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Utility of Insects for Studying Human Pathogens and Evaluating New Antimicrobial Agents

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Abstract Insect models, such as *Galleria mellonella* and *Drosophila melanogaster* have significant ethical, logistical, and economic advantages over mammalian models for the studies of infectious diseases. Using these models, various pathogenic microbes have been studied and many novel virulence genes have been identified. Notably, because insects are susceptible to a wide variety of human pathogens and have immune responses similar to those of mammals, they offer the opportunity to understand innate immune responses against human pathogens better. It is important to note that insect pathosystems have also offered a simple strategy to evaluate the efficacy and toxicity of many antimicrobial agents. Overall, insect models provide a rapid, inexpensive, and reliable way as complementary hosts to conventional vertebrate animal models to study pathogenesis and antimicrobial agents.

Keywords Antimicrobial efficacy · *Drosophila* · *Galleria* · Infection · Insect · Pathogen · Pathogenesis

Abbreviations

MRSA	Methicillin-resistant *Staphylococcus aureus*
RNAi	RNA interference

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1 Introduction

Pathogenic microbes cause a variety of infectious diseases in human hosts, and the threat from these pathogens has never faded in human history [1–6]. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) alone infects more than 94,000 people and kills nearly 19,000 in the United States every year, more deaths than those caused by HIV/AIDS, Parkinson's disease, emphysema, and homicide combined [7, 8]. Also, in addition to bacterial infections, the frequency, spectrum, and associated cost of opportunistic invasive fungal infections have significantly increased over the past two decades and accounted for the rapidly growing populations of immunosuppressed and debilitated patients [9–11]. The substantial disease burden of infectious diseases in humans underscores the need for better understanding of the pathogenicity and virulence of human pathogens.

Pathogenesis, immunology, and pharmacology research have traditionally relied on mammalian models such as mice, rats, rabbits, and guinea pigs, but such experiments are costly, time consuming, and require full ethical consideration. Hence, cheaper and ethically more acceptable insect models of infection have been introduced, including the larvae of the greater wax moth *Galleria mellonella*, *Drosophila melanogaster*, and other insects. Accumulating data indicate that the virulence of many human pathogens is comparable in insects and mammals, and often identical virulence factors are used by human pathogenic microbes to infect insects and mammals. Moreover, insects have an immune system that is functionally similar to the innate immune system of mammals, which offers a simple model to understand innate immunity better. Furthermore, the insect infection models provide a rapid, inexpensive, and reliable evaluation of the efficacy and toxicity of new antimicrobial agents in vivo. In this chapter, we discuss how the insects *G. mellonella*, *D. melanogaster*, and other insects can be employed to study various human pathogens and to evaluate new antimicrobial agents.

2 Use of Insects for Studying Human Pathogens

Galleria mellonella and *Drosophila melanogaster* have emerged at the forefront of host–pathogen interaction research and show promise for identification of novel virulence genes and deciphering conserved innate immunity mechanisms. Insects have both cellular and humoral immune response to infection [12–14], making them attractive models to study pathogen–host interactions. Although adaptive immunity is unique to vertebrates, the innate immune response seems to be well conserved between vertebrates and invertebrates [15–18]. Moreover, insects have immune responses mediated by antimicrobial peptides [14], which also play a crucial role in human immunity. Assays using insects as the infection host are usually inexpensive, simple to perform, and yield results within a short timeframe [19, 20]. These advantages make insects attractive hosts for studying human pathogens.

2.1 Galleria mellonella Infection Model

The greater wax moth *G. mellonella* (Lepidoptera: Pyralidae) is found in most of the world [19] and has many advantages over other invertebrate hosts [18, 19, 21]. Firstly, *G. mellonella* is easy to work with and the larvae are inexpensive [19]. The larvae do not require any specialized equipment; they are housed in petri dishes and can be kept in an incubator or at room temperature [22, 23]. Also, *G. mellonella* larvae are a convenient size (2–3 cm in length) to work with and a large number of larvae may be inoculated in a short period of time [20]. Second, the *G. mellonella* larvae can be maintained at temperatures between 15 and 37 °C [22, 24]. This makes the larvae well suited to study pathogens at human body temperature. Other invertebrate hosts, such as *D. melanogaster*, cannot be maintained at temperatures over 30 °C [18, 19, 25]. This is significant because it affects the pathogenicity of organisms inasmuch as virulence factors are known to be regulated by temperature [19, 22, 25–27]. Third, quantifying the infecting inoculum is accurate in *G. mellonella*. Precise infection inoculum can be delivered to *G. mellonella* by injection into the larva's hemocoel [22]. This is notable because an accurate dose of inoculated pathogen partly contributes to the reproducible disease progression and survival outcomes [19].

The *G. mellonella* pathosystem has been widely used in the virulence study of pathogenic microbes, especially in the identification of novel virulence genes through comparing the virulence of the mutant and the wildtype strains. Interestingly, this model was previously mainly used to characterize virulence factors in *Bacillus cereus* and *Bacillus thuringiensis* [28–30]. More recently, *G. mellonella* has been employed to investigate a variety of pathogens (Table 1), including Gram-negative bacteria such as *Acinetobacter baumannii* [24, 31–38], *Burkholderia* spp. [39–56], *Listeria monocytogenes* [57–61], *Pseudomonas aeruginosa* [58, 62–67],

Table 1 Examples of human microbial pathogens that have been studied in insects for their virulence/pathogenicity

	Galleria mellonella (Ref[a])	*Drosophila melanogaster* (Ref[a])	Other insects (Ref[a])
Gram-negative bacteria			
Acinetobacter baumannii	√ [24, 31–38]	×	*Acanthamoeba castellanii* [178]
Aeromonas veronii group	√ [179]	×	×
Burkholderia spp.	√ [39–56]	√ [180]	×
Campylobacter jejuni	√[49, 72, 73]	×	*Acanthamoeba castellanii* [181–183]
Francisella tularensis	√ [117]	√ [184]	*Acanthamoeba castellanii* [185, 186]
Listeria monocytogenes	√ [57–61]	√ [138, 139, 155]	*Acanthamoeba castellanii* [187]
Pandoraea spp.	√ [56]	×	×
Pseudomonas aeruginosa	√ [58, 62–67]	√ [15, 63, 132–137]	*Bombyx mori* [188]; *Dictyostelium discoideum* [189]
Stenotrophomonas maltophilia	√ [190]	×	*Bombyx mori* [191]
Yersinia pseudotuberculosis	√ [68–71]	√ [192]	*Dictyostelium discoideum* [193]
Gram-positive bacteria			
Bacillus anthracis	√ [58]	√ [194]	*Acanthamoeba castellanii* [195]
Bacillus cereus	√ [28, 29]	√ [196]	*Dictyostelium discoideum* [197]; *Bombyx mori* [198, 199]
Bacillus weihenstephanensis	√ [200]	×	×
Enterococcus faecalis	√ [58, 80–86]	√ [201]	*Tribolium castaneum* [202]
Group A Streptococci	√ [114]	√ [203]	*Bombyx mori* [204]
Streptococcus mutans	√ [205]	×	×
Staphylococcus aureus	√[13, 87–89]	√ [206]	*Bombyx mori* [207]
Fungi			
Aspergillus fumigatus	√[97, 101–111]	√ [126, 129, 161]	*Acanthamoeba castellanii* [208]
Candida spp.	√ [22, 90–95]	√ [92, 125, 128, 131, 156–158]	*Bombyx mori* [209]
Cryptococcus neoformans	√ [19, 93–97]	√ [99, 159, 160]	*Bombyx mori* [210]
Microsporum spp.	√ [116]	×	×
Pneumocystis murina	√ [115]	√ [211]	×
Trichophyton spp.	√ [116]	×	×

[a] Example references

Yersinia spp. [68–71], *Campylobacter jejuni* [49, 72, 73], *Proteus mirabilis* [74–77], *Escherichia coli* [78], and *Legionella pneumophila* [79], Gram-positive bacteria such as *Enterococcus faecalis* [58, 80–86] and *Staphylococcus aureus* [13, 87–89], and fungi such as *Candida albicans* [22, 90–95], *Cryptococcus neoformans* [22, 96–100], and *Aspergillus* spp. [97, 101–111]. Infections caused by *A. baumannii* [24, 31–38], *Burkholderia* spp. [39–56], *L. monocytogenes* [57–61], *C. albicans* [22, 90–95], and *C. neoformans* [22, 96–100] have been particularly well characterized in the *G. mellonella* infection model; the *G. mellonella* pathosystem was efficient especially in Gram-negative bacteria and in fungi. It is important to note that the virulence determinants of most pathogens are similar in the *G. mellonella* larvae and mammals [20, 39, 42, 50, 65, 68, 98, 107, 112–114]. Nevertheless, the *G. mellonella* model is not appropriate for studying infections caused by *Pneumocystis murina* [115] or *Dermatophytes* [116], as the *G. mellonella* larva is resistant to these fungi.

Many studies have characterized the immune defense responses of the *G. mellonella* larvae [59, 68, 72, 90, 96, 98, 114, 115, 117–119]. There are considerable similarities between the systemic cellular and humoral immune responses of the *G. mellonella* larvae and the innate immune responses of mammals [12, 120–122]. Both the *G. mellonella* and mammals have many immune recognition proteins [12, 121, 122]. After pathogen recognition, both the insect and mammalian immune defenses rely on phagocytosis, the production of reactive oxygen species, the expression of antimicrobial peptides, and clotting cascades to combat invasive pathogens [12, 25, 123]. However, the *G. mellonella* larvae also form melanin during infection, but this process does not occur in mammals. In addition, the *G. mellonella* larvae form pathogen microaggregates that ultimately lead to hemocyte nodule or capsule formation [12], which is also different from mammalian immune responses. Compared with *D. melanogaster*, a notable disadvantage of the *G. mellonella* model is that the *Galleria* genome sequence has not been completed yet, although a huge array of genetic tools has been used in the *D. melanogaster* model. The recent characterization of the *Galleria* immune gene repertoire and transcriptome by next generation sequencing and traditional Sanger sequencing has led to the design of gene microarrays and paves the way for further use of *Galleria* for elucidation of innate antimicrobial immune mechanisms [124].

2.2 *Drosophila melanogaster* Infection Model

Drosophila melanogaster is a species of *Diptera* in the *Drosophilidae* family. The species is known generally as the common fruit fly. Beginning with Charles W. Woodworth, this species has been a widely used model organism for biological research in studies of genetics, physiology, and microbial pathogenesis. It is typically used because it is an animal species that is easy to handle and breed. The most important advantage of *D. melanogaster* as a mini-host is that the fruit fly is amenable to forward and reverse genetics and large collections of *Drosophila* mutants and transgenic cell lines are commercially available (http://flybase.org).

The *Drosophila* genome sequence has been completed and is among the most fully annotated eukaryotic genomes. Thus, gene microarrays have been generated, double-stranded RNA has been synthesized for all genes (www.flyrnai.org), and RNA interference technology is commercially available for conditional inactivation of any gene at the whole-animal or tissue levels (http://stockcenter.vdrc.at/control/main) [124].

Drosophila melanogaster requires a more significant commitment than the *G. mellonella* model. Working with *D. melanogaster* as a host to study human pathogens requires considerable experience and specialized equipment such as microinjectors [125–131] to infect it with a certain infecting inoculum. In addition, because wildtype *D. melanogaster* are resistant to infection with some pathogens, Imd or Toll pathway-deficient flies need to be used, which in some cases requires a fly genetic cross [125, 126, 128–130]. Nevertheless, the *D. melanogaster* pathosystem is still among the simplest infection models.

The *D. melanogaster* pathosystem has also been widely used to identify virulence determinants of pathogenic microbes, including *P. aeruginosa* [15, 63, 132–137], *Streptococcus pneumoniae* [138, 139], *Serratia marcescens* [140–154], *L. monocytogenes* [138, 139, 155], *C. albicans* [92, 125, 128, 131, 156–158], *C. neoformans* [99, 159, 160], and *Aspergillus fumigatus* [126, 129, 161] (Table 1), and this pathosystem is promising for large-scale studies. Note that there is also a significant concordance for virulence of most pathogens in *D. melanogaster* and mammals. Three infection assays have been used for assessment of fungal virulence in insects: injection, rolling, and ingestion assays. Although quantification of the infecting inoculum is feasible only in the injection assay, the availability of different routes of infection permits comparative analyses of virulence and host–pathogen interactions between an acute infection introduced directly into the hemolymph (injection assay) versus more protracted infections originating from epithelial surfaces [i.e., skin (rolling assay) or gastrointestinal mucosa (ingestion assay)]. Interestingly, the *alb1*-deficient *A. fumigatus* mutant was found to be hypovirulent in *D. melanogaster* when introduced via epithelial surfaces but not by injection [130].

Drosophila melanogaster has been a major tool for studying innate immunity [124] inasmuch as they mount a highly efficient innate immune defense, the first line of which consists of epithelial responses that prevent infections. When physical barriers are breached and pathogenic microbes invade within the insect body, insects induce a highly coordinated immune response that has both cellular and humoral constituents. In the case of fungal infection, the immune responses at the epithelial level are Toll-independent, which is opposed to the requirement of intact Toll signaling for defense against systemic fungal infection. Consistently, the epithelial antifungal immune responses in the fruit fly are mediated by the dual oxidase (DUOX), JAK-STAT, and immune deficiency (imd) pathways [147, 162, 163] instead of Toll. Notably, the epithelial immune responses are conserved in the fruit fly and mammals, and the similarity in the intestinal epithelium anatomy and

regeneration time between flies and mammals [164] supports the utility of *Drosophila* for examining immunological mechanisms of mucosal infection.

Some *Drosophila* strains have been employed to study cellular and humoral immunity. With regard to cellular immunity, a phagocytosis-defective eater-null *Drosophila* strain was used to reveal that phagocytosis is indispensable for fly survival against zygomycosis [165]. A *Drosophila* S2 phagocytic cell line was used to describe a macroglobulin complement-related protein; the protein bound specifically on the surface of *C. albicans* and enhanced phagocytosis. Also by using the S2 phagocytic cell line, some autophagy host factors (e.g., Atg2, Atg5, Atg9, Pi3K59F) were identified; the autophagy molecules were induced after exposure to *C. neoformans* in the fly and were also required for cryptococcal intracellular trafficking and replication within murine phagocytes [166]. With regard to humoral immunity, the Toll signaling cascade in *D. melanogaster* is crucial for host defense against systemic infection via induction of antifungal peptide [167, 168], the role of which is similar to the Toll/IL-1β receptor signaling in mammals.

Of note, comparative analyses of immune responses using more than one insect host and more than one inoculation assay could be enlightening for dissecting pathogen- and tissue-specific innate immune mechanisms [169], because insects have differential susceptibility to some infections (e.g., wildtype *Galleria* is susceptible to *Candida* or *Cryptococcus* injection whereas wildtype *Drosophila* is not) [20, 98, 125, 159], and because an insect may exhibit differential susceptibility to a specific pathogen depending on the route of inoculation (e.g., *Cryptococcus* ingestion but not injection kills wildtype *Drosophila* [98], and *Candida* injection but not ingestion kills adult Toll-deficient flies [125]).

2.3 Other Insect Infection Models

In addition to *D. melanogaster* and *G. mellonella*, the red flour beetle *Tribolium castaneum* (*Coleoptera*, *Tenebrionidae*) has previously been used to investigate host–pathogen interactions with a wide array of pathogenic bacteria, sporozoa, cestoda, nematoda, mites, and hymenopterous parasites [170]. The *Tribolium* genome has been sequenced by the Human Genome Sequencing Center, Baylor College of Medicine, USA (Tribolium Genome Sequencing Consortium 2008). Similar to *D. melanogaster*, *Tribolium* is also amenable to systemic RNAi-mediated gene silencing and other genetic tools for functional gene analyses [171–177]. Moreover, the soil-living amoebas *Acanthamoeba castellanii* and *Dictyostelium discoideum*, the *lepidopteran* insect silkworm *Bombyx mori*, the mosquito *Culex quinquefasciatus*, and the German cockroach *Blattella germanica* have attracted interest due to their potential as good model systems for the screening of virulence factors of pathogenic microbes (Table 1).

3 Use of Insects for Evaluating New Antimicrobial Agents

Host-based antimicrobial drug discovery is important because efficacy of potential antimicrobial agents might be altered by in vivo factors such as pH, enzymatic degradation, or binding to molecular components within the host [19, 212]. Moreover, some compounds without in vitro activity may be antimicrobially effective because of their immune regulating role or the production of effective metabolites in vivo. With regard to the in vivo studies, animal infection models not only provide data on effectiveness of antimicrobial agents in vivo and their toxicity, but also enable dose and medication schedule recommendations for use in the first human dose. Using insect models for initial toxicity and efficacy screening is financially and ethically more acceptable in the early stages of antimicrobial discovery and development [19]. As a consequence, interest in using insect models to evaluate compounds before testing in mammalian species has increased [213].

3.1 Galleria mellonella Infection Model for Evaluating New Antimicrobial Agents

Infection of *G. mellonella* is amenable to antimicrobial treatment [19, 98, 117]. This makes the *G. mellonella* model highly suitable for evaluating the efficacy and toxicity of potential antimicrobial agents in vivo prior to testing in mammalian species.

A key benefit of using *G. mellonella* larvae to assess antimicrobial efficacy is that, as with administration of the infectious inoculum, an accurate dose of antimicrobial can be delivered directly into the hemocoel by injection [19, 22]. This is not always possible in other invertebrate models [23]. Moreover, this model simulates the intravenous systemic administration of antimicrobial agents in a relative dose and schedule that would be used in patients. The *G. mellonella* model is useful for testing different treatment regimens. Furthermore, the experimental course of therapy can be varied according to the dose of antimicrobial agent administered, the number of doses, and the timing of the first and successive administrations [19].

The *G. mellonella* model has been used to study the efficacy of many antimicrobial agents against a multitude of bacterial and fungal pathogens (Table 2). For example, streptomycin, ciprofloxacin, and levofloxacin were evaluated for treating *Francisella tularensis* live vaccine strain (LVS) [117]; gentamicin, meropenem, tetracycline, and cefotaxime were evaluated for treating *A. baumannii* infection [24]; amphotericin B and other antifungal agents were evaluated for treating *C. neoformans* infection in this *G. mellonella* model [98].

Note that the antimicrobial drug susceptibility profiles of pathogens in *G. mellonella* larvae are largely the same as those in vitro studies [5, 15, 41–44]. Peleg et al. infected *G. mellonella* larvae with a lethal dose of a reference strain of

Table 2 Antimicrobial agents tested in insect pathosystems that increased insect's survival significantly compared to controls

Pathogen	Agent	*Galleria mellonella* (Ref[a])	*Drosophila melanogaster* (Ref[a])	Other insects (Ref[a])
Acintobacter baumannii	Colistin	√ [35]	×	×
	Gentamicin	√ [24, 35]	×	×
	Meropenem	√ [24]	×	×
	Vancomycin	√ [35]	×	×
Burkholderia cenocepacia	Baicalin hydrate (antibiofilm compound)	√ [220]	×	×
	Cinnamaldehyde	√ [220]	×	×
	Tobramycin	√ [220]	×	×
	Bacteriophages: KS4, KS4-M, KS12, KS14	√ [39]	×	×
Burkholderia multivorans	Baicalin hydrate (antibiofilm compound)	√ [220]	×	×
	Cinnamaldehyde	√ [220]	×	×
	Tobramycin	√ [220]	×	×
Francisella tularensis	Azithromycin	√ [221]	×	×
	Ciprofloxacin	√ [117]	×	×
	Levofloxacin	√ [117]	×	×
	Streptomycin	√ [114]	×	×
Staphylococcus aureus	Daptomycin	√ [214]	×	×
	Hamamelitannin (antibiofilm compound)	√ [220]	×	×
	Penicillin	√ [220]	×	×
	Vancomycin	√ [220]	×	*Bombyx mori* [222]
Candida albicans	1,10-Penanthroline	√ [223]	×	×
	$[Ag_2(mal)(phen)_3].2H_2O$	√ [223]	×	×
	Caspofungin	√ [224]	×	×
	Silver nitrate ($AgNO_3$)	√ [223]	×	×
	Fluconazole	√ [217]	√ [125]	×
Cryptococcus neoformans	Amphotericin B	√ [98]	×	*Bombyx mori* [210]
	Fluconazole	√ [98]	×	*Bombyx mori* [210]
	Flucytosine	√ [98]	×	*Bombyx mori* [210]
	Ketoconazole	×	×	*Bombyx mori* [210]

[a] Example references

A. baumannii, which was susceptible to gentamicin and meropenem but resistant to tetracycline and cefotaxime in vitro [24]. Survival was significantly higher in the groups receiving gentamicin and meropenem ($p < 0.001$) compared to the infected untreated control group. Treatment with tetracycline and cefotaxime had no effect on survival. Also, Hornsey et al. demonstrated the same phenomenon using a strain of *A. baumannii* susceptible to gentamicin and colistin but resistant to teicoplanin [35]. Another notable example is focused on the treatment of *S. aureus* infection using this *G. mellonella* model. When the larvae were infected with a penicillin-susceptible strain, larvae could be protected significantly by penicillin; when the larvae were infected with a penicillin-resistant strain, larvae could not be protected by penicillin. Of note, many studies have showed that effective weight-based doses of antibiotics used in *G. mellonella* larvae are similar to recommended doses used in human subjects [24, 35, 98, 117, 214]. Dose data from this model are likely to provide a more precise estimate of doses in subsequent mammalian studies than in vitro data based on minimum inhibitory concentration values [19].

Nowadays combination antibiotic therapy is widely used to prevent the emergence of resistant strains of pathogens [215]. The *G. mellonella* model is well placed to test the efficacy of combination antimicrobial therapy and drug interactions in vitro (Table 3). Mylonakis et al. demonstrated the benefits of combination antifungal therapy in larvae infected with *C. neoformans* [98]. Larvae treated with combination amphotericin B (1.5 mg/kg) and flucytosine (20 mg/kg) had significantly higher survival rates than those treated with amphotericin B alone. Vu and Gelli showed significantly higher survival in *C. neoformans*-infected larvae treated with combination flucytosine (53 mg/kg) and astemizole (53 mg/kg), an antihistamine, or an astemizole homologue compared to monotherapy with these agents alone [166, 216]. Similarly, Cowen et al. demonstrated higher survival in treating *C. albicans* and *A. fumigatus* infections in this model using a combination of Hsp90 inhibitors with fluconazole or caspofungin, respectively, than with monotherapy with these agents [217]. These data imply that insects are promising hosts for assessing the efficacy of innovative therapeutic strategies such as a combination of antifungal agents with immune- or virulence-modulating drugs.

Although larval survival is the most common used measurable endpoint to assess antimicrobial efficacy, the microbial burden in larvae can also be used to quantify antimicrobial efficacy [39, 50, 65, 68, 96, 98, 117]. The microbial burden may be a better indicator of the more subtle effects of antimicrobial agents where larval survival is less indicative of antimicrobial efficacy [19]. It can also be used to confirm the complete treatment of infection after eradication of pathogens or to study the dynamics of infection during treatment [19, 39, 65]. There is generally an inverse relationship between the microbial burden and larval survival, although this is not always the case. The treatment of infection and efficacy of antimicrobial agents can also be assessed using hemocyte counts in hemolymph or changes in larval gene expression [93, 118, 119, 218]. Hemocyte counts should be interpreted with caution because counts may be lower in more virulent infections or remain

Table 3 Combination antimicrobial therapy tested in insect pathosystems that increased insect's survival significantly compared to controls

Pathogen	Agent(s)	*Galleria mellonella* (Ref[a])	*Drosophila melanogaster* (Ref[a])	Other insects (Ref[a])
Acintobacter baumannii	Colistin + teicoplanin	√ [35]	×	×
	Colistin + vancomycin	√ [35]	×	×
Burkholderia cenocepacia	Cinnamaldehyde + tobramycin	√ [220]	×	×
Burkholderia multivorans	Cinnamaldehyde + tobramycin	√ [220]	×	×
	Hamamelitannin (antibiofilm compound) + vancomycin	√ [220]	×	×
Aspergillus fumigatus	Caspofungin + geldanamycin	√ [217]	×	×
	Voriconazole + terbinafine	×	√ [130]	×
Candida albicans	Fluconazole + 17-(allylamino)-17-demethoxygeldanamycin (AAG; Hsp90 inhibitor)	√ [217]	×	×
	Fluconazole + 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (DMAG; Hsp90 inhibitor)	√ [217]	×	×
Cryptococcus neoformans	Amphotericin + flucytosine	√ [98]	×	×
	Amphotericin B + fluconazole + flucytosine	√ [98]	×	×
	Fluconazole + astemizole (antihistamine)	√ [19, 216]	×	×
	Fluconazole + astemizole analogue: 1*H*-benzimidazole-2-amine,1-[2-(4-methoxyphenyl)ethyl]-4-piperidinyl]	√ [19, 216]	×	×
	Fluconazole + sertraline (selective serotonin reuptake inhibitor antidepressant)	√ [19, 215]	×	×

[a] Example references

unchanged if the interaction is nonpathogenic [118]. This depends upon the pathogen under investigation.

Also, the *G. mellonella* model provides a unique opportunity to identify toxic effects of antimicrobial agents during an infection, which is not possible with in vitro testing [19]. In standard in vivo testing, the median lethal dose (LD_{50}) is established by administering various doses to otherwise healthy animals. It is possible, however, that toxicity of some antimicrobial agents may only become apparent once administered to sick animals. As reviewed by Desalermos et al., certain doses of antimicrobial agents that were nontoxic in standard in vivo testing in healthy animals can cause greater or faster mortality in infected animals compared to a control group of infected animals administered carrier solvent only [219]. The *G. mellonella* model is ideal for this type of study, which can only be achieved using an in vivo infection model. Prescreening the promising antimicrobial agents using the *G. mellonella* model before progressing to the mammals will save time, money, and needless experimentation in mammals [19].

3.2 *Drosophila melanogaster Infection Model for Evaluating New Antimicrobial Agents*

The *D. melanogaster* infection model is not often used to evaluate antimicrobial agents (Table 2). Some studies have used the *D. melanogaster* infection model to study the efficacy of some licensed antifungal agents and shown remarkable correlation between in vitro susceptibility testing results and in vivo drug efficacy in both insects and mammals [125, 130]. Notably, the synergy between voriconazole and terbinafine against *A. fumigatus* was demonstrated in this model [130], which is consistent with the synergistic effect in vitro and in mammals, thus further providing evidence that the *D. melanogaster* model may be used as a complementary assay to evaluate antimicrobial agents.

Of note, pharmacology studies in insects also have limitations despite their potential. Although both *Drosophila* and *Galleria* can be used for testing orally absorbable compounds, the exact ingested drug dose per insect is difficult to quantify precisely. Testing of parenteral antimicrobial compounds also has constraints as repeated drug injections lead to injury, especially in *Drosophila*.

3.3 *Other Insect Infection Models for Evaluating New Antimicrobial Agents*

The silkworm *B. mori* infection model is useful for evaluating the efficacy, pharmacokinetics, and toxicity of antifungal drugs, similar to the *G. mellonella* model [210]. Antifungal drugs, amphotericin B, flucytosine, fluconazole, and

ketoconazole showed therapeutic effects in silkworms infected with *C. neoformans* [210]. However, amphotericin B was not therapeutically effective when injected into the *B. mori* intestine, comparable to the fact that amphotericin B is not absorbed by the intestine in mammals [210].

Despite the potential of insect models, pharmacokinetic analyses are problematic in insects and it is technically challenging to measure drug levels inside the insects. It is important to note that it is difficult to rely on insect models for critical pharmacological parameters such as drug absorption, distribution, metabolism, excretion, and drug–drug interactions, and therefore testing is necessary in mammalian hosts that are phylogenetically closer to humans.

4 Conclusion

Drosophila melanogaster and *G. mellonella* have emerged at the forefront of host–fungal interaction research and show promise for evaluating antimicrobial agents. Because no single nonvertebrate organism fully reproduces all aspects of mammalian infection, comparative research in these hosts is required and should be complemented by studies in mammalian models of infection.

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