Animal models of hospital-acquired pneumonia: current practices and future perspectives

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Abstract: Lower respiratory tract infections are amongst the leading causes of mortality and morbidity worldwide. Especially in hospital settings and more particularly in critically ill ventilated patients, nosocomial pneumonia is one of the most serious infectious complications frequently caused by opportunistic pathogens. *Pseudomonas aeruginosa* is one of the most important causes of ventilator-associated pneumonia as well as the major cause of chronic pneumonia in cystic fibrosis patients. Animal models of pneumonia allow us to investigate distinct types of pneumonia at various disease stages, studies that are not possible in patients. Different animal models of pneumonia such as one-hit acute pneumonia models, ventilator-associated pneumonia models and biofilm pneumonia models associated with cystic fibrosis have been extensively studied and have considerably aided our understanding of disease pathogenesis and testing and developing new treatment strategies. The present review aims to guide investigators in choosing appropriate animal pneumonia models by describing and comparing the relevant characteristics of each model using *P. aeruginosa* as a model etiology for hospital-acquired pneumonia. Key to establishing and studying these animal models of infection are well-defined end-points that allow precise monitoring and characterization of disease development that could ultimately aid in translating these findings to patient populations in order to guide therapy. In this respect, and discussed here, is the development of humanized animal models of bacterial pneumonia that could offer unique advantages to study bacterial virulence factor expression and host cytokine production for translational purposes.

Keywords: Ventilator-associated pneumonia; cystic fibrosis; animal models; *Pseudomonas aeruginosa*

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Hospital-acquired pneumonia

Epidemiology of hospital-acquired pneumonia

Hospital-acquired pneumonia (HAP) is the second most common nosocomial infection (1), and is characterized by high morbidity and mortality (2). HAP is frequently caused by either multidrug-resistant nosocomial bacteria or by opportunistic pathogens, i.e., microorganisms that usually do not cause an infection in healthy individuals but can typically colonize and infect critically ill patients. HAP is especially a serious threat to patients hospitalized in the intensive care unit (ICU) and receiving mechanical

ventilation. This so called ventilator-associated pneumonia (VAP) is defined as a pneumonia that typically develops more than 48 hours after endotracheal intubation and initiation of mechanical ventilation (3,4). Mechanical ventilation significantly increases the risk for infections resulting in a 20-fold increased risk for developing pneumonia as compared to non-ventilated patients in the ICU (5,6). VAP is the most common nosocomial infection in ICU settings (7,8), and after controlling for other variables, patients developing VAP have a considerably higher mortality, reaching up to 50% in some studies (9,10), compared to non-VAP pneumonia patients (11,12).

Etiology of hospital acquired pneumonia

HAP is mostly caused by opportunistic pathogens such as *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* and *Enterobacteriaceae* that tend to colonize patients very quickly once admitted to the hospital (13,14). In particular, infections caused by Gram-negative multidrug-resistant organisms, including *P. aeruginosa* and extended-spectrum β-lactamase-producing or carbapenemase-producing *Enterobacteriaceae*, are increasingly being reported worldwide (15). Especially in VAP, *P. aeruginosa* is one of the main etiologic agents responsible for a global prevalence rate of >25% (16) and is associated with development of other serious complications such as septic shock and multiple organ dysfunction (17).

For this review, we consider animal cystic fibrosis (CF) models as highly relevant to understand the pathophysiology of HAP because of their shared etiology. *P. aeruginosa* is, as in VAP, a major cause of pulmonary infection in CF patients (18), along with other pathogens known for their biofilm producing capacity such as *Staphylococcus aureus* and *Burkholderia cepacia* (19). Cystic fibrosis is the most common and fatal autosomal-recessive disease in the Caucasian population affecting ≈70,000 individuals worldwide (20) and is caused by a dysfunctional CF transmembrane conductance regulator (CFTR) (21,22) resulting in increased mucous secretion in the alveolar spaces that provide an ideal environment for bacterial colonization and biofilm formation (23). This biofilm protects bacteria from host immune cells and antibiotics by encapsulation and sequestration (24,25) and thus co-induces the typically persistent type of lung inflammation observed in CF patients (26). Moreover, VAP pathogenesis is also closely linked to biofilm forming organisms colonizing the endotracheal tube (ETT) such as *P. aeruginosa*, and the presence of *P. aeruginosa* in the biofilm on the ETT microbiome negatively correlates with patient prognosis (27).

Animal models of hospital-acquired pneumonia

Need for animal modeling

For many decades now, animal models of infection are increasingly being utilized in medical research and are responsible for accelerated progress in various fields such as cancer, neuroscience and most importantly, infectious diseases and drug development. The aim of developing an animal model to study pneumonia is to mimic the pathophysiologic and phenotypic characteristics seen in humans in a more controlled setting. Such models give a more accurate control of significant variables through the course of infection by minimizing confounders like co-morbidities or antibiotic use. Studying animal models also has other advantages including circumventing sampling limitation issues that are commonly encountered with human subjects. Precise control over timing of the infectious challenge in animal models also allows for a better understanding of temporal evolution of the disease and development of complications that are most likely related to an altered immune-inflammatory response of the host. Partial or isomorphic induced animal models of pneumonia are usually designed with the purpose of studying specific phenotypic aspects of diseases while lacking other clinical signs or etiology. Nonetheless animal models of pneumonia, and more specifically rodent pneumonia models, have aided considerably in our understanding of disease pathomechanisms and shown their utility in pre-clinical drug testing.

As discussed above, although other pathogens including *Enterobacteriaceae*, *S. aureus* and *A. baumannii* are important causes of HAP, *P. aeruginosa* is one of the most common HAP etiologies and therefore the most studied organism. Thus, in this review, the main characteristics of the most used *P. aeruginosa* pneumonia animal models for acute pneumonia, VAP and chronic pneumonia occurring in CF patients are summarized and compared (*Table 1*). Several different species have been used to model human pneumonia including piglets (28-30), rodents (31-33), primates (34,35), sheep (36,37), dogs (38,39) and rabbits (40,41) and these models have proven instructive in studies of disease mechanisms and in antibiotic testing. Nonetheless, rodents have been the preferred choice in translational pulmonary

Table 1 Summary of different animal models of HAP

Animal model	Model of human pathology	Primary endpoints	Animal species	Important considerations	Relevant characteristics
One-hit acute model	Acute pneumonia, hospital-acquired pneumonia (depending on the choice of pathogen)	Histopathology: multi-lobar confluent pneumonia Mortality: high	Rat Mouse	High bacterial dose used Aerosol, intranasal, intra-tracheal or endotracheal inoculation	Rapid clearance and risk for sepsis development Different accuracy for dose delivery depending on method
VAP model	Ventilator-associated pneumonia model using MV as additional insult	Histopathology: multi-lobar confluent pneumonia Mortality: very high	Piglet Rat Mouse	MV parameters (protective or injurious) have major impact on model Bacterial inoculation before or after MV Prolonged MV is challenging in rodents	Spontaneous pneumonia (piglet): polymicrobial etiology of common airway colonizing organisms Induced oropharyngeal aspiration model (Piglet) MV itself causes inflammation that impacts the disease pathogenesis
Pa agar bead model	Cystic fibrosis (chronic) pneumonia using agar beads to mimic biofilm matrix	Histopathology: bronchopneumonia Mortality: moderate	Rat Mouse	Bacteria loaded beads or Free-living bacteria mixed with sterile beads Use of mucoid strains	Sterile beads induce infiltration of neutrophils and eosinophils Airway obstruction can occur in mice

VAP, ventilator-associated pneumonia; MV, mechanical ventilation; Pa, *P. aeruginosa*.

research as they not only are in accordance with the 3R principles of animal experimentation (42) but also offer specific advantages such as the potential to validate key findings or elucidate distinct pathogenic steps in a wide range of developed transgenic rodent models that are available to the scientific community.

Types of pneumonia models

One hit acute pneumonia model

This simple model of pneumonia is established by administering a bacterial inoculum into the lungs and different methods of bacterial delivery have been described (*Figure 1*). Intratracheal instillation is the most used method in HAP research and involves injecting the bacterial suspension directly into the trachea or lungs followed by air for dispersion of the bolus (33,43-50). This method gives the most precise control on the delivered dose. However, surgically exposing the trachea and then suturing the incision provokes an inflammatory response in the target organ, the lung, which can have an effect on the measured endpoints. Endotracheal inoculation (*Figure 2A* upper panel), on the other hand, involves intubating the animal to facilitate instillation of the bacterial solution in the lungs and is as precise as the intratracheal instillation method (31,32,51). Less invasive methods include intranasal administration, whereby bacterial dose is administered in droplets through the nostrils followed by aspiration by the animal (52), or aerosol administration, that can be performed in unrestrained animals (53). However, the exact dose that reaches the lower respiratory tract in both methods is uncontrolled and animals frequently develop upper respiratory tract infections (52) or infections other than pneumonia (53). One method to control dose delivery with aerosolization is to immediately sacrifice a few (sentinel) animals after aerosolization for quantitative colony counts performed on bronchoalveolar lavage fluid or lung tissue lysates (53).

Acute *P. aeruginosa* pneumonia, both in rodents and humans, is characterized by a high 1- to 3-day mortality (33,50) and histologically presents as multilobar confluent pneumonia (54) causing high alveolar neutrophilic infiltration along with vascular congestion, alveolitis and alveolar collapse (*Figure 2B,C*). The inflammatory response towards acute *P. aeruginosa* pneumonia is chiefly governed by expression of proinflammatory cytokines including TNFα, IFNγ, IL-1α, IL-1β and IL-12 as well as chemotactic molecules such as IL-8 secreted by innate

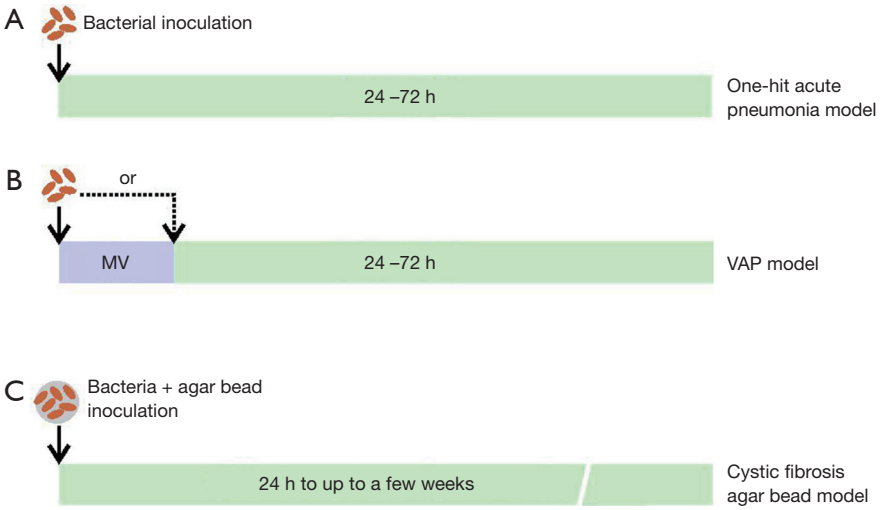

Figure 1 Overview of different *P. aeruginosa* experimental pneumonia models. (A) One-hit acute pneumonia model where different inoculation routes (intratracheal, endotracheal, intranasal, or aerosolization) are employed; (B) VAP models where animals receive bacterial inoculation, prior to or after mechanical ventilation (MV). In spontaneous piglet VAP model, no bacterial inoculations are performed; (C) *P. aeruginosa* agar bead models wherein bacteria are either entrapped within agar beads or free-living bacteria are mixed with sterile agar beads prior to animal inoculation.

immune cells, epithelial cells and alveolar macrophages that result in neutrophil recruitment to the site of infection (54-57). However, the choice of inoculum dose is of high importance because a relatively high dose of *P. aeruginosa* causes a more marked increase in production of cytokines in the early time-points compared to lower doses (50) and thus has important consequences for studying pneumonia development.

Ventilator-associated pneumonia model

As described above, mechanical ventilation by itself is an important component in the pathogenesis of VAP, however, spontaneous development of pneumonia that occurs naturally in patients, is induced in mechanically ventilated animals most frequently by co-challenging with a bacterial inoculum. Interestingly, mechanical ventilation has been shown to cause a sterile inflammatory response in the lung leading to tissue damage caused by different mechanisms such as overstretching of the lung, barotrauma and volutrauma, leakage of air due to disruption of the airspace wall, pulmonary edema and atelectrauma (repeated opening and closing of alveoli) (58-61). Mechanical ventilation-associated lung inflammation is marked by an upregulation of proinflammatory cytokines such as TNFα, IFNγ, IL-6 and IL-1α and IL-1β (62-68) combined with chemokine release including IL-8 and CXCL-1 (69). However, mechanical ventilation also has been shown to cause a lowered natural killer cell activity and reduced MIP-2 and IL-10 expression in splenocyte proliferation assays from mechanically ventilated rats (67). Moreover, host immune response alters when different ventilation strategies are used (70) and therefore, clinically relevant ventilation strategies similar to those applied in patients of 8 mL/kg tidal volume or less should be used in animals to accurately mimic lung inflammation caused by mechanical ventilation.

To establish VAP animal models, two main strategies have been used. In the first strategy, bacteria are injected into the lungs prior to mechanical ventilation (71,72) (*Figure 1*). A second strategy uses bacterial instillation after ventilation and mimics the more natural disease evolution (31,32) where mechanical ventilation precedes bacterial infection occurring in VAP patients. VAP models display similar histological features as compared to the direct acute pneumonia model (*Figure 2C*), however, VAP animal model show an increased bacterial lung burden, a more severe disease progression and a higher mortality compared to animals that received the same bacterial dose without prior ventilation (31,32) (*Figure 2B*).

In piglets, it has been shown that experimentally-induced tracheal stenosis along with prolonged mechanical ventilation of up to 4 days causes spontaneous pneumonia development with endogenous microbiota as primary

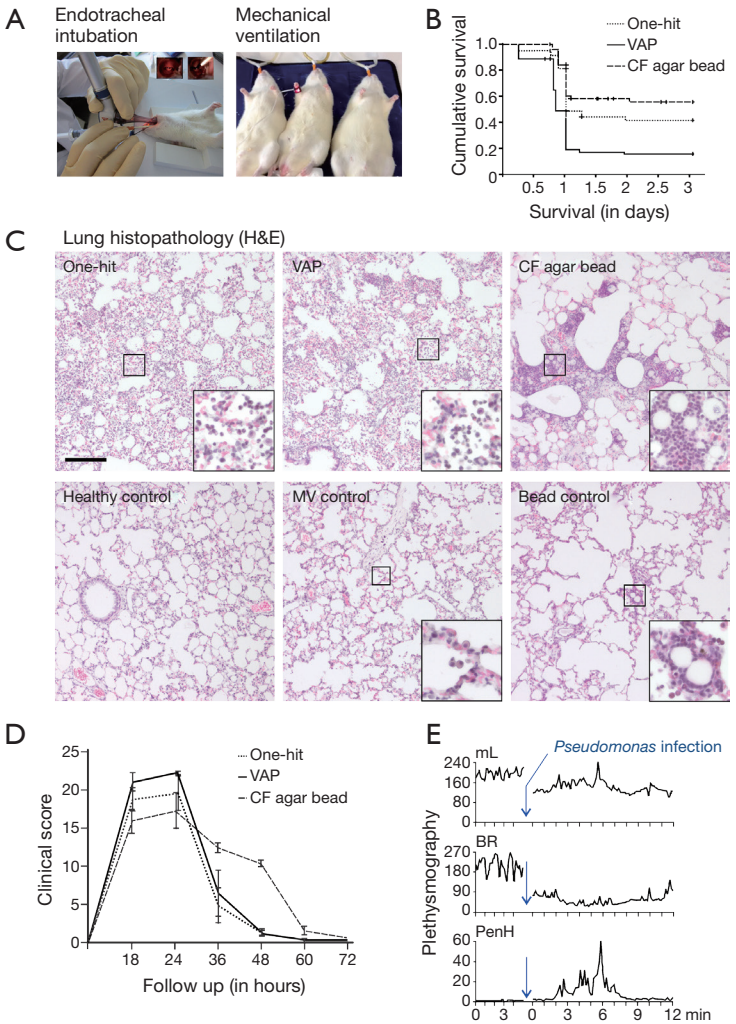

Figure 2 Characteristics and endpoints of animal models of hospital-acquired pneumonia. (A) Left image shows endotracheal intubation technique illustrated here on a Wistar rat. Animal is fixed on a tilting platform by front incisors. Using a speculum mounted on an otoscope, an endotracheal tube is advanced towards the vocal cords with the aid of a guide wire. The right image shows animals receiving parallel mechanical ventilation. A pressure-controlled ventilation setting was used that allows for equal volume distribution between the different animals and small Y-shaped adaptors decrease the dead space to a minimal. Animals are placed on a heating blanket to maintain body temperature and blood oxygenation is measured using pulse-oximetry (red adaptor around paw of middle animal); (B) Kaplan-Meier survival analyses of rodent pneumonia models show highest mortality for VAP model while CF agar bead chronic pneumonia model shows highest survival; (C) representative images from H & E stained lung paraffin sections from rat pneumonia models. One-hit and VAP models cause confluent multilobar pneumonia with distinct signs of vascular congestion, alveolitis and alveolar collapse along with high infiltration of neutrophils (see insets). CF agar bead model shows more intense inflammation around areas that contain beads (inset) mostly restricted to the larger bronchioles resembling diffuse bronchopneumonia. The control MV and sterile beads only cause a mild inflammation evidenced by increased cellular infiltrates that for sterile bead inoculated animals are restricted to areas surrounding the beads (see insets). Scale bar represents 200 µm; (D) clinical follow-up of VAP and one-hit rodent pneumonia models show characteristic sharp increase in pneumonia clinical parameters up to 24 h post infection followed by a sharp decline to baseline levels 3 days post-infection. CF agar bead chronic pneumonia model shows a progressive pneumonia development with clinical signs of pneumonia present up to 3 days post-infection; (E) plethysmography recording of animal pre- and 18 hours post- *P. aeruginosa* endotracheal inoculation (blue arrow). Upper panel shows drop in tidal volume (mL), middle panel shows drop in breathing frequency (breath/min) and lower panel shows increase in Penh (non-invasive extrapolation to indicate bronchoconstriction). (B,C,D,E) Data presented here are from meta-analyses of our own results. MV and VAP models were ventilated using 8 mL/kg tidal volume and 4 cm H_2O positive end expiratory pressure. All animals received same bacterial load of 2E7 CFU in 500 µL.

etiology, although known opportunistic human VAP pathogens including *Pseudomonas* and *Klebsiella* species have also been isolated in this piglet VAP model (29). Recently, a porcine *P. aeruginosa* VAP model was established via oropharyngeal challenge immediately after intubation and a second challenge 4 hours into ventilation using a ceftriaxone-resistant *P. aeruginosa* strain to ensure pulmonary aspiration of oropharyngeal secretions caused by the desired organism (28). Using 40 cm H_2O pressure in the endotracheal cuff, the authors limited direct bacterial inoculation but rather mimicked aspiration of oropharyngeal secretions that build up behind the cuff, as similarly occurs in VAP patients (28,73). This resulted in localized lung pathology in distinct lobes, as also observed in VAP patients (28,73). However, in this model, the authors did not use a lung-protective ventilation strategy as currently used in patients and animals, where, as discussed above, lungs are typically ventilated with approximately 8 mL/kg tidal volumes accompanied by positive end-expiratory pressure to mitigate end-expiratory alveolar collapse. Perhaps this or less control on etiology leads to more variability and a clinical course of experimental VAP that appears slightly different from that observed in VAP patients (73).

Agar-bead (chronic) pneumonia model

This model is used extensively in the field of cystic fibrosis research and was originally developed in rats (74). To mimic biofilm, agar or seaweed alginate beads are used as extracellular polymeric substances which are loaded with bacteria in a process that requires mixing with mineral oil (55,75) and addition of an emulsifying agent, sorbitan-monooleate, to increase uniformity of the beads (75) (*Figure 1*). For sham animals, sterile beads are prepared using PBS or saline, however, instillation of sterile beads itself incites an inflammatory response resulting in increased cellular infiltrates in lungs (55,75) (*Figure 2C*) and increased release of inflammatory cytokines that, depending on the precise clinical endpoints utilized in the study, could obscure the potential beneficial treatment effects (75).

Interestingly, although bacteria can migrate from the agarose beads *in vivo*, bacterial growth is slow and is limited to the beads, a situation that is similar to what has been observed in bacteria existing in a biofilm phenotype (76). Also, clearance of bacteria is impaired and animals are less likely to develop acute sepsis that occurs more frequently with free-living bacterial inoculation (54). Interestingly, *P. aeruginosa* loaded agar beads induce several of the main characteristics of the chronic lung infection observed in CF patients including lung histopathology and elevation in lung neutrophils and increased cytokines (22). In particular, rodents, when inoculated with *P. aeruginosa* loaded beads, highly reproduce human pathology by developing a diffuse bronchopneumonia type of lung histopathology (*Figure 2C*). This is accompanied by a higher production of proinflammatory cytokines and a more significant weight loss compared to animals receiving free-living bacteria with equal bacterial titers (54). Although the precise reasons for this remains unknown, extensive neutrophil influx in response to the *P. aeruginosa*-loaded beads has been noted and is proposed to cause severe airway obstruction and limited gas exchange (22), especially in mice (54). Additionally, few studies have shown that mixing free-living bacteria with sterile beads produces similar pathological changes as observed with bacteria-loaded beads (54,77) (*Figure 1*). However, *P. aeruginosa*-loaded agarose beads better resemble the chronic lung infection observed in CF patients with regards to histopathological features, elevation in lung neutrophils and the accumulation of cytokines in epithelial lining fluid (54).

Unmet pre-clinical need in pneumonia animal modeling

A better control on etiology and administered dose

Of utmost importance in developing animal models of infection is to accurately estimate the bacterial dose given to each animal and to keep this dose consistent between different independent experiments. Consistent culturing methods along with predetermined standards for optical density measurements that correlate with colony forming unit (CFU) counts is, in that respect, one of the most accurate methods that does not require costly equipment such as flow cytometers. Nonetheless, it is recommended that, for every inoculum used for an experiment, the actual bacterial load be validated using quantitative culture followed by (CFU) counts (54).

Besides a good control on administered dose, the choice of a particular strain can have profound effects on the phenotype of the pneumonia model. For example, naturally occurring *P. aeruginosa* isolates can lack ExoU expression, an important toxin secreted by the type 3 secretion system (T3SS). These isolates induce a reduced pneumonia phenotype in animal models (78,79). Furthermore, strains isolated from CF patients have reduced T3SS expression (80) while biofilm formation in these strains

is enhanced resulting in a mucoid phenotype due to high alginate expression (81). Therefore, mucoid strains like PAO1 should be preferred for developing CF models of chronic pneumonia. However, a precise bacterial count for highly mucoid strains is more difficult and leads to high inter-experiment variance.

An important issue that also needs to be addressed is the fact that different bacterial strains are host-specific (82) and only express certain virulence factors depending on the particular host niche (83). In that respect, clinical isolates might not be ideally suited to establish pneumonia in a rodent model, but, on the other hand, rodent adapted bacterial strains might lack expression of specific key virulence factors and thus provoke an altered immune response (84,85). To circumvent this limitation, studies have frequently resorted to supra-physiological high dose inoculation in animals, using, for example, initial doses ranging from 1E7-1E8 CFU in rats (86-88). This, however, does not resemble the common pathophysiological mechanisms observed in human pneumonia where colonization and micro-aspiration are the main initial events leading to infection. However, to study specific aspects of the disease, i.e., the effect of different pathogenic strains or antibiotic testing, high dose inoculation remains the most used method to create an experimental animal pneumonia model (6).

Disease endpoints for translational studies

In vivo non-invasive disease monitoring

In order to accurately follow-up animal disease progression, detailed non-invasive *in vivo* monitoring is required. One approach is to simply observe the animals for signs of pain, discomfort or distress (*Figure 2D*), as has been previously utilized (89). In our experience and also of others (89), pneumonia progression can be categorized into 3 distinct stages each containing signs resembling a specific stage during acute lung inflammation/infection. Stage 1 presents signs of early infection/inflammation in the lung, stage 2 presents distinct signs of severe pneumonia and stage 3 presents signs of severely compromised lung functions, as seen in HAP patients when infection overwhelms the host immune system and patients tend to succumb to the disease. The specific signs from stage 2 or stage 3 could be weighted more than those from stage 1 in order to create a clinical scoring scheme.

Recently, unrestrained whole body lung plethysmography has been shown to be able to monitor progressive pneumonia robustly utilizing tidal volume, breathing frequencies, and enhanced pause (Penh) as measures of lung function (*Figure 2E*) (90,91). While tidal volume and breathing frequencies are being commonly utilized, Penh can only be used in unrestrained plethysmography if the gas in the plethysmograph is pre-conditioned to body temperature and humidity, however, this lung function parameter as a measurement for airway restriction is much debated (92,93).

Other methods for non-invasive disease monitoring include the use of genetically engineered bioluminescent bacteria (94,95). The *Photorhabdus luminescence* lux operon is one of the best-studied operons to be utilized as a marker (96-98). Luminescent detection, however, requires sufficient signal emission and therefore highly virulent rodent strains that need to be used in lower doses might not be suited for this model as well as larger animal species that do not allow enough transmitted light to be detected by the camera (95).

Bacterial enumeration

As explained above, accurate bacterial enumeration to estimate bacterial load is of utmost importance in animal infection modeling. This becomes especially important when testing antibacterial compounds in animal models. Quantitative lung bacteriology can be performed on BAL fluid, or on homogenized lung tissue, collected post-euthanasia. Additionally, detection of nucleic acids by PCR can also be used to estimate bacterial lung burden (53), however, these assays will also quantify dead bacteria that might not be directly involved in the infectious process.

Lung histopathology

Besides lung bacteriology, histology is one of the key primary endpoints post- euthanasia (*Figure 2C*). Different lung pathology scoring schemes have been used previously (98), however, the American Thoracic Society has developed a dedicated lung pathology-scoring scheme for animals (99). Next to standard lung histopathology on H&E stained sections, immunohistochemistry for main cell types of the innate immune system including macrophages and neutrophils can aid in grading pneumonia severity.

Inflammatory parameters

Serum samples taken serially from tail vein can be used to monitor the systemic response against developing pneumonia. Acute phase proteins including CRP, serum amyloid protein A, haptoglobin and α2 macroglobulin (100) as well as proinflammatory cytokines including TNFα, IFNγ, IL-1α, IL-1β and IL-6 can be measured in rodents

by antibody based detection methods. While ELISAs are being commonly employed, availability of multiplexing platforms such as Luminex and Mesoscale, allowing simultaneous measurement of different proteins from single sample analysis, are highly beneficial when sample amounts are limited. Post-euthanasia, inflammatory profiles can also be established using lung transcript analysis, however, proper handling of tissue prior to RNA extraction is vital. Also other considerations when targeting lung transcript studies have to be taken in account. For instance, when BAL is being performed, the major proportion of alveolar macrophages as well as neutrophils is washed out from airways that can have a drastic effect on total lung transcript readouts. Also, improper washing of tissue can result in mixed lung/blood causing more difficulty in interpreting results. In our experience, snap-freezing lung tissue in liquid nitrogen allows for both downstream protein and RNA studies and gives more flexibility compared to using RNA protection media.

Humanized models

Although humans and rodents belong to the monophyletic group of *Mammalia*, considerable differences exist between rodents and humans including their immune system. For instance, mice have fewer circulating neutrophils compared to humans (101), have different Toll-like receptor expression patterns in specific cell subsets (102), and respond differently towards specific chemotactic molecules (102). In addition, IL-10 is believed to have a predominant Th2 anti-inflammatory function in rodents, while in humans, both Th1 (proinflammatory) and Th2 cells can secrete IL-10 and serves as an immunomodulatory cytokine (103). Moreover, activation and proliferation of Th17 cells, a T-cell subset important in defense against Gram-negative bacterial pneumonia (104,105), is predominantly induced by IL-1β, IL-6, and IL-23 in humans compared to IL-6 and TGFβ that are the main drivers of Th17 differentiation in rodents (106). Even within the same species, notable differences occur that can influence experimental outcomes. For example, BALB/c mice are classified as a Th2 responder strain compared to C3H/HeN mice that are Th1 responders (26); and establishment of infection using the same bacterial dose led to higher mortality in the Th2 responding strain (26).

In the last decade, these inherent differences between rodents and humans have most likely contributed to the high number of failures observed in human clinical trials (107-109). For example, one meta-analysis study has shown that in the period 2008–2010, 51% of phase II clinical trials failed due to insufficient efficacy of the compound in human pathology (109). In this context, humanized mouse models that have a closer resemblance to the human immune system could offer great benefits in pre-clinical research by lessening type 1 and type 2 errors made by a wrongful extrapolation of results obtained from common animal models. Humanized mouse models have already been extensively employed in research of cancer and human-specific viruses, such as HIV and herpes (110-112), however, their use in bacterial pneumonia models is not well established. A humanized bacterial pneumonia model could offer significant advantages over wild type animals. A recent study using NSG (NOD *scid* gamma) background mouse strain grafted with human CD34 cells showed increased susceptibility towards clinical isolate USA300 *S. aureus* strain compared to wild type animals (113). Furthermore, this study identified specific bacterial toxins that were more effective in the humanized mouse strain (113). This indicates that specific host-pathogen interactions drive pneumonia development and illustrates the importance of humanized animal models to study HAP. Additionally, an adult, immunocompetent humanized HAP animal model that mimics "human" host cytokine response would generate data and mechanisms with more translational value in directly investigating more clinically relevant infectious processes of human pneumonia (114). Besides these potential major advantages of utilizing a humanized immune system to study bacterial pneumonia, the commonly used wild-type animal models described above are currently easier to create and allow for more flexibility in the experimental setup and will therefore remain an important tool to tackle the problems surrounding hospital-acquired pneumonia.

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Footnote

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