MINIREVIEW

Small animal models for the study of Clostridium difficile disease pathogenesis

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Abstract

Clostridium difficile is the leading cause of bacterial antibiotic-associated diarrhoea in hospitals in the developed world. Despite this notoriety, the complex mechanisms employed by this pathogen to overcome innate host defences and induce fulminant disease are poorly understood. Various animal models have been used extensively for C. difficile research to study disease pathogenesis. Until recently, the most commonly used C. difficile disease model has utilised hamsters; however, mouse and pig models have now been developed that unravel different aspects of C. difficile pathology. This review summarises key aspects of the small animal models currently used in C. difficile studies with a specific focus on major differences between them. Furthermore, this review highlights the advantages and disadvantages of each model and illustrates that careful consideration is required when selecting models for use in C. difficile research.

Introduction

Clostridium difficile infection (CDI) is primarily associated with the use of antibiotics, which disrupt the resident gut microbiota and permit colonisation by C. difficile (Bartlett et al., 1978b, c; George et al., 1978). The scope of disease induced by CDI varies from mild diarrhoea to more severe conditions such as pseudomembranous colitis and toxic megacolon (Kelly & LaMont, 2008). The intestinal pathology accompanying CDI is caused by the major exotoxins, toxin A and toxin B, which inactivate Rho family GTPases, ultimately leading to disorganisation of the actin cytoskeleton, cell rounding and cell death (Voth & Ballard, 2005; Jank & Aktories, 2008). Primary CDI treatments include vancomycin or metronidazole; however, disease recurrence following cessation of antibiotic therapy is increasing (Kelly & LaMont, 2008). Thus, the development of novel therapeutics is imperative for the prevention and treatment of CDI. This urgent requirement, combined with the need to better understand C. difficile pathogenesis, has resulted in the development of various C. difficile experimental animal models.

Animal models of C. difficile infection

Several animal models have been developed to study various aspects of CDI, including colonisation, disease pathophysiology, intoxication, transmission, recurrence, efficacy testing of potential therapeutics and the impact of strain variability on all of these factors. Small animals that have been utilised in these studies include mice, hamsters, rats, rabbits, hares, guinea pigs, prairie dogs and quails (Best et al., 2012; Lawley & Young, 2013). More recently, zebrafish embryos have been used to study and compare the effects of C. difficile variant toxin B derivatives isolated from a number of C. difficile strains (Hamm et al., 2006; Lanis et al., 2010). A recent review provides details of other studies that have used larger animals such as foals, Rhesus monkeys and gnotobiotic piglets to study CDI (Best et al., 2012).

Each of the available C. difficile animal models has intrinsic advantages and disadvantages. The utility of a given model will therefore depend on multiple factors, including the complexity and convenience of performing the model and the scientific question that is being addressed. Of particular importance is the relevance of
the model to human disease and whether the disease pathological features observed in infected animals mirror the key pathological features seen in humans. Previous studies have suggested, for example, that the host response to CDI varies between animals (Keel & Songer, 2006); thus, the disease that manifests in the different animal models will undoubtedly be influenced by the immune response to infection. Importantly, many of the animal models used for C. difficile research require pretreatment with antibiotics to induce CDI. However, it is critical that the susceptibility of C. difficile isolates to the various antibiotics used in any model be tested as this may have significant implications on the disease outcome and should be taken into consideration when selecting a model. It is therefore imperative that each animal model continues to be critically evaluated and refined as relevant information is gathered so that the most appropriate and reliable model can be selected for future studies involving C. difficile infections. This is especially true when C. difficile interactions with the host are under examination. In the following review, we describe the small animal models currently used in C. difficile research, with a particular focus on the most recent mouse models which have been developed.

**Hamster models of CDI**

In the 1960s, hamsters were shown to develop fatal enterocolitis following lincomycin or clindamycin treatment (Small, 1968; Bartlett et al., 1977a), but the aetiologic agent remained unknown until the 1970s when C. difficile was identified as the causative agent (Bartlett et al., 1978b; George et al., 1978; Larson et al., 1980). Early work in which healthy hamsters developed disease following the intracecal administration of caecal contents from antibiotic-treated, diseased hamsters showed that disease was transmissible (Bartlett et al., 1977b). Later studies showed a link between toxins and C. difficile disease, with the intracecal or intragastric administration of purified C. difficile toxins inducing disease pathology (Bartlett et al., 1978a; Libby et al., 1982; Lyerly et al., 1985). Other studies have examined the role of toxins in disease, by comparing isogenic mutants in infection studies (Lyras et al., 2009; Kuehne et al., 2010, 2014).

The hamster model has been refined and used extensively for studies relating to C. difficile pathogenesis, as the pathology observed during hamster infection mirrors many clinical aspects of human CDI (Price et al., 1979). After pretreatment with clindamycin and challenge with toxigenic strains of C. difficile, hamsters develop a haemorrhagic caecitis which presents as diarrhoea or ‘wet tail’ as well as other fulminant disease symptoms including ruffled fur, hunching and lethargy, leading to death (Bartlett et al., 1977b; Price et al., 1979). Histopathological analysis of caecal tissue isolated from diseased hamsters shows mucosal ulceration associated with polymorphonuclear leucocyte (PMN) influx and tissue haemorrhage (Fig. 1). However, the site of C. difficile infection in hamsters differs to that in humans, as infection occurs in the caecum of hamsters but in the colon of humans (Price et al., 1979). Nevertheless, despite this anatomical variation in the pathology, hamsters are an important and relevant model of C. difficile disease.

The hamster model has been integral in understanding C. difficile pathogenesis. This model has been used to study multiple aspects of disease including colonisation, transmission, virulence factors, vaccine development and for testing of a variety of therapeutics (recently reviewed in Best et al., 2012). It has also been used to assess the virulence of various animal and human C. difficile isolates. Notably, early infection studies aimed at explaining the spectrum of human disease caused by C. difficile showed that strains could be classified into highly virulent or less virulent categories based on how quickly the infected hamsters succumbed to disease (Borriello et al., 1987). Similarly, infection of hamsters with C. difficile strains belonging to different serogroups revealed differences in pathogenicity among strains which could be correlated with a strain’s capacity to cause disease in humans (Delmee & Avesani, 1990). In addition, prior colonisation of hamsters with nontoxigenic, avirulent strains of C. difficile was protective against disease mediated by toxigenic strains (Wilson & Sheagren, 1983; Borriello & Barclay, 1985). Since then, many studies have focussed on characterising the pathogenicity of different C. difficile isolates, including historical and epidemic strains, by comparing their ability to cause disease in hamsters (Sambol et al., 2001; Razaq et al., 2007; Goulding et al., 2009; Buckley et al., 2011, 2013). Nevertheless, one limitation of this model is the lack of immunological reagents available to study host responses to CDI. To overcome this constraint, mouse models of infection have been developed and are being used to increase the understanding of C. difficile pathogenesis.

**Mouse models of CDI**

The use of mouse models to study CDI is increasing, mainly due to improved methods of inducing disease susceptibility in mice and the greater availability of mouse-specific reagents to perform detailed host tissue analysis. Untreated mice are relatively resistant to infection with C. difficile and do not develop fatal infections (Lawley et al., 2009). This is most likely due to the colonisation resistance provided by the resident microbiota, although these mice can become asymptomatic carriers that
persistently shed low numbers of spores (Lawley et al., 2009). Similar to human CDI, susceptibility of mice to infection must be induced by disrupting the microbiota through antibiotic treatment. The effect of antibiotics on the microbiota and the role of commensal organisms in colonisation resistance to CDI have been reviewed in detail elsewhere (Lawley & Young, 2013). Recently, a mouse model involving the intrarectal administration of Clostridium difficile toxins was described, whereby toxin delivered directly into the colon resulted in inflammation, upregulation of cytokines/chemokines and increased tissue damage (Hirota et al., 2012). This model is similar to the hamster model mentioned earlier in this review, where toxins were administered intragastrically to assess their individual roles in disease (Lyerly et al., 1985). In addition to this intoxication model, three different mouse C. difficile infection models have been described. The first employs gnotobiotic/germ-free mice (Pawlowski et al., 2010; Reeves et al., 2012), the second uses a cocktail of antibiotics to disrupt the normal gut microbial communities and predispose the mice to infection (Chen et al., 2008), and the third uses a single antibiotic to induce susceptibility to CDI (Theriot et al., 2011; Buffie et al., 2012). Table 1 details the pretreatment regime used in these various models as well as the infecting strains used and the histopathological outcomes of infection.

Gnotobiotic mouse models used to study CDI

The use of germ-free mice has the obvious advantage of not requiring antibiotic pretreatment of animals to disrupt the resident gut microbiota. One such study describes the use of aged (7–14 months) gnotobiotic...
C57BL/6 mice to study fulminant CDI and to elucidate the host immune response to acute infection (Pawlowski et al., 2010). These mice were shown to develop symptomatic disease similar to that seen in mice treated with an antibiotic cocktail prior to infection (Pawlowski et al., 2010). Additionally, the infected germ-free mice

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Antibiotic pretreatment and route of administration</th>
<th>C. difficile strains used for infection</th>
<th>Histopathological outcomes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic cocktail model</td>
<td>Mice receive the antibiotic cocktail for 7 days in the drinking water (metronidazole (0.215 mg mL$^{-1}$), vancomycin (0.045 mg mL$^{-1}$), kanamycin (0.4 mg mL$^{-1}$), gentamicin (0.035 mg mL$^{-1}$) and colistin (850 U mL$^{-1}$)). Two days after cessation, a single intraperitoneal injection of clindamycin (10 mg kg$^{-1}$) is administered (1 day prior to infection)</td>
<td>VP110463, B11, B17, K14</td>
<td>VP110463 Proliferative ulcerative enteritis with superficial epithelial necrosis and release of inflammatory exudate and necrotic material into the intestinal lumen. Extensive submucosal oedema without submucosal inflammation, mucosal proliferation and inflammatory cell influx B11, B17 or K14</td>
<td>Chen et al. (2008)</td>
</tr>
<tr>
<td>Aged gnotobiotic mouse model</td>
<td>No antibiotics required</td>
<td>UVA13, VP11186</td>
<td>UVA13 Caecal and colonic ulceration, loss of mucosal architecture, epithelial exfoliation, minor neutrophilic infiltration, oedema and haemorrhage in the lamina propria VP11186 (nontoxigenic) No significant histopathological changes</td>
<td>Pawlowski et al. (2010)</td>
</tr>
<tr>
<td>Young gnotobiotic mouse model</td>
<td>No antibiotics required</td>
<td>VP110463, 630</td>
<td>VP110463 Severe colonic pathology with oedema within the submucosa and the lamina propria as well as neutrophilic inflammation 630 No histology was shown as these mice did not exhibit clinical signs of disease</td>
<td>Reeves et al. (2012)</td>
</tr>
<tr>
<td>Clindamycin model</td>
<td>Clindamycin via oral gavage (1 mg day$^{-1}$) or in the drinking water (0.25 mg mL$^{-1}$) for 4 days A single dose of clindamycin (200 µg) by intraperitoneal injection 1 day prior to infection</td>
<td>M68</td>
<td>M68 Moderate intestinal inflammation, oedema, epithelial damage and immune cell infiltrate in the caecal tissue</td>
<td>Lawley et al. (2009)</td>
</tr>
<tr>
<td>Cefoperazone model</td>
<td>Mice given cefoperazone (0.5 mg mL$^{-1}$) in the drinking water for 10 days followed by 2 days with regular water prior to infection with C. difficile</td>
<td>VP110463, 630, B11, F200</td>
<td>VP110463 or B11 Severe colonic pathology, with high levels of inflammation, submucosal oedema and epithelial damage 630 or F200 Minimal inflammation and slight oedema observed</td>
<td>Theriot et al. (2011)</td>
</tr>
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demonstrated an increase in the production of pro-inflammatory cytokines such as KC, the murine equivalent of human interleukin-8 (IL-8), which is increased in the faeces of patients with severe CDI (Steiner et al., 1997). A separate study utilised 6- to 8-week-old germ-free Swiss Webster mice to examine the role of murine Lachnospiraceae and Escherichia coli strains, both part of the normal microbiota of mice, in colonisation resistance against C. difficile (Reeves et al., 2012). Analogous to the aged, gnotobiotic model, mice infected in this study developed clinically severe disease, with diarrhea, hunched posture, weight loss, mortality and histopathological changes recorded (Table 1). However, while these infection models appear to result in clinically relevant host innate immune responses and disease symptoms, animals that are deficient in resident microbiota do not develop all of the normal immune and mucosal responses (Macpherson & Harris, 2004), which may influence the natural course of CDI in these models. Despite the similarities in disease pathology observed between the germ-free models and the antibiotic cocktail model, the total absence of microbiota in the former does not reflect the normal situation in humans and animals. This may explain why models involving antibiotic-induced susceptibility to C. difficile disease are more widely used to study CDI. Nevertheless, the use of a germ-free mouse model can have important applications as recently shown by Janoir et al. (2013). These researchers utilised germ-free C3H mice to study the C. difficile transcriptome over the course of infection (Janoir et al., 2013). When comparing the regulation of genes in vivo to that seen during in vitro studies, this approach revealed a large number of genes that were differentially regulated during the early stages of mouse colonisation, suggesting a role for these genes in virulence (Janoir et al., 2013). Indeed, this study resulted in the identification of a novel colonisation gene, CD1581. Studies such as these pave the way for the development of exciting new tools to increase our understanding of CDI.

Antibiotic cocktail mouse model of CDI

The first published mouse model was the antibiotic cocktail model, which elegantly demonstrated that a mixture of antibiotics (Fig. 2) administered to mice prior to C. difficile infection reproducibly induces disease (Chen et al., 2008). This model mirrors key features of CDI in humans, including diarrhea, weight loss and histological damage (Fig. 1), characterised by the severe colonic pathology described in Table 1, and allows the entire human disease spectrum to be reproduced by varying the infectious spore dose or by using different strains (Chen et al., 2008). The antibiotic cocktail mouse model has been developed further to include a disease relapse component (Table 2), which mimics a common aspect of human disease (Chen et al., 2008). The antibiotic cocktail model has also successfully been used to explore the dynamics of the gut microbiome during CDI, with mice that developed severe clinical disease shown to harbour a different gut microbial community compared to mice that did not succumb to disease (Reeves et al., 2011). Additionally, this model has been extensively used to investigate the therapeutic potential of novel vaccines and treatments for CDI as well as to improve the understanding of current treatments.

Fig. 2. Antibiotic regimes used for the small animal models of Clostridium difficile infection. (a) Golden Syrian hamsters were orogastrically administered 30 mg clindamycin phosphate kg⁻¹ 5 days before infection and monitored for signs of disease (Buckley et al., 2011). (b) C57BL/6 mice were administered an antibiotic mixture of Kanamycin (0.4 mg L⁻¹), gentamicin (0.035 mg L⁻¹), colistin (850 U ml⁻¹), metronidazole (0.215 mg L⁻¹) and vancomycin (0.045 mg L⁻¹) in the drinking water for 7 days. Following this, mice were given normal water for 2 days and administered clindamycin (10 mg kg⁻¹) intraperitoneally 1 day before C. difficile challenge. Mice were then monitored for diarrhoea, hunched posture, wet tail and weight loss (Chen et al., 2008). (c) C57Bl/6j mice received a single dose of clindamycin (200 μg) by intraperitoneal injection 1 day prior to infection with C. difficile and were subsequently monitored for signs of diarrhoea and weight loss (Buffie et al., 2012). (d) C57Bl/6 mice were administered cefoperazone (0.5 mg ml⁻¹) in the drinking water for 10 days. Following this, the mice were given regular drinking water for 2 days prior to infection with C. difficile and were monitored for inappetence, diarrhoea and hunching (Theriot et al., 2011).
Single antibiotic mouse models of CDI

In humans, treatment with a single antibiotic is the most common factor predisposing patients to CDI (Johnson et al., 1999; Gerding, 2004). Similarly, CDI can be induced in mice by the use of a single antibiotic to alter the gut microbiota, for example clindamycin (Lawley et al., 2009; Buffie et al., 2012) or the third-generation cephalosporin, cefoperazone (Theriot et al., 2011; Fig. 2). The use of clindamycin and the cephalosporins are key...
risk factors for human CDI (Sullivan et al., 2001). Pretreatment of mice with either antibiotic induces susceptibility to CDI and results in disease characterised by diarrhoea, weight loss, mortality and colonic or caecal pathology (Fig. 1; Table 1; Theriot et al., 2011; Buffie et al., 2012; Lawley et al., 2012). When directly compared to mice on the antibiotic cocktail, cefoperazone-treated mice exhibited heightened susceptibility to C. difficile, probably due to the more severe effect of cefoperazone on the composition of the gut microbiota (Reeves et al., 2011). Interestingly, the disruption to the gut microbiota caused by cefoperazone was of a longer duration than that caused by the antibiotic cocktail (Reeves et al., 2011). Thus, it is possible that the increased time required for recovery of the normal microbiota may lead to increased susceptibility to relapse in these mice. Similarly, another study showed that a single dose of clindamycin resulted in a rapid loss in the diversity of the resident microbiota and that mice remained susceptible to CDI for at least 10 days after administration of the antibiotic, suggesting that clindamycin-sensitive organisms may be responsible for resistance to CDI (Buffie et al., 2012). By contrast, however, an earlier study showed that clindamycin only had a transient effect on the composition of the resident microbiota, with recovery of the microbiota occurring 7 to 10 days after cessation of antibiotic treatment (Lawley et al., 2009). These contrasting results may reflect differences in the existing microbiota of experimental mice prior to antibiotic treatment. Additionally, in the hamster model, clindamycin has been shown to remain in the caecal contents, at levels sufficient to inhibit growth of clindamycin-sensitive strains of C. difficile, for up to 11 days following the cessation of treatment (Larson & Borriello, 1990). It has also been shown that clindamycin-sensitive strains behave differently in hamsters pretreated with cefoxitin compared to those administered clindamycin, with delayed faecal colonisation observed in hamsters treated with the latter. This delay in colonisation suggests that residual clindamycin may have an impact on the kinetics of infection (Delmee & Avesani, 1990). Thus, testing C. difficile strains for susceptibility to clindamycin prior to infection may be useful if clindamycin is to be used in a model. Importantly, when the antibiotic cocktail without clindamycin, the cocktail with clindamycin, or clindamycin alone was compared, it was shown that the combination of the antibiotic cocktail and clindamycin was the most effective at suppressing colonisation resistance to C. difficile in mice (Reeves et al., 2011), demonstrating that the specific changes in microbiota profiles resulting from treatment with particular antibiotics determine the susceptibility level of mice to CDI (Reeves et al., 2011).

While the use of a single antibiotic such as clindamycin or cefoperazone to induce disease susceptibility may be a less expensive alternative to using the antibiotic cocktail for experimental models, it is important to consider that mice obtained from different vendors harbour diverse populations of intestinal microbiota (Ivanov et al., 2008). Thus, an individual antibiotic may induce susceptibility in mice obtained from one source but be less effective on mice obtained from another. For this reason, susceptibility to C. difficile colonisation following single antibiotic use and experimental reproducibility upon infection needs to be determined independently on mice obtained from different sources. The use of a mixture of antibiotics, which target a broader range of microbial species, may result in more consistent and reproducible results. Nonetheless, if colonisation resistance to C. difficile is overcome by pretreatment with a single antibiotic, the latter model provides the advantage of not only being less costly, but also offers the advantage of being a less acute disease model that allows the more subtle aspects of disease to be studied (Lawley et al., 2009). These include C. difficile interactions with the microbiota, colonisation and transmission as well as investigating the efficacy of treatments such as faecal transplantation, which may otherwise be difficult to measure due to the speed at which the mice succumb in the acute disease model.

**Mouse models to study transmission of C. difficile and disease relapse**

The clindamycin mouse model has been adapted to investigate the transmission of C. difficile (Lawley et al., 2009, 2012). An advantage of the transmission model is that it allows for the study of carriage, shedding and natural host-to-host transmission of C. difficile (Lawley et al., 2012). The use of this model has shown that brief exposure to environmental spore contamination is sufficient for transmission of C. difficile to naïve but susceptible mice. Compared to artificial challenge of mice via spore or vegetative cell gavage, this model may equate to a more natural and less acute disease model which may be more representative of typical human infection (Lawley et al., 2009). Interestingly, in the context of human infection, the CDI transmission model has been used to demonstrate that clindamycin treatment of asymptomatic carriers of C. difficile can inadvertently trigger the excretion of high levels of spores (Lawley et al., 2009). Furthermore, this mouse model was recently used to show that the spo0A gene is an important factor in C. difficile persistence and transmission (Deakin et al., 2012). The development of the transmission model marks an important step in developing tools that will allow a better understanding of the complexity of C. difficile.
It is important to note that the various animal CDI models considered when using the small animal CDI models

transmission and will allow therapeutics to be assessed for their utility as preventive measures.

In addition to this, the antibiotic cocktail model has recently been used to investigate disease relapse (Chen et al., 2008; Sun et al., 2011). Disease relapse resembling that seen in humans was successfully induced in mice by administering an antibiotic cocktail to mice that had previously recovered from a primary infection (Table 2; Chen et al., 2008; Sun et al., 2011). Mice were re-challenged with spores at the time of antibiotic treatment and subsequently developed severe disease symptoms similar to those seen during primary infection (Sun et al., 2011). In humans, however, relapse usually occurs following treatment with a single antibiotic such as vancomycin (Johnson, 2012) and so the antibiotic cocktail model may not accurately reflect human disease recurrence. For this reason, the relapse model has been further refined to include vancomycin-related relapse (Sun et al., 2011; Lawley et al., 2012). In concordance with patient treatment, mice were administered vancomycin postinfection in order to suppress CDI. Following cessation of antibiotic treatment, mice were either re-challenged or not with spores and were monitored for signs of relapse. Importantly, in the absence of spore re-challenge, mice were shown to shed increased numbers of spores (Lawley et al., 2012) or to develop mild, fast-resolving diarrhoea in 30% of mice (Sun et al., 2011). The incidence of diarrhoea increased to 80% following re-challenge with C. difficile (Sun et al., 2011). Collectively, these results suggest that by contrast to human relapse, vancomycin alone does not sufficiently disrupt the resident microbiota of mice to allow fulminant CDI to re-establish. Nevertheless, the milder disease accompanying vancomycin treatment still offers a valuable mouse relapse model, offering important insights into the prevention of recurrence which are less easily discerned using the rapidly fatal hamster or mouse antibiotic cocktail models. The refinement of the mouse model to include a relapse component provides the opportunity to investigate potential therapies for this important disease aspect, for which no effective treatments are currently available. To this end, the relapse model has recently been used to explore the use of a mixture of different bacterial species to re-establish the gut microbiota and subsequently clear the mice of CDI, preventing relapse (Lawley et al., 2012).

Considerations when using the small animal CDI models

It is important to note that the various C. difficile strains used in these models can result in different degrees of disease pathology (Table 1). For example, Chen et al. (2008) showed that challenge of mice with strain VP10463 resulted in severe disease symptoms, with the majority of mice succumbing to infection, while infection with strain B11 did not result in the death of mice and less severe enteritis was observed. Likewise, in the hamster model, more severe differences in pathology were observed when animals were infected with C. difficile strain B1 compared with those infected with strain 630, when compared to uninfected animals (Goulding et al., 2009). Therefore, it is important to take into account the strains used in any given model, and caution must be used when drawing conclusions from models using a single strain for infection studies.

Variation can also be observed when mice are infected with the same strain, with not all mice in a given group showing the same disease outcome. For example, Reeves et al. (2011) reported that infection with VP10463 (10⁵ CFU of spores) led to colonisation of all mice; however, only a proportion (7/12 mice) displayed overt signs of disease. An alternative study, using the same infectious dose but with strain UK1, reported a mortality rate of 30% and diarrhoea in 70% of mice (Sun et al., 2011). This variation can be overcome by modifying the infectious dose administered. For example, when mice are administered low doses of C. difficile inoculum, there is often variability in the disease outcomes observed within a given group. However, when larger infectious doses are administered, this can result in more consistent disease pathology consequences, although this outcome still depends on the strain used for infection or the mouse model used to induce susceptibility to CDI (Chen et al., 2008; Theriot et al., 2011; Reeves et al., 2012). For this reason, it is imperative that researchers determine the optimum inoculum required for any given strain in their particular model, under the experimental conditions used. Studies such as those described in this review highlight the complexity of C. difficile infection and the difficulty in establishing a mouse model that results in consistent disease outcomes. Nonetheless, these models are providing vital insights into the complex pathogenesis of CDI.

Conclusion

The understanding of C. difficile pathogenesis has steadily increased since the development of the hamster and mouse models of CDI. While the hamster model provided the foundation for C. difficile research and continues to be a useful and important model for studying C. difficile, the development of new mouse models, combined with wide access to mouse-specific reagents and tools, is offering new opportunities to study subtle features of disease. However, due to the notable interspecies differences in susceptibility to CDI and the severity of disease outcomes, caution should be used when
comparing results obtained using different animal models or when extrapolating those results to humans. Thus, the choice of model is critical when endeavouring to study CDI and it is likely that in coming years the use of the various animal models will be re-evaluated, particularly as more become known about the complex relationship between C. difficile, host cell receptors, the gut microbiota and the host immune system. Until then, the development of multiple mouse models as well as the hamster, pig and zebrafish models means that there are now different models available to investigate the various aspects of CDI. Thus, the use of a wide range of models is providing new and exciting opportunities to understand disease pathogenesis and to test novel therapeutics for the prevention and treatment of CDI.

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