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1 ***Drosophila melanogaster* as an organism model for studying cystic fibrosis**  
2 **and its major associated microbial infections**

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13 **Abstract**

14 Cystic fibrosis (CF) is a human genetic disease caused by mutations in the *cystic fibrosis*  
15 *transmembrane conductance regulator* gene that encodes a chloride channel. The most  
16 severe clinical manifestation is associated with chronic pulmonary infections by pathogenic  
17 and opportunistic microbes. *Drosophila melanogaster* has become the invertebrate model of  
18 choice for modelling microbial infections and studying the induced innate immune response.  
19 Here, we review its contribution to the understanding of infections with six major pathogens  
20 associated with CF (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia*  
21 *cepacia*, *Mycobacterium abscessus*, *Streptococcus pneumoniae*, and *Aspergillus fumigatus*)  
22 together with the perspectives opened by the recent availability of two CF models in this  
23 model organism.

24 **Keywords:** *Drosophila*, cystic fibrosis, CFTR, ENaC, *Staphylococcus aureus*, *Pseudomonas*  
25 *aeruginosa*, *Mycobacterium abscessus*

26

## 27 **1. Bacterial infections in Cystic fibrosis**

28 Cystic fibrosis (CF) is a human genetic disease with a recessive autosomal transmission. It is  
29 the most common genetic disease among Caucasians and affects approximately  
30 7,97/100.000 persons in the USA and 7,37/100.000 in the European Union (1). Although the  
31 pulmonary form is the most severe clinical manifestation, other exocrine organs may also be  
32 affected (*e.g.*, the pancreas and intestine). The disease is caused by loss-of-function  
33 mutations in the *cystic fibrosis transmembrane conductance regulator (cftr)* gene (2–4),  
34 which encodes a member of the ATP-binding cassette (ABC) protein superfamily (3). CFTR  
35 is an ATP-gated ion channel that conducts chloride ions across epithelial cell membranes (5,  
36 6), as well as glutathione thiocyanates and bicarbonates.

37 In addition to modulating the chloride transport, it regulates the activity of other ion channels  
38 such as the trimeric epithelial sodium channel (ENaC), which consists of the sub-units  $\alpha$ ,  $\beta$   
39 and  $\gamma$ . How CFTR negatively regulates ENaC is still controversial. According to König and  
40 collaborators, this regulation occurs indirectly through the accumulation of intracellular  
41 chlorine (7). However, conflicting results have shown that inhibition of ENaC by CFTR is  
42 independent of the direction and extent of chloride transport (8). Studies have shown that  
43 CFTR inhibits ENaC through a direct physical interaction (9) or by regulating ENaC subunit  
44 quantities (10).

45 In any case, CFTR dysfunction leads to an excessive activity of the trimeric ENaC channel,  
46 causing uncontrolled sodium and excessive water entry into the epithelial cells following the  
47 osmotic gradient. This leads to dehydration of the intraluminal surface and an increase in the  
48 thickness of the mucus bordering the epithelium (11). In the lungs, the accumulation of thick  
49 viscous secretions causes obstruction and inflammation of the airways. These prevent the  
50 proper functioning of the mucociliary barrier, which is the primary protective barrier against  
51 many pathogens (12). In addition, this mucus has poor antibacterial activity owing to its  
52 reduction in acidity. Indeed, CFTR dysfunction prevents the exit of bicarbonate ions. This  
53 modified mucus constitutes the ideal environment for the accumulation, proliferation and  
54 persistence of pathogenic and/or opportunistic microorganisms.

55 Chronic and recurrent infections and persistent inflammation cause airway damage that can  
56 lead to bronchiectasis and thus, a decline in respiratory function (13). The ensuing  
57 respiratory failure is the cause of more than 90% of the recorded deaths (14). According to  
58 the 2021 report of the French Cystic Fibrosis Registry, these infections are mainly due to  
59 *Staphylococcus aureus* (60.6%), *Pseudomonas aeruginosa* (34%), *Aspergillus fumigatus*  
60 (21.6%), *Haemophilus influenzae* (10.1%), *Stenotrophomonas maltophilia* (9.3%),  
61 *Achromobacter xylosoxidans* (6.4%), *Streptococcus pneumoniae* (1.7%), non-tuberculous  
62 mycobacteria (3,7%) and *Burkholderia cepacia* (2%). The prevalence of these pathogens  
63 vary according to the geography. For comparison, in the USA, *S. aureus*, *P. aeruginosa* and  
64 non-tuberculous mycobacteria have approximately 63%, 24% and 9.6% respectively of the  
65 overall prevalence according to the 2021 report of the Cystic Fibrosis Foundation (CFF). The  
66 dynamics of the prevalence of the isolated pathogens also changes with time. For example,  
67 the respective trends in the prevalence of *S. aureus* and *P. aeruginosa* have taken opposite  
68 trajectories over time in the USA. While the former is becoming increasingly prevalent (29%  
69 in 1991 vs. 63% in 2021), the latter is less prevalent over time (61% in 1991 vs. 24% in  
70 2021). The same trend is observed in Europe (15).

## 71 **2. *Drosophila*, an established organism model for the study of pathogens**

72 *Drosophila melanogaster* is a century-old organism model that is used in various aspects of  
73 life sciences such as genetics, developmental biology, cellular biology, neurobiology, and  
74 immunity. The constant development and availability of different genetic tools have facilitated  
75 its genetic manipulation, making *Drosophila* central to the study of responses to infection and  
76 host-pathogen interactions in the last three decades. In their own natural environment, fruit  
77 flies face a panel of viruses, bacteria, fungi, and parasites (e.g., wasp (16)). In the laboratory,  
78 *Drosophila* is used as an experimental host to study infection with its natural pathogens as  
79 well as human ones. Indeed, *Drosophila* has become an attractive and emergent model for  
80 studying host response, virulence factors and pathophysiology of pathogens associated with  
81 human infectious diseases, such as those caused by Zika Virus, *Mycobacterium marinum*,  
82 *Listeria monocytogenes*, and *Candida albicans* (17–21).

83 *Drosophila* is a dipteran with three larval stages and a complete metamorphosis. In  
84 laboratory, third instar larvae and adults are usually infected either orally, locally by wounding  
85 or systemically by injecting the microorganism.

86 *Drosophila* lacks an adaptive immune response but has innate immunity involving conserved  
87 signaling pathways. In both mammals and flies, the JNK, JAK-STAT and NF $\kappa$ B signaling  
88 pathways are critical for immune response regulation (22). To note, the response mediated  
89 by Toll-like receptors was discovered in this organism (23). *Drosophila* pattern recognition  
90 receptors (PRRs) recognize the pathogen-associated molecular patterns (PAMPs) of  
91 microbes, such as peptidoglycan (PGN), or lipoteichoic acid (LTA) (24). They induce an  
92 adequate immune response involving both cellular and humoral response (25).

93 The cellular response is based on blood cells (hemocytes) which are equivalent to  
94 mammalian monocytes and macrophages. Until recently, three morphologically distinct types  
95 of hemocytes have been identified: plasmatocytes, crystal cells (involved in wound healing,  
96 reactive oxygen species production and hypoxic response) and lamellocytes (involved in  
97 response to wasp parasitization) (26). The most abundant hemocytes are plasmatocytes that  
98 respond to wound signals and control the coagulation response. They also phagocytose and  
99 encapsulate invading pathogens and clear apoptotic bodies (27). However, this simplistic  
100 classification of hemocytes into three subtypes has been reviewed thanks to recent studies  
101 based on single-cell sequencing on either larval (28–31), adult (32) or pupal (33) hemocytes.  
102 Collectively, these studies have identified at least eight distinct specialized hemocyte  
103 subpopulations waiting to be functionally characterized.

104 The existence of an inducible humoral response in fruit flies was first reported in 1972 (34).  
105 This response is mainly based on the production of antimicrobial peptides (AMPs), primarily  
106 by the fat body, which is functionally homologous to mammalian adipocytes and liver. AMPs  
107 can also be produced locally by epithelial cells or hemocytes. Two conserved NF $\kappa$ B  
108 signalling pathways, Toll and Immune deficiency (Imd), mediate AMP production. The former  
109 is implicated in response to both bacteria with Lys-type PGN (mainly Gram-positive) and  
110 fungal infections, while the second is involved in responses to infection by bacteria with DAP-

111 type PGN (mainly Gram-negative) (22, 23, 35, 36). In addition to AMPs production, humoral  
112 response also includes the generation of reactive oxygen species (ROS) by DUOX proteins  
113 locally at the epithelial level (37, 38).

### 114 **3. Lessons from *Drosophila* infections with some CF major pathogens**

115 *Drosophila* is commonly used to study infections with a single pathogen associated with CF  
116 or co-infection. Here, we review the contributions of this model organism to the identification  
117 of host receptors, *in vivo* validation of virulence factors, and to the screening of effective  
118 drugs. We will follow the prevalence of these pathogens as reported by the French Cystic  
119 Fibrosis Registry in 2021, and the number of relevant publications. An overview is provided  
120 by the Table 1.

121 *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* were excluded for the  
122 following reasons. *Stenotrophomonas maltophilia* has been isolated at the surface and in the  
123 gut of wild female *Drosophila* captured in Puerto Rico (39). Its intestinal presence was  
124 confirmed in laboratory strains (40). *Achromobacter xylosoxidans*, has been reported to be  
125 pathogenic for *Drosophila*, as its injection in adult males leads to rapid dose-dependent  
126 death (41).

#### 127 ***Staphylococcus aureus***

128 Infections with the Gram-positive bacterium *Staphylococcus aureus* (*S. aureus*) are among  
129 the most prevalent in CF patients. Injection of live *S. aureus* into *Drosophila* leads to an  
130 important transcriptional response and a systemic infection resulting in a reduction in fly life  
131 expectancy (42, 43). Phagocytosis plays a major role in the response to *S. aureus* infection  
132 as flies devoid of plasmatocytes succumb more rapidly (44, 45). *Drosophila* Schneider 2 (S2)  
133 cells were used as a surrogate for hemocytes. Indeed, this widely used cell line, derived from  
134 late embryos, is phagocytic. Genetic screening of S2 cells identified Eater and Croquemort,  
135 as *S. aureus* scavenger receptors (44). This was confirmed in adult hemocytes (44) and  
136 mammalian macrophages (46). Croquemort is the first CD36 family member to be described  
137 as being involved in bacterial recognition. Eater does not recognize lipoteichoic acid (LTA), a

138 cell wall polymer found in Gram-positive bacteria. Indeed, the *ltaS* mutant strain (deficient in  
139 LTA synthesis) was phagocytosed less by wild-type hemocytes than the wild-type *S. aureus*  
140 strain. Moreover, the *ltaS* mutant strain was equally phagocytosed by wild-type and Eater-  
141 lacking larval hemocytes (47). However, this was not the case for hemocytes lacking the  
142 receptor Draper, whose extracellular region binds LTA, strongly suggesting that this cell wall  
143 component is its ligand, contrary to Eater (47).

144 The integrin  $\beta v$  is also involved in *S. aureus* recognition by the hemocytes but through  
145 peptidoglycan. Indeed, a mutant bacterial strain that produces reduced levels of PGN, due to  
146 defective UDP-N-acetylenolpyruvylglucosaminereductase, was less efficiently phagocytosed  
147 by integrin  $\beta v$ -deficient hemocytes (48).

148 PGN Recognition Proteins (PGRP), such as PGRP-SA and PGRP-SC1a, are also important  
149 for the recognition and phagocytosis of *S. aureus* (49). However, wall teichoic acids (WTA),  
150 which are covalently linked to PGN, mitigate *S. aureus* recognition by these *Drosophila*  
151 immune receptors. Indeed, infection with strains with defective WTA production led to a  
152 reduction of *S. aureus* virulence. This loss of pathogenicity is due to increased PGN binding  
153 and detection by PGRP-A (50). Complementary to inducing a cellular response, *S. aureus*  
154 PGRP-SA-mediated recognition systemically activates the Toll pathway leading to AMPs  
155 production (e.g., Drosomycin, Defensin and Metchnikowin) (51). Although Imd-related AMPs  
156 are not induced, this pathway is required for effective clearance of the infection (52).

157 Moreover, fly infections have been used to validate known virulence factors, such as  
158 hemolysin  $\alpha$  (53), as well as to identify new ones. An example is the production of D-  
159 alanylated teichoic acid, which reduces PGN recognition by host receptors and thus  
160 interferes with the host humoral response to *S. aureus* infection (51).

161 *Drosophila* infections have confirmed that Methicillin-Resistant *S. aureus* (MRSA) isolates,  
162 notably the USA300 and PFGE strains, were less virulent than non-MRSA isolates (54).  
163 Correlations with clinical observations were found for the community-associated MRSA  
164 strains USA300, USA400 and CMRSA2. Indeed, the latter are more virulent than the



165 hospital-associated strain CMRSA6 (53). Recently, a model of oral USA300 infection in  
166 *Drosophila* larvae showed that bacterial catalase neutralizes a DUOX-mediated oxidative  
167 response that promotes AMPs production through Toll pathway activation (55).

168 To identify drugs effective against these MRSA, a panel of antibacterial peptides was  
169 screened *in vivo*. Two antibiotics, nisin and NAI-107, have been shown to have the ability to  
170 rescue adult flies from fatal infections with the USA300 strain. NAI-107 presented an efficacy  
171 equivalent to that of vancomycin, a widely applied antibiotic for the treatment of serious  
172 MRSA infections (56).

173 The antimicrobial activity of plumbagin, a phytochemical, was also validated with the  
174 *Drosophila* systemic infection model, whether with *S. aureus* alone or in co-infection with *C.*  
175 *albicans*, as is often observed in the urinary tract in humans. Plumbagin (5-hydroxy-2-methyl-  
176 1,4-naphthoquinone) has been identified *in vitro* as a potent antimicrobial agent against *S.*  
177 *aureus* and *C. albicans* (57).

### 178 ***Pseudomonas aeruginosa***

179 *Drosophila* is susceptible to both oral and systemic infections by the Gram-negative  
180 bacterium *P. aeruginosa*. This leads to the invasion of host tissues, then their degradation  
181 and ultimately death through the bacterial spread in the hemolymph (34, 58–60).

182 *P. aeruginosa* infections induce systemic AMP production mediated by both the Toll and Imd  
183 pathways (60, 61), a local epithelial Imd-dependent one and a cellular response (59). More  
184 recently, a novel and evolutionarily conserved defence mechanism has been reported (62).

185 *P. aeruginosa* infection induces the overexpression of the iron transferrin 1-encoding gene in  
186 the fat body. The consequence is sequestration of iron from the hemolymph and its  
187 relocation to the fat body. The importance of the competition for iron between *P. aeruginosa*  
188 and its host is further supported by the reduced pathogenicity of a siderophore-defective  
189 strain of *P. aeruginosa* in *Drosophila* (62).

190 Fruit flies have been used to screen *P. aeruginosa* mutants and thus to validate (63) and  
191 identify new virulence factors (e.g., *relA*) (64). Similarly, the contribution of certain virulence

192 factors has been characterized *in vivo* in fruit flies. Examples include the oxylipins involved in  
193 biofilm formation and virulence (65), glutathione biosynthesis genes *gshA* and *gshB* (66),  
194 transcriptional regulators PA1226 and PA1413, which modulate the virulence (67), reactive  
195 chlorine species resistance factor RcsA (68), glucose transport regulator GltB (69) and the  
196 nitrite reductase NirA (70). The essential role of the *P. aeruginosa* respiratory chain in  
197 virulence and pathogenicity has also been demonstrated in *Drosophila*. Indeed, a PA4427-  
198 PA4431 operon mutant strain, defective for respiratory chain complex III (*cytobc1*), induces  
199 less mortality in *Drosophila* than the PAO1 reference strain (71).

200 Many pathogenic Gram-negative bacteria, including *P. aeruginosa*, possess a type III  
201 secretion system (T3SS), which injects virulence factors into their host (72). The presence  
202 and activation of T3SS are required in *P. aeruginosa* to induce fly death (73). The exotoxin  
203 ExoS, whose injection into the host cell cytoplasm is mediated by T3SS, interferes with  
204 bacterial phagocytosis by hemocytes (74). ExoS is known to target host Rho GTPases and  
205 the contribution of different fly Rho GTPases to *P. aeruginosa* infection resistance has been  
206 assessed *in vivo*, revealing that Rac2 is the main target of ExoS to prevent engulfment (75).  
207 ExoS can also induce apoptosis at least in *Drosophila* S2 cells (76), similar to Exotoxin A  
208 (77).

209 *P. aeruginosa* uses quorum sensing (QS) to regulate and adapt its gene expression. During  
210 infection, the QS signaling molecule N-3-oxododecanoyl homoserine lactone (3OC12-HSL)  
211 is essential for the bacterial virulence in flies. *Drosophila* lacks Paraoxonases (PONs) which  
212 are able to degrade 3OC12-HSL *in vitro*. Transgenic expression of human PON1 protects  
213 flies against *P. aeruginosa* infection lethality by interfering with 3OC12-HSL-dependent QS  
214 (78). The QS transcription factor RhIR interferes with the host's cellular immune response  
215 during the early stages of infection (59). *P. aeruginosa* can also inhibit the host response by  
216 suppressing AMP production (61).

217 Chronic *P. aeruginosa* infection in patients with CF is associated with the formation of  
218 mucoid micro-colonies called biofilms. These are observed in the *Drosophila* crop, the  
219 functional equivalent of the mammalian stomach, after oral infection. Bacteria recovered from

220 this *in vivo* biofilm present an increased antibiotic resistance and less virulence than the  
221 planktonic bacteria (79). Transcriptional regulator PA3898 controls biofilm formation and  
222 virulence in *Drosophila* (80). Furthermore, oral infection with *P. aeruginosa* leads to midgut  
223 hyperplasia. This is due to activation of the stress response JNK pathway in enterocytes,  
224 leading to their apoptosis and indirectly to the overproliferation of intestinal stem cells (81).

225 Fruit flies can help to find alternative effective therapeutic strategies against *P. aeruginosa*  
226 infections, in addition to antibiotics. Indeed, the *in vivo* antibacterial efficacy of *P. aeruginosa*-  
227 targeting lytic phages, such as MPK1 and MPK6, has been assessed and proven in  
228 *Drosophila* (Heo *et al.*, 2009; Lindberg *et al.*, 2014; Jang *et al.*, 2019). Moreover, Baicalin,  
229 has been validated *in vivo* in *Drosophila* (84). This extract from the Chinese herb *Scutellariae*  
230 *radix* has been proposed as an alternative anti-*P. aeruginosa* compound targeting bacterial  
231 T3SS.

### 232 ***Aspergillus fumigatus***

233 Immuno-compromised patients as well as those living with CF are prone to invasive  
234 aspergillosis. In order to examine the conserved Toll pathway associated with the response  
235 to fungal infection in *Drosophila*, including *A. fumigatus* (85) and the virulence of different  
236 strains of the cosmopolitan filamentous fungus *A. fumigatus* was assessed using Toll-  
237 deficient flies (86). Infections were induced by injecting, feeding or rolling flies with conidia  
238 (87). Concordance with results obtained in mammalian models was observed with either the  
239 hypovirulent strain  $\Delta alb1$  (88) or other *A. fumigatus* mutant strains defective in siderophore  
240 biosynthesis, starvation stress response (89), or Glicotoxin production (90).

241 Toll-deficient *Drosophila* have also been used to assess the *in vivo* efficacy of orally  
242 absorbed antifungal agents such as voriconazole and posaconazole, which are commonly  
243 used as prophylaxis and treatment for the fungus (88, 91). An *in vitro* pre-exposure of *A.*  
244 *fumigatus* to these molecules was performed before *Drosophila* infection did not affect the  
245 fungal virulence or the efficacy of these molecules to clear the infection *in vivo* (88, 91).

246 Synergistic effects have been observed when voriconazole was combined with terbinafine  
247 (87, 91, 92).

248 *In vivo* toxicity of volatile organic compounds (VOCs) produced by filamentous fungi (e.g.,  
249 alcohols, aldehydes, thiols, esters...) has been explored in flies. Exposition of *Drosophila*  
250 larvae to VOCs emitted by living fungi delayed metamorphosis towards the pupae stage and  
251 subsequently to the adult stage. In addition, this exposure was detrimental to both larval and  
252 adult survival (Inamdar *et al.*, 2014; Zhao *et al.*, 2017; Al-Maliki *et al.*, 2017). This toxigenic  
253 effect suggests that VOCs may contribute to the fungal pathogenesis, at least in flies.

#### 254 ***Burkholderia cepacia* complex**

255 *Drosophila* is an established model for studying systemic infections caused by species of  
256 opportunistic Gram-negative bacteria belonging to the *Burkholderia cepacia* complex (Bcc). It  
257 has been used to characterize the virulence of different strains (96), the phenotype of some  
258 mutants (97–99) and also to identify virulence factors of strains isolated from CF patients  
259 (100).

260 In response to *B. cepacia* infection, fruit flies produce AMPs, such as Drosomycin and  
261 Dipteracin, via both the Toll and Imd pathways (101). We recently demonstrated that the  
262 induced AMPs are crucial for *Drosophila* survival against *B. cepacia* infection (102).

263 *Drosophila* mutants for the *period* gene, whose circadian rhythm is altered, are more tolerant  
264 to Bcc infection (101). This study also revealed that both glucose and amino-acid intake  
265 improved host tolerance to infection and that the TOR pathway mediates both resistance and  
266 tolerance to Bcc infections (101).

#### 267 ***Mycobacterium abscessus* and the non-tuberculous mycobacteria**

268 *Drosophila* is also a validated model for studying mycobacterial infections. As recently  
269 reviewed, most studies have focused on the pathogenic slow-growing  
270 *Mycobacterium marinum* to model tuberculosis (103). The most frequently isolated non-  
271 tuberculous mycobacteria (NTM) in patients with CF are species of *M. abscessus* and *M.*

272 *avium* complexes, *M. fortuitum* being rarely found (104, 105). In a study including French  
273 patients, *M. abscessus* accounted for more than half of the NTMs isolated (104). This  
274 bacterium causes the most deleterious pulmonary infections in patients with CF (106). *M.*  
275 *abscessus* belongs the group of fast-growing mycobacterium which are predominantly  
276 saprophytic. It is considered the most pathogenic species within this group (107).

277 After systemic injection, *M. abscessus* can proliferate within *Drosophila*, leading to severe  
278 tissue damage and, ultimately, death (Oh *et al.*, 2013). It is recognized by PGRP-SA and  
279 activates the production of Drosomycin, a Toll-mediated AMP (108). Recently, we confirmed  
280 and extended this observation. Indeed, *M. abscessus* injection induced the expression of  
281 AMPs encoding genes, either Toll or Imd-regulated and showed that these AMPs did not  
282 seem to play a major role for *Drosophila* survival during *M. abscessus* infection, as indicated  
283 by the similar survivals of wild-type and AMP-deficient flies (102). We therefore hypothesized  
284 and demonstrated that the intracellular localization of *M. abscessus* protects it from AMPs,  
285 particularly Defensin, which we have shown to have a direct bactericidal action against  
286 extracellular *M. abscessus* (109). Indeed, after its injection, *M. abscessus* is rapidly  
287 internalized by *Drosophila* plasmatocytes in which it grows (102), as observed during fly  
288 infection with *M. marinum* (18).

289 Fly infections have been used to validate mutants for genes encoding known virulence  
290 factors, such as the  $\Delta 0855$  and  $\Delta 4532c$  strains, both defective for intracellular growth (110,  
291 111), as well as to identify some new genes such as *MAB\_0471*, *MAB\_0472* and  
292 *MAB\_3317c* (112).

293 *Drosophila* have also highlighted *M. abscessus* resistance to host innate cytotoxic  
294 responses. Indeed, thanocytes, a newly described hemocyte subpopulation identified by  
295 single-cell sequencing (29), induce caspase-dependent apoptosis in *M. abscessus*-infected  
296 plasmatocytes through the action of two serine proteases, encoded by *CG30088* and  
297 *CG30090*. However, *M. abscessus* resists this lysis and spreads systemically, leading to  
298 bacteremia and subsequent death of infected flies. The resistance of *M. abscessus* to  
299 cytotoxic lysis of phagocytes was validated in a mammalian model after contact of infected

300 murine primary macrophages with autologous natural killer cells. This propensity of  
301 *M. abscessus* to resist the host cytotoxic innate response, typical of strict pathogenic  
302 mycobacteria such as *M. tuberculosis*, could partially explain its superior pathogenicity  
303 among fast-growing mycobacteria.

304 *M. abscessus* is also multi-resistant to antibiotics, including most of the anti-tuberculosis  
305 drugs (113), making it difficult to treat its infections in patients with CF (114). *Drosophila* have  
306 been used to test the effectiveness of antibiotics against *M. abscessus in vivo*. Tigecycline  
307 treatment was the most efficient and its potency was increased when combined with linezolid  
308 (115).

### 309 ***Streptococcus pneumoniae***

310 Injection of the Gram-positive bacterium *S. pneumoniae* in *Drosophila* causes lethal  
311 infections. Fly exposure to sublethal doses primes resistance to subsequent infections by *S.*  
312 *pneumoniae* (116). Phagocytosis by plasmatocytes is crucial for resistance to streptococcal  
313 infections (116–118). It is activated by Eiger, a *Drosophila* homolog of humans TNF $\alpha$ , (119).  
314 Hemocyte activation requires increased consumption of energy, which is obtained by a  
315 systemic metabolic switch involving the release of glucose from glycogen. This is mediated  
316 by adenosine signalling and is modulated by adenosine deaminase ADGF-A to prevent the  
317 loss of energy reserves during chronic infection (118). Interestingly, this effect of adenosine  
318 has also been observed in a mice lung streptococcal infection model in which it regulates  
319 pulmonary neutrophil recruitment (120).

320 The *Drosophila* response to a systemic infection with *S. pneumoniae* is not limited to the  
321 immune cellular response because it also includes the production of AMPs, mediated by both  
322 Toll and Imd pathways (118).

323 *S. pneumoniae* infections have been used to assess whether interactions between circadian  
324 rhythm and immunity exist in flies, as observed in mammals (121). Infected wild-type flies  
325 lose circadian regulation of locomotor activity, whereas mutant flies for *timeless* or *period*,

326 which encode components of the central circadian clock, were more sensitive than wild-type  
327 flies to *S. pneumoniae* infection (122).

#### 328 **4. Coinfection models**

329 Most patients with CF are prone to polymicrobial infections. *Drosophila* has been used to  
330 study such interactions between pathogens as well as those with the host microbiome.  
331 Indeed, flies were orally infected with a combination of *P. aeruginosa* and strains isolated  
332 from the oral flora of patients with CF to compare bacterial virulence genes and host AMP  
333 gene expression with mono-infections. Thus, it was observed that coinfection with  
334 *Streptococcus sp.* and *P. aeruginosa* increased the production of the flagellar filament  
335 protein fliC in *P. aeruginosa*, most likely to increase its motility (123). Upon co-infection with  
336 Gram-positive bacteria, *P. aeruginosa* also presents an increased virulence, due to the  
337 production of antimicrobials and toxins that kill the other bacteria as well as the host cells.  
338 The latter is induced by the detection by *P. aeruginosa* of Gram-positive bacteria PGN (124).  
339 Conversely, *Streptococcus parasanguinis*, a Gram-positive colonizer of the airway of patient  
340 with CF, hijacks *P. aeruginosa* exopolysaccharide alginate production to form a biofilm that  
341 limits *P. aeruginosa* growth. This biofilm contains streptococcal adhesins, which are also key  
342 factors for fly colonization and mortality (125). Nitrite reductase production is crucial for *P.*  
343 *aeruginosa* virulence (126).

344 A more recent model of co-infection with two common pathogens found in patients with CF  
345 was based on the co-injection with *S. aureus* and *P. aeruginosa* in adult *Drosophila* (127).

#### 346 **5. Modelling CF in *Drosophila***

347 Two CF-like models have been proposed in *Drosophila*. The first consists of mutant flies for  
348 the *bereft* gene which encodes *miR-263a*, a microRNA which negatively regulates the  
349 quantity of transcripts encoding the  $\alpha$  and  $\beta$  subunits of ENaC (*ppk4* and *ppk28* respectively).  
350 Thus these flies are a model of ENaC hyperactivity model. Indeed, phenotypes in their  
351 midgut are similar to those observed in epithelia of patients with CF. It was observed that

352 there was excessive sodium entry within enterocytes, the most abundant intestinal cells,  
353 leading to an incoming flow of water following the osmotic gradient and to a dehydration of  
354 the intraluminal area bordering the epithelium (128).

355 These phenotypes are also observed in the second gastro-intestinal *Drosophila* CF, which  
356 has been more recently reported model (129). It is a CFTR mutant model obtained by  
357 depleting in enterocytes of the transcripts of *CG5789/Cftr*. This gene encodes the *Drosophila*  
358 structural and functional equivalent of human CFTR. Indeed, the expression of human *CFTR*  
359 in this CF model rescued gastro-intestinal phenotypes. Partial suppression of these  
360 phenotypes as also observed upon overexpression of *miR-263a*, suggesting that ENaC may  
361 act downstream of CFTR, as in humans (129).

362 Both models exhibit increased levels of antimicrobial peptides due to the activation of the Imd  
363 pathway in response to increased bacterial accumulation in the midgut. Moreover, they are  
364 more susceptible to oral infections with *Pseudomonas aeruginosa* (128, 129). Here again,  
365 *human CFTR* expression rescued this phenotype in flies depleted of *Cftr* transcripts,  
366 establishing a new model to study CF pathophysiology, particularly in respect to the  
367 susceptibility to pathogen infections (129).

368 However, such phenotypic rescue experiments have not been reported in *miR-263a* mutant  
369 flies. Thus, whether hyper-susceptibility to bacterial infections is due to increased levels of  
370 ENaC of remains to be determined. Similarly, even if it is likely, it would be interesting to  
371 determine the susceptibility to other major pathogens found in CF. To note, ENaC has been  
372 proposed to be involved in airway liquid clearance (130). One may wonder whether the CF  
373 phenotypes observed in *miR-263a* mutant flies are only restricted to the midgut and whether  
374 this model is more susceptible to systemic infections.

## 375 **6. Concluding remarks**

376 The recent use of certain CFTR modulators has brought relief to many CF patients; but  
377 unfortunately, not to all. The development of relevant models is crucial for understanding CF  
378 pathophysiology and consequently for searching for effective molecules that can be



379 beneficial in all kinds of *cftr* mutations leading to CF. *Drosophila* can meet this need, all the  
380 more so as CFTR and ENaC channels are present and their deregulation leads to a CF  
381 phenotype. As we have shown in this review, fruit flies have already allowed the identification  
382 of many virulence factors of the most common pathogens in patients with CF, as well as  
383 numerous host factors required to counter these infections. *Drosophila* use should make it  
384 possible to study and understand host resistance factors that are modulated in the context of  
385 CF. In the long term, treatments based on the modulation of the evolutionarily conserved  
386 susceptibility and predisposition factors could reduce CF-associated infections.

387

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791 **Table 1:** Modeling the infections by major CF pathogens in *Drosophila*

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Pathogen	Host immune response				Other response(s)	Host manipulation by the pathogen	<i>In vivo</i> validated antimicrobial
	Cellular response		Humoral response				
	Phagocytosis	ROS production	Toll	IMD			
<i>S. aureus</i>	✓ <u>Receptors:</u> Croquemort, Draper, Eater	✓	✓	✗	?	- Neutralization of host oxidative and antimicrobial responses by catalase  - Reduction of PGN detection and humoral response by producing D-alanylated teichoic acid	- Nisin - NAI-107 - Plumbagin
<i>P. aeruginosa</i>	✓ <u>Receptors:</u> ?	?	✓	✓	- Activation of JNK pathway in enterocytes during oral infection - Nutritional immunity: iron sequestration from the hemolymph and relocation to the fat body	- Prevention of phagocytosis by hemocytes by RhIR and the exotoxin ExoS  - Induction of apoptosis of S2 cells by ExoS and Exotoxin A  - Suppression of AMP production	- Lytic phage MPK1 - Lytic phage MPK6 - Baicalin
<i>B. cepacia</i>	? <u>Receptors:</u> ?	?	✓	✓	Activation of TOR pathway for tolerance and resistance	?	?
<i>M. abscessus</i>	✓ <u>Receptors:</u> ?	?	✓	✓	Granzyme-mediated cytotoxic response by thanocytes	?	- Tigecycline - Linezolid
<i>S. pneumoniae</i>	✓ <u>Receptors:</u> ?	?	✓	✓	Activation of adenosine signaling for metabolic switch	- Loss of circadian regulation of locomotor activity	?
<i>A. fumigatus</i>	? <u>Receptors:</u> ?	?	✓	✗	?	?	- Voriconazole - Posaconazole - Terbinafine

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