 signaling pathways (Williams and Schumacher 2018).

*C. elegans–P. aeruginosa* model is developed for host–pathogen interaction studies. *P. aeruginosa* strain PA14 causes disease in both animals and plants through

a shared set of virulence factors. This bacterium kills the worms either by fast killing or by slow killing method. In slow killing mechanism, PA14 in a low salt medium kills worms within a period of 2–3 days through the accumulation of pathogen inside the intestine of worms. In addition, in a high salt medium PA14 adopts a fast killing mechanism within 4–24 h through the production of diffusible toxins. *P. aeruginosa* PA01 strain kills the worm through a rapid paralysis mechanism after 4 h (Tan 2000).

## 20.4 Amoeba

Amoeba is a eukaryotic microorganism with great diversity. They belong to different taxonomic group such as fungi, algae, and protozoa. They stand along among all microorganisms due to their amoeboid lifestyle. Amoeboid lifestyle is characterized by their capacity to change shape by forming pseudopods. Amoeba is considered as professional phagocytes owing to their ability to feed on bacteria and other microbes by phagocytosis. Even then, amoeba cannot degrade all microbes. Some of the microorganisms are able to resist the digestion by amoeba and even can use them as host cell for their activities. There are several studies of using amoebae as host models for analyzing the pathogenicity of pathogens. Most commonly used amoebae belong to the phylum Amoebozoa, which is closest to the phylum fungi and animals and the predominant representatives are from the genera *Dictyostelium* and *Acanthamoeba*. Several pathogenic fungi (*Cryptococcus*, *Candida*, and *Aspergillus*) and bacteria (*Mycobacterium*, *Legionella*, *Francisella*, and *Salmonella*) are known pathogens of these amoebae. Hence, amoeba models are useful for studying the complex interactions with the above pathogens (Thewes et al. 2019).

*Acanthamoeba* and *Dictyostelium* are identified as the natural and versatile host model for *Legionella* infection. Etiology and cellular host interactions of *L. pneumophila* have been particularly viewed in these amoebas. Owing to their similarity in causing infection in macrophages and amoebae, amoeba is used as a powerful model to study bacteria–macrophage interactions. Among these, *Acanthamoeba* are observed in habitats with *Legionella* positive isolates and are widely distributed. Yet, for laboratory purposes axenic growth of *Acanthamoeba* strains are mostly favored. This amoeba implements a biphasic life cycle comprising a trophozoite stage and cyst stage. A particular strain, *A. castellanii* adopts a diverse repository of pattern recognition receptors, which are thought to have orthologous roles in the innate immunity of higher organisms (Swart et al. 2018). *A. castellanii* can be used to study the molecular basis of different pathogen interactions as this amoeba interacts with wide variety of pathogens. The role and characterization of arsenal receptors utilized by this strain to engulf the pathogens would extend knowledge in how pathogenicity could be enhanced. Virulence gene expression in *A. castellanii* and mammalian cells would draw information regarding how these pathogens evolved and got adapted to different hosts (Guimaraes et al. 2016).

One of the merits of using this amoeba over other nonmammalian system is that they can be grown at 37 °C, which is the optimal temperature for most of the deadly

pathogens. This will allow setting up of conditions in the laboratory that is more similar to the natural pathology of human pathogens and offers a great relevance. Complex mechanisms of host defense against pathogens can dissect from the *Acanthamoeba* due to their unicellular nature compared to the metazoal-nonvertebrate hosts. Information regarding the novel genes that are involved in mammalian pathogenesis caused by bacterial pathogens can be predicted using the amoeba model. Also, host cell components employed to respond to the pathogen attack can be identified using the unicellular amoeba compared to mammalian hosts (Sandstrom et al. 2011). There is some spine-like structures found on the surface of *Acanthamoeba* spp. known as acanthopodia. *A. castellanii* are known as simple, rapid, and low-cost model for studying host–pathogen interactions. Some of the pathogens are able to grow and internalize the amoeba. This may lead to the transmission to other susceptible hosts and exerts pathogenicity. These amoebas harboring the human pathogen act as Trojan horses and thus protect them from antimicrobial effectors and other environmental circumstances, which provide conditions for its survival and growth. Amoeba and macrophages are thought to share similar ability to ingest particles into the phagosomes. Presence of lysosomes makes both the cells hostile to infection by the pathogen. This model is attractive for phagocytosis of several pathogens such as *H. capsulatum*, *Sporothrix schenckii*, and *Blastomyces dermatitidis* (Singulani et al. 2018).

The interaction between *Legionella pneumophila* pathogen and the social amoeba has been explored using biochemical, cell biological, and genetical approaches with a focus on their small and large GTPases, autophagy components, phosphoinositide lipids, retromer complex, autophagy components, and bacterial effectors attacking these host factors. The genome of *D. discoideum* is having a size of 34 Mb and along with six chromosomes (size of 3.5–8.6 Mb). Studies related to genes can be performed using the model system where the genes in their mutant nature are able to cause disease in humans. In brief, complete information on genome of amoeba enhances the application of *D. discoideum* as an outstanding collection of genetic tools to evaluate their fundamental cellular functions. It is important to modulate various host cell processes through qualitative and quantitative approaches for an efficient replication and establishment of infection by *L. pneumophila* in *D. discoideum* and macrophages infection model. DNA microarray analysis comprising the half of the genome of amoeba identified around 371 genes that are regulated during an infection with *pathogenic L. pneumophila* Philadelphia-1 strain JR32 after 48 h of infection. Transcriptional analysis of *D. discoideum* infection model was revealed vital aspects of host–pathogen cross talk (Swart et al. 2018).

The genetically tractable, cooperative, and haploid social amoeba serves as a host for diverse pathogens such as *Legionella pneumophila*, *Mycobacterium* spp., *Pseudomonas aeruginosa*, and *Cryptococcus neoformans*. The studies on this amoeba enlighten more on host–pathogen interactions which include: (1) use of wild-type amoeba as a screening platform for extracting information regarding the virulence factors of intracellular, extracellular, and mutant pathogens; (2) mutants of this amoeba to classify the host susceptible and resistance determinants to infection; and (3) introduction of reporter strains of amoeba to understand in detail about

the mechanism of host–pathogen cross talks (Steinert and Heuner 2005). This social amoeba is proven as a tool for finding several bacterial and fungal virulence factors. Specifically, tagging of genes with some markers such as green fluorescent protein allows the real and in vivo monitoring of unique virulence and host cell factors. Most of the assays that decipher host–pathogen interactions in amoeba integrate infection assay, phagocytosis assay, and confocal assay for in vivo monitoring of fluorescence (Ünal and Steinert 2006).

## 20.5 The Honeycomb Moth *Galleria mellonella*

Greater wax moth, *G. mellonella* are widely used for evaluating host–entomopathogenic microbe interactions. Not only for entamopathogenic microorganisms, *Galleria* can effectively employed as a reliable model to study the pathogenesis that exerted by many of the human pathogens. Vast opportunities open with the honeycomb wax host model to study the host–pathogen cross talks owing to their low rearing costs, ranking as an ethically acceptable model and their convenience in injection feasibility. Apart from this, growth of moth at 37 °C which is similar to that of human pathogens allows them to produce various pathogenic factors. Researchers have found a correlation between virulence of pathogen in mammals and this model. *Galleria* produces a complex innate immunity toward their pathogen. The multicomponent immune response produced in moth involves cellular phagocytosis, phenol oxidase-based melanization, and hemolymph coagulation. Pathogens will be destroyed by the production of lysozymes, antimicrobial peptide like defensins, and reactive oxygen species, which is similar to the mechanisms observed in mammals. *Galleria* can also recognize molecular patterns associated with nonself microbes through their germ line encoded receptors such as peptidoglycan and Toll recognition proteins. *Galleria* also employs danger signaling for detection of pathogens either through the sensing of peptides resulted from a protein cleavage process by metalloproteinase or nucleic acids produced by damaged cells (Mukherjee et al. 2010).

*Galleria mellonella*, a caterpillar of the wax moth are utilized in the host–pathogen interactions of *Burkholderia mallei*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Burkholderia cepacia*, *Bacillus cereus*, *Francisella tularensis*, and several pathogenic fungi. *C. elegans* infection models for the study of *Acinetobacter* pathogenesis showed some limitations that recovered with the use of *Galleria*. This model is effectively used for the evaluation of efficacy of antimicrobial agents as the model is amenable to antibiotic treatment (Peleg et al. 2009). This model is considered as ethically acceptable owing to their ability to enhance reproducibility by introducing larger group sizes. This simple invertebrate animal is a promising infection model for *M. tuberculosis* complex. In the first time of *Galleria* infection model for tuberculosis pathogen showed replicate features of pathogenesis through the induction of granuloma-like structures and inclusion of lipid bodies, which are the unique features of infection. Use of this model has markedly reduced the use of more expensive and time-consuming mycobacterial infection models. Model is an effective tool

for the assessment of unusual antimycobacterial drugs and novel vaccine entrants in vivo. Future studies of mycobacterium infection models with *Galleria* include the optimization studies with the pathogenic, nonpathogenic, drug-resistant, and drug-sensitive *M. tuberculosis* isolates (Li et al. 2018).

Greater wax moth belong to the Lepidoptera family has successfully used as a model to study the virulence of pathogenic fungi (*Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans*). Model is useful for the evaluation of antifungal drugs in the treatment of fungal infections. Hemocytes present in the hemolymph of *Galleria* presents a phagocytic effect against its pathogens. Another important role of immune system in pathogen defense is through the stimulation of melanization and encapsulation of foreign particles. Virulence of fungal pathogen can be assessed by the microbial burden, hemocyte density, and induction of microbial morphological changes in the moth. There are reports stating the killing effect of various *Candida* species in *G. mellonella*. Host–pathogen interactions of *C. tropicalis* and *Galleria* were fully characterized recently. Results indicated that *G. mellonella* is a nonconventional host to study the virulence of human fungal pathogen *C. tropicalis*. Also, this offers a feasible and simple model system for analyzing the antifungal drug efficacy and their protective role during *C. tropicalis* infection (Mesa-Arango et al. 2013).

## 20.6 The Fruit Fly—*Drosophila melanogaster*

Genetically tractable fruit fly, *D. melanogaster*, has delivered remarkable views into the host–pathogen interactions. This model provided that many of the aspects related to these host–pathogen interactions are conserved in higher organisms. Fruit fly possesses a well-established stand in the evaluation of host interactions with bacterial, viral, and fungal pathogens. Previously, host response of malarial parasite has been studied using *D. melanogaster* model prior to the sequencing of mosquito genome (Igboin et al. 2012). Fruit fly is used as a model of innate immune system owing to their simplicity and ease in which they can be applied in both forward and reverse genetics. Forward and reverse genetics allows the characterization and identification of innate immune responses produced against microbial pathogens that are preserved across evolution. Fruit fly generates the immune responses with three effector arms such as an inedible antimicrobial peptide response, a reactive oxygen response (by the enzyme phenoloxidase) and a cellular immune response through which foreign particles are phagocytized in fly hemocytes and accumulation of melanin pigment. Humoral antimicrobial peptide response is studied and controlled primarily by two pattern recognition pathways. The two pathways include Toll and Imd. Regulatory mechanisms of melanin deposition and cellular immunity are not fully explicated and studied widely only in recent years (Moule et al. 2010).

*Drosophila* recognize various Gram-negative and Gram-positive pathogens by sensing the specific forms of peptidoglycan present in the bacterial cell wall using peptidoglycan recognition proteins (PGRPs). These peptidoglycans found only in cell membrane of bacteria are essential glucopeptidic polymers (Lemaitre and

Hoffmann 2007). *D. melanogaster* is one the most and well-studied organism for a century of genetic work including RNA interference–hairpin constructs, reporter genes studies, and targeted gene expression to overexpress the recombinant proteins. This invertebrate organism provides an interesting alternative as host model for evaluating pathogenesis owing to their powerful genetics. Previously, genome-wide screen in fruit fly enabled us to identify the genes that are involved in the virulence of *Serratia marcescens* in the host infection model. A similar study was conducted to identify the genes associated with *P. aeruginosa* mutant virulence in fruit fly infection model. Unlike other invertebrate models, *D. melanogaster* is not useful for the high throughput screening of antimicrobial drugs but better designed for understanding the host–pathogen interactions in detail (Limmer et al. 2011).

*Drosophila* has been used for probing the mechanisms behind the interactions between *P. aeruginosa* virulence factors and host cells. It has been proven that Toll signaling pathway is induced in response to the *Pseudomonas* infection in the host cell that provided the insights how these virulence factors cause resistance in pathogen. One of the advantages of using *P. aeruginosa*–fruit fly infection model over human pathogenesis model is the manipulation of genome of host and pathogen (Lau et al. 2003). Recently, fruit fly is employed as a model system for host–symbiotic microbiota interactions other than the typical host–pathogen studies. Most commonly found gut microbiota of fruit fly are from the families of Lactobacillales, Enterobacteriaceae, and Acetobacteraceae. Gut microbiota communities are strongly dependent on the diet of the model system. Oxygen will be able to enter into the entire diameter of fruit fly gut as these gut flora are aero tolerant or obligate aerobes. It is possible to virtually culture all the gut microbiota in laboratory owing to the aerobic growth of this flora and their relative taxonomic simplicity. *Drosophila* possess a large potential to enable the better understanding of host–symbiont interactions due to the culturing of large proportions of fruit fly gut microbiome along with the rapid growth, wide collections of mutant flies, and their high reproductive capacity (Kostic et al. 2013).

Reports say that *P. aeruginosa* and *Plasmodium gallinaceum* can infect *D. melanogaster* but the former kills the model system where as latter one proliferate and develop within the fruit fly. Owing to the low cost of model system screening methodologies offer to unravel the mechanisms behind the host–pathogen interactions which could reduce the use of expensive or laborious vertebrate hosts. Also, the genetically tractable infection model allows a quick and possibly unbiased identification of host factors affected during the pathogenesis. In one study, *Mycobacterium marinum* causes systemic disease in fruit fly which is closely similar to the human tuberculosis. This bacterium had killed *Drosophila* with a lethal dose of 5 CFU. Also, adult flies or larvae can be easily infected with injected doses of bacteria for evaluating the pathogenesis. Genetic tools available in the fruit fly infection model are unparalleled and stand as a best studied model among all animal model. Like other vertebrates, *Drosophila* has bactericidal phagocytes called as hemocytes to fight with the pathogen attack (Dionne et al. 2003). *Drosophila* has been successively employed as a systemic infection model for evaluating antibacterial efficacy of phages toward the secondary opportunistic human pathogen, *P. aeruginosa*. Unlike

antibacterial agents, phages can be easily and delicately counted by employing simple assays to study the pharmacokinetic properties of injected phages in the small-scale infection model. In order to address the bioactivity of antibacterial agents in this small-scale infection model, therapeutic phages would be transferred to the flies by placing starved flies in the media harboring appropriate number of phages. Routes of antibacterial administration can be consecutively exploited here to assess the antibacterial efficacy of bacteriophages against *P. aeruginosa* infection (Jang et al. 2019).

## 20.7 The Zebra fish *Danio rerio*

Zebra fish gained much interest as infection model for developmental biologists. It is a teleost fish which belongs to the family of Cyprinidae. They can breed easily and a single female can lay eggs up to a number of 200 per week. This fish was found as an amazing developmental model 30 years ago. Future expectations of zebra fish as model systems relies purely on different studies regarding the complete genome sequencing and expressed sequence tags sequencing projects to identify different zebra fish genes. Preliminary studies revealed that zebra fish shares many orthologous genes and conserved synteny with mammals (van der Sar et al. 2004). This model holds a position in the high throughput screening of drugs for inflammatory diseases, cancer, and infectious diseases. Zebra fish can be effectively used for genomic and mutant analysis with excellent opportunities of in vivo imaging. It is possible to study the different types of immune cell types synthesized toward disease progression with help of developing embryo immune system. In addition, zebra fish embryos and larvae are suitable for dichotomizing the innate immunity host factors related to the pathology owing to their temporal separation of innate responses from adaptive immunity responses. Moreover, immune systems of zebra fish and human share a remarkable similarity, which possess central role in biomedical applications. Current knowledge on the downstream signaling and signaling components involved in the innate immune responses of embryo are important to decipher the mechanism of host–pathogen interactions. Zebra fish larvae and embryo holds a position to fill the gap generated between the cell- and rodent-based model systems. This transparent model system also provides several advantages in drug trafficking and drug administration studies. Zebra fish has developed as an extremely powerful model over the past years for studying vertebrate host immune response and interaction with bacterial virulence factors, in vivo imaging, and genetic analysis and drug screening in fish larvae and embryos (Meijer and Spaink 2011).

In the present century, zebra fish larvae and embryo are accepted as genetically tractable and optically accessible with fully functional immune system that comprises macrophages and neutrophils, which mimic the mammalian counter parts. Several pathogenic interactions have been investigated to provide unprecedented resolution of cellular responses to the infections in vivo zebra fish model. This fish model has been proposed for several bacterial, viral, and fungal pathogens. Zebra fish and larvae models are utilized to understand the pathogenesis and cell biology

rather than focusing the whole field of fish–human pathogen interactions. This model has effectively used for pathogen studies of several Gram-negative and Gram-positive bacteria such as *Salmonella typhimurium*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Mycobacterium marinum*, *Mycobacterium abscessus*, and *Mycobacterium leprae* (Torraca and Mostowy 2018). Recently, infection course of pathogen studied using fluorescence-tagged pathogen allowing it to visualize through wild field epi-fluorescence microscopy due the transparency at larval stages. Further, recently, a method was introduced to monitor the *Salmonella typhimurium* infection progression using epi-fluorescence microscopy. Study also allowed visualizing the free-swimming bacteria through the circulatory system, phagocytosis of bacteria, and heterogeneous gene expression activation using a nontoxic inducer (Medina and Royo 2013).

Zebra fish embryos are well-known model for in vivo pathogenic studies of *P. aeruginosa*. There are two methods for zebra fish–pathogen studies where infection is achieved either by microinjection into the larvae or by static immersion method. A report was found with *P. aeruginosa* infection to zebra fish through both microinjection method and immersion method. Proteomics pathways affected by infection also evaluated both in pathogen and in host using non-isotopic metaproteomics methods. They found that metabolic pathways of fish such as hypoxia through HIF pathway was enriched by immersion method whereas inflammatory pathways mediated by chemokine and cytokine signaling molecules were enriched in infected larvae exposed to injection methods. They demonstrated the fitness of embryos as a model for assessing proteomic studies after infection (Díaz-Pascual et al. 2017).

Other than the conventional models, zebra fish xenografts are coming into the picture as a useful disease models and for translational research. Recently, scientists developed novel mouse–zebra fish hematopoietic tissue chimeric embryos for host–pathogen studies and hematopoiesis. Both the mouse and human hematopoietic tissues can be engrafted into the fish embryos for studies. Authors predicted that the chimeric embryos could be amended to study in vivo and real visualization of and analysis of host–pathogen interactions. Here, the zebra fish xenografts of murine tissue eliminates the generation of chimeric animals for different studies. Then, it also expands its area of studies that can be studied in zebra fish chimeras such as the murine cell behaviors (Parada-Kusz et al. 2018). Zebra fish continues to a model organism for disease and provides new insights into the disease mechanisms and its therapy (Patton and Tobin 2019).

## 20.8 Primates

Nonhuman primates used for host–pathogen studies are rhesus macaques (*Macaca mulatta*) and bonnet macaques (*Macaca radiata*). These nonhuman primates and humans are not studied widely for host–pathogen cross talks. These models are used only for evaluating specific pathogens (Burt et al. 2017). Early development and pathogenesis studies are performed in small mammals even though there is a

constant pressure for the employment of nonhuman primates. Despite of their cost and relative scarcity, ethical concerns over their use often limits use in host–pathogen studies. There is always an intuitive feeling that only these models can provide more reliable data despite of their genetic and morphological similarities between primates and humans. Baboons are thought to be better primate model for evaluating course of pathology and disease. Exciting studies performed using baboons for evaluating schistosomiasis and periportal fibrosis (Druilhe et al. 2002). Diverse mammalian species are applied as experimental models to study infectious diseases caused by *P. aeruginosa* such as chinchilla to swine and up to nonhuman primates. These models served as suitable hosts to analyze the infections associated with *Pseudomonas* such as biofilm-associated infections (Lorenz et al. 2016).

### 20.8.1 Mouse

Based on a number of practical reasons, mice are preferred over other animal models. Major advantages of mouse animal models for host–pathogen studies are small size, cost effectiveness of maintenance in laboratory, availability of immunological tools for mice, and availability of genetically modified mouse strains. However, there are some criticisms of mouse models. Always, research with mice should be validated with other model systems to confirm the verdicts. Mouse model always stands as an important model of infection (Fonseca et al. 2017; Lowe et al. 2018). Inbred mouse strains are used past many years for studying the degree of susceptibility of different types of infectious agents. In order to gain more knowledge about the host responses toward these infections, a genetic approach in mice is adopted. Present advances such as germ line modification (BAS transgenics) which provided with positional cloning approach have made the studies easier. Quantitative trait loci and additional novel genetic loci, which play a vital role in host responses toward infections have been recognized. Thus, cloning and characterization of novel loci approaches would light the future years to unfold the story of genes and proteins involved in the host–pathogen interactions that eventually lead to onset and progression of an infection (Fortier et al. 2005).

Years ago, molecular and genetic toolbox created for mouse models empower the scientists manipulates and study the genes in vivo. Even though, mice are employed extensively to study the pathogenesis of human infections, these models summarizes many aspects of human infections as incorrect. Briefly, mouse is generally resistant to infections caused by HIV, *Plasmodium falciparum*, and *Shigella flexneri*. Host tropism or host restriction toward infections often stand as a hindrance for using mice as experimental model. In such situations, mice can be genetically engineered so that it strictly resembles to humans in all means of host–pathogen interactions (Coers et al. 2009). Another mouse pharyngeal colonization model is an inexpensive and available experimental model, which permits to evaluate broader pathogens. There is a great similarity between human and murine immune factors involved in pharyngeal colonization. In order to avail the pinpoint elements related to murine immune system responses towards pharyngeal colonization, humanized

mice could be adopted. However, inbred mouse appears to be appropriate in those cases related to bacterial and host immune factors (Gogos and Federle 2019). Other than mouse models, humanized models are created through the reconstitution of immunocompromised mice with hematopoietic cells of different organs. In a study, humanized mice is employed to study pathogenesis of HIV/tuberculosis such that model could fully reflect its human immunity to tuberculosis pathogen (Fonseca et al. 2017).

*Salmonella typhi* is known to infect humans exclusively and owing to the lack of animal models host–pathogen studies related to typhoid fever has hampered. Currently, murine models with oral and systematic inoculation of streptomycin are used for evaluating intestinal pathology and inflammatory responses in patients with typhoid fever (de Jong et al. 2012).

Interaction of Toll-like receptor 4 and surface protein A during *Pseudomonas aeruginosa* lung infection in mouse model was evaluated. It has concluded that Toll-like receptor interaction with surface protein advances the host defense and relieves the tissue injury in a mouse model of bacterial lung infection. Also, pro-phagocytic and anti-inflammatory responses were studied in JAWS II dendritic cells and primary alveolar macrophages. Therapeutic potential of surface protein A-4 decreases bacterial burden, intracellular signaling, lactate levels, lung edema, and production of inflammatory cytokines and chemokines in infected mouse model. Altogether, this peptide may be helpful in reducing the bacterial load, tissue damage, and inflammation in bacterial infected murine model (Awasthi et al. 2019).

## 20.9 Large Models

Large animals such as swine, horse, cattle, sheep, and deer might be used as good experimental model for studying several human infectious diseases such as viral diarrhea, asthma, Crohn's disease, tuberculosis, and influenza. Use of large experimental animals provided numerous advances in developmental immunology studies. Over millennia, large animals and humans had established as out bred populations and their size also adds several advantages. Hence, it is credible that how their immune responses are sculpted by exposure to a similar range of pathogens (Conti et al. 2014).

## 20.10 Future Perspectives and Conclusion

Although humans are the best suitable model for evaluating host–pathogen interactions, other vertebrate and invertebrate models are preferred as laboratory animal models owing to the ethical concerns associated with humans. Animal models paved the way for much of the studies related to infectious disease to understand its pathogenicity. These models are one of the well-known factors involved in the current needs to study and develop antimicrobial therapy to combat human pathogens. There are some models which entirely reflect the host–pathogen cross talk in humans other than

the models that based on the type of host and its specificity for pathogen. Yet, there are some poorly adapted models, which provide contradictory information regarding pathogenesis mechanisms. Hence, there is a need to develop advanced animal models for improved studies in biomedical research. Scientists are engaged in the development of novel model systems for the improved evaluation and understanding of pathogenicity in hosts. Novel models to be developed could better define the relevance of laboratory hosts in pathogen studies and to understand the mechanisms of virulence. In near future, combination of different animal models could provide new insights into the understanding of pathogenesis.

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