Modelling the in-host dynamics of *Neisseria gonorrhoeae* infection

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Abstract

The bacterial species *Neisseria gonorrhoeae* (NG) has evolved to replicate effectively and exclusively in human epithelia, with its survival dependent on complex interactions between bacteria, host cells and antimicrobial agents. A better understanding of these interactions is needed to inform development of new approaches to gonorrhoea treatment and prevention but empirical studies have proven difficult, suggesting a role for mathematical modelling. Here we describe an in-host model of progression of untreated male symptomatic urethral infection, including NG growth and interactions with epithelial cells and neutrophils, informed by *in vivo* and *in vitro* studies. The model reproduces key observations on bacterial

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load and clearance and we use multivariate sensitivity analysis to refine plausible ranges for model parameters. Model variants are also shown to describe mouse infection dynamics with altered parameter ranges that correspond to observed differences between human and mouse infection. Our results highlight the importance of NG internalisation, particularly within neutrophils, in sustaining infection in the human model, with \(~80\%\) of the total NG population internalised from day 25 on. This new mechanistic model of in-host NG infection dynamics should also provide a platform for future studies relating to antimicrobial treatment and resistance and infection at other anatomical sites.

**Introduction**

Gonorrhoea is a sexually transmitted infection caused by the bacterial species *Neisseria gonorrhoeae* (NG). The incidence of gonorrhoea is increasing worldwide (CDC 2017; Kirby Institute 2017), and the World Health Organization estimated that in 2012, 78 million cases of infection occurred worldwide (Newman, et al. 2015). The male urethra and the lower female genital tract are the predominant sites of infection with NG (Edwards 2008; Miller 2006), which can result in serious sequelae including neonatal blindness, epididymitis, pelvic inflammatory disease, infertility and ectopic pregnancy (Holmes, et al. 2008; Stevens and Criss 2018; Unemo and Shafer 2014). Infection also commonly occurs at the pharynx and rectum through a diverse range of sexual practises (Edwards and Apicella 2004). With the emergence of multi-drug resistant NG strains being reported in several countries, including strains that exhibit high-level resistance to all extended-spectrum cephalosporins, our last remaining proven options for gonorrhoea monotherapy, there is increasing concern that NG may become untreatable in the near future (Eyre, et al. 2018; Golparian, et al. 2018; Poncin, et al. 2018; Regan, et al. 2018; Whiley, et al. 2018).

NG is highly adapted to establish infection and survive within the human host. In order to establish infection, NG must attach to mucosal epithelial cells. This process is
facilitated by surface components, including pili and opacity associated proteins (Opa). Once attached, NG can be internalized within epithelial cells (Apicella, et al. 1996) where they can replicate (Criss and Seifert 2006; Shaw and Falkow 1988), evade the immune system, delay apoptosis of epithelial cells and infect cells deeper within the epithelium (Binnicker, et al. 2003; McGee, et al. 1983; Mosleh, et al. 1997). The innate immune system is triggered in response to NG infection by the elevation of pro-inflammatory cytokines and chemokines, which lead to the recruitment of polymorphonuclear leukocytes (PMN, or simply neutrophils) to the infection site. Despite rapid recruitment of PMN, NG are able to resist killing by PMN (Criss and Seifert 2012; Simons, et al. 2005; Simons, et al. 2006) to the extent that viable NG are commonly found within PMN in exudates examined from natural human infections (Casey, et al. 1980; Veale, et al. 1979). Furthermore, NG has evolved to avoid and suppress the adaptive immune response (Edwards, et al. 2016; Liu, et al. 2014; Liu and Russell 2011). Although IgG, IgM and IgA antibodies have been found in human mucosa in response to NG infection (Ison, et al. 1986), these antibody response levels are relatively weak and short lived (Holmes, et al. 2008). As such, the adaptive immune response against infection with NG is considered to be, at best, only weakly effective and reinfection is common (Schmidt, et al. 2001; Stupiansky, et al. 2011).

Experimental NG infection models in humans have been limited to males, as infection in females can result in serious reproductive complications including pelvic inflammatory disease and infertility. Even in the case of experiments conducted in men, prolonged infection without provision of treatment is considered unethical and therefore treatment is provided when symptoms appear, typically 5-7 days post infection (Hobbs, et al. 2011; Schmidt, et al. 2001; Schneider, et al. 1995). These limitations have hampered our ability to study the natural course of infection in vivo and our current understanding is largely derived from in vitro studies and animal models of infection.
In particular, mouse models have been used to understand within-host dynamics to a certain extent (Francis, et al. 2018; Jerse 1999; Li, et al. 2011; Packiam, et al. 2010). These are typically models of vaginal infection in female mice, which require hormonal treatment with 17β-estradiol to allow prolonged infection (Jerse, et al. 2011; Rice, et al. 2017), and most closely resemble human asymptomatic vaginal infection (Francis, et al. 2018). However, there are limitations to the mouse model, due to the specificity of several gonococcal proteins for human specific targets, including receptors required for adherence and invasion of epithelial cells, as well as iron sources required by NG for survival (Jerse, et al. 2011; Rice, et al. 2017).

Although mathematical models have been developed to describe transmission of NG infection at a population level (for example, Chan, et al. 2012; Fingerhuth, et al. 2016; Hui, et al. 2015; Hui, et al. 2013), there has been very little focus on developing models capturing the course of NG infection at a within-host level. Such within-host models have been developed for other pathogens, describing the interaction between pathogen, host cells, and host immune response (for example, Colijn and Cohen 2015; Nowak and Bangham 1996; Smith, et al. 2011; Wilson, et al. 2003). However, the infection processes, immune responses and mechanisms describing the acquisition of resistance related to infection described by these models differ from those that are essential for NG infection. To the best of our knowledge, the only published within-host model of NG infection is Mao and Lu (2016). In that study, horizontal gene transfer by natural transformation is modelled by considering the interaction of NG with a genomic pool without specific consideration of interaction with host cells. Although not specifically a within-host model, the theoretical NG vaccine study by Craig, et al. (2015) described infectiousness of individuals using a parametric function for the within-host NG load.
An improved understanding of the within-host dynamics of NG infection offers the potential to gain insights about the immune response, development of antibiotic resistance and potential mechanisms for vaccine action. However, the existing in-vivo or in-vitro studies are unable to fully capture the long-term infection dynamics of NG infection. To address this, we have developed a model that captures the natural course of untreated symptomatic urethral NG infection in men, which will assist in understanding the within-host factors that govern the ability of NG infection to persist and the ability of the immune system to clear infection. In concert with multivariate sensitivity analysis we have also sought to constrain plausible ranges for relevant biological parameters. In addition, due to the absence of time-course data on untreated human NG infection, we attempted to validate the modelling approach by fitting the model to time course data from a mouse model of NG infection described by Jerse (1999).

**Materials and Methods**

**Model structure and formulation to describe human urethral NG infection**

A compartmental mathematical model was developed to capture the time course of urethral NG infection in men by considering the interaction between bacteria (NG), epithelial cells, and the PMN subset of the innate immune response. The model has five compartments (or states). Four of the compartments describe interactions between NG and the host: NG unattached ($B$) or attached ($B_a$) to epithelial cells, NG internalized within epithelial cells ($B_i$) and NG surviving within PMN ($B_s$). The fifth compartment represents the activated PMN cells ($N$). Transitions between the five compartments are illustrated schematically in Fig. 1. An overview of the modelling approach follows with a complete and detailed description provided in the Supplementary File.

The model is formulated as a system of ordinary differential equations as follows:
The model initial conditions are given in Table 1 and the model parameters, including transition rates are provided in Table 2. Key study sources that inform their assigned values are described in more detail in Table 3.

\[
\frac{dB}{dt} = \left(1 - \frac{B + B_a}{k_1}\right)\left(\tau_1 B + d_3 B_s + \eta B_i\right) - d \frac{B N}{c N + B} - d_2 B - a_4 B \left(1 - \frac{B_a}{k_1 a_2}\right)
\]

\[
\frac{dB_a}{dt} = \tau_1 B_a \left(1 - \frac{B + B_a}{k_1}\right) + a_1 B \left(1 - \frac{B_a}{k_1 a_2}\right) - d \frac{B_a N}{c N + B_a} - \eta B_a
\]

\[
\frac{dB_i}{dt} = \left(1 - \frac{B_i}{k_1 a_2}\right)\left(\eta B_a + r_2 B_i\right) - \eta B_i
\]

\[
\frac{dB_s}{dt} = \left(1 - \frac{B_s}{N k_2}\right)\left(p d \frac{B N}{c N + B} + p d \frac{B_a N}{c N + B_a} + r_3 B_s\right) - d_3 B_s
\]

\[
\frac{dN}{dt} = \mu (N_{max} - N) (B + B_a) - d_3 N
\]
**Bacteria**

Growth of the NG population occurs through bacterial replication (Apicella, et al. 1996; Criss and Seifert 2006; Shaw and Falkow 1988; Simons, et al. 2005) at the rates $r_1$ for both unattached NG ($B$) and NG attached to epithelial cells ($B_a$), $r_2$ for NG internalized in epithelial cells ($B_i$) and $r_3$ for NG surviving in PMN ($B_s$). NG growth in unattached and attached NG states was bounded by a maximal urethral infection capacity ($k_1$) (based on the approximate surface area of the urethra and the cross-sectional area of NG). In addition, as several NG can attach to the surface of a single epithelial cell (Gubish, et al. 1979; Heckels, et al. 1976), the rate of attachment to epithelial cells was limited by the maximal attachment capacity ($k_1 a_2$), where $a_2$ was the maximal NG attachment capacity per epithelial cell. NG surviving within PMN can delay PMN apoptosis to provide NG with time for replication within PMN (Simons, et al. 2006). In the study by Simons, et al. (2005) it was observed that PMN with delayed apoptosis had less than 10 associated NG per PMN and based on this we limited the maximum number of NG that can survive within PMN to delay apoptosis ($k_2$).

Engulfment of non-internalised NG (unattached NG and NG attached to epithelial cells) by PMN (at rate $d$) was modelled in a manner corresponding to a ratio-dependent predator-prey interaction (Arditi and Ginzburg 1989; Getz 1984). When the number of NG is small relative to the number of PMN, the engulfment rate per NG approaches a maximum constant level $\frac{d}{c}$, while when the NG population is large, the engulfment rate of bacteria is directly proportional to the number of PMN. The ratio dependent constant $c$ reflects the reduction in NG engulfment by PMN as the NG population decreases (Getz 1984; Getz 1998). NG in the internalised ($B_i$) state were considered to be inaccessible to killing by PMN.

Unattached NG were assumed to be washed away (e.g., by passive efflux from the urethra and through urination) at the rate $d_2$ (Burgess 1971; Pelouze 1939; Schneider, et al.)
NG in the internalized state ($B_i$) exit from epithelial cells at rate $e$ (Criss and Seifert 2006; Mosleh, et al. 1997) and are then available again to further infect epithelial cells. NG that survive within PMN ($B_s$) were assumed to exit that state at the same rate as for PMN death ($d_3$). However, the number of NG that can exit from the $B_i$ and $B_s$ states and move onto the unattached state is constrained through the urethral carrying capacity term ($k_1$). NG that could not move into the unattached state due to this capacity restriction were assumed to be washed away.

When referring to the NG load we use the term “bacteria” throughout, which refers to modelled numbers of bacteria and also to empirical data reported as number of colony-forming units (CFU).

**Immune response: PMN**

The total number of PMN ($N_{max}$) was assumed to remain constant over time, with the immune response assumed to be triggered by their activation. Inactivated PMN were assumed to be activated at a rate $\mu$ multiplied by the number of non-internalized NG. During infection, engulfed NG have been observed to prolong the lifespan of PMN and, based on Simons, et al. (2006), it was assumed that PMN were apoptotic at 24 hours ($d_3$).

In studies of infection in humans, cytokines were observed to be elevated two hours following inoculation (Ramsey, et al. 1995; Ramsey, et al. 1994), and the PMN response was therefore assumed to be initiated early in the infection. However, it was not established whether this early cytokine response in experimental models was a result of the inoculation procedure itself or occurred in response to gonococcal infection (Ramsey, et al. 1995) as several studies had indicated a 2-3 day delay in PMN response (Cohen, et al. 1994; Criss and Seifert 2012; Seifert, et al. 1994). We focus on the model without a PMN delay term in our
main analysis but present results including a delay in Supplementary Fig. S8 in the Supplementary File Section 3.

**Model parameters**

Initial conditions for model states are listed in Table 1, while the parameters used in this study and their assigned values are listed in Table 2. Where possible, parameter values were based on estimates found in the published literature. Where parameter values could not be informed directly by the literature, they were estimated by fitting simplified versions of the model (sub-models) to relevant *in vitro* data (Further details provided in Supplementary File, Section 1.2). These sub-models reflect the fact that the *in vitro* studies used for parameter estimation do not consider NG interactions with both epithelial cells and PMN simultaneously and hence, some model states and interaction terms were set to 0 as part of these parameter estimation exercises. There is a lack of published empirical data to inform the values for the two parameters, wash out rate of unattached NG ($d_2$) and exit rate of internalized NG ($e$). For $d_2$, we assigned a value around the median of the retained samples in the multivariate sensitivity analysis (described below), and for $e$ a value near the mode, to ensure point estimate values that are consistent with an infection duration of 75 days. Finally, the bacterial engulfment rate ($d$), proportion of NG surviving within PMN ($p$), ratio dependent constant ($c$), the replication rate of non-internalised NG ($r_1$) and the PMN activation rate ($\mu$) were estimated by fitting the model to simulated data sets that were generated based on the known qualitative features of the infection. These qualitative features used to generate data are described below in the section ‘*Qualitative features of the time-course of infection*’.
Parameters derived from published literature

The replication rate of NG surviving within PMN ($r_3$) was based on the intracellular replication of NG within PMN that was measured in the \textit{in vitro} study by Simons, et al. (2005) over a time period of 5 hours. The urethral carrying capacity ($k_1$) was estimated based on the approximate surface area of the urethra and the cross-sectional area of NG (NG has a diameter of 0.5 - 1 µm (Herz, et al. 1996; Westling-Haggstrom, et al. 1977); the length of the entire male urethra is around 20 cm (Moore 2006) with a diameter of 8-9 mm (Talati 1989)). The total number of PMN in the body ($N_{\text{max}}$) was based on estimates of the normal range of PMN in the body (2.5-7.5 x 10$^9$/L) from the study by von Vietinghoff and Ley (2008), and an average blood volume in adults of approximately 5L (Wei, et al. 1995).

Parameter estimation through model fitting

In this section, we summarise parameter estimation via fitting of sub-models to data from \textit{in vitro} studies using the MATLAB (MathWorks, Natick, MA) nonlinear least squares solver 'lsqcurvefit'. Further details of the study data and sub-models are provided in Supplementary File Section 1.2 and summarised in Fig. 2.

The \textit{in vitro} data used to estimate these parameters are summarised in Table 3. A function of the form $a_2(1 - e^{-a_1t})$ was fitted to data in Gubish, et al. (1979) to estimate the bacterial attachment rate ($a_1$) and maximal NG attachment capacity of an epithelial cell ($a_2$). In order to estimate the replication rate of internalized NG ($r_2$) and the rate of internalization ($\eta$), the sub-model described in the Supplementary File Section 1.2.2 was fitted to data from Shaw and Falkow (1988). Data in the \textit{in vitro} study by Rest, et al. (1982) were used to obtain estimates of the bacteria engulfment rate ($d$), the proportion of NG surviving within PMN ($p$) and the ratio dependent constant ($c$) by fitting the sub-model described in the Supplementary File Section 1.2.3. Credible intervals around both sets of \textit{in vitro} estimates were developed by
varying the fixed parameters within specified ranges. In the case of $r_2$ and $\eta$, this involved varying the urethral carrying capacity ($k_1$) and maximal NG attachment capacity of an epithelial cell ($a_2$) (details in the Supplementary File Section 2.2.1). For $d$, $c$ and $p$ this involved varying $r_1$, $r_3$ and $k_2$ (described in the Supplementary File Section 2.2.2).

Model fitting to estimate bacterial engulfment rate ($d$), proportion of NG surviving within PMN ($p$), ratio dependent constant ($c$), PMN activation rate ($\mu$) and replication rate of non-internalized bacteria ($r_1$).

When the values for $d$, $p$ and $c$ obtained from least squares minimization by fitting sub-models to the data in the study by Rest, et al. (1982) were used as parameters in the full model, the duration of untreated infection obtained was well below the desired range (Fig. 5(b)). We investigated whether adjustment of epithelial internalisation parameters might resolve this issue but the results did not match other observations (see Supplementary File Section 2.3). Additional sensitivity analyses around in vitro estimates are described in Section 2.2.2 of the Supplementary File but did not support use of the in vitro estimates in the full model.

As we lacked other experimental evidence on which to base these parameters and to obtain a point estimate for the parameters $\mu$ and $r_1$, we fitted the total NG load ($B + B_a + B_t + B_s$) obtained from our human infection model to 1000 simulated data sets, consisting of total bacterial load values at 5 time points. These were generated based on the qualitative features of the time-course of infection described in the next paragraph, while the data generation and fitting procedure is described in detail in the Supplementary File Section 1.2.4. The median of the 1000 estimates of each parameter was used as the respective point estimate of $d$, $c$, $p$, $\mu$ and $r_1$. 

Qualitative features of the time-course of infection

Human experimental studies suggested a peak NG load of $10^6 - 10^8$ bacteria reached at around 2-5 days into infection (Ramsey, et al. 1995; Schmidt, et al. 2001; Schneider, et al. 1995; Schneider, et al. 1991; Schneider, et al. 1996). In these studies, it was observed that the bacterial load reached a plateau level of above $10^6$ bacteria from around day 1-2 to the initiation of treatment usually at around days 5-7. Based on pre-antibiotic era empirical studies (Hill 1943; Pelouze 1939) and theoretical estimates (Johnson, et al. 2010; Korenromp, et al. 2002), the expected duration of untreated male symptomatic infection is considered to be in the range of 1-6 months. The infection is considered to be cleared once the NG load falls below 10 bacteria (Schneider, et al. 1995).

Initial conditions

The number of unattached NG at time 0 ($B(0) = 1000$) was taken from the study by Schmidt, et al. (2001) and Schneider, et al. (1996). We assumed that initially there were no attached NG ($B_a(0) = 0$) or internalised NG ($B_i(0) = 0$). The latter assumption is supported by Shaw and Falkow (1988), where internalized NG were not observed until > 6 hours after the start of the experiment. We also assumed no initial NG internalised in PMN ($B_\gamma(0) = 0$), supported by the cytokine response being elevated at >2 hours after inoculation (Criss and Seifert 2012; Ramsey, et al. 1995). The ratio-dependent term in our equations does not allow solution when the activated PMN value is exactly 0 and hence we assumed a small positive initial PMN value ($N(0) = 10^{-8}$ cells).

Multivariate sensitivity analysis

We conducted a multivariate sensitivity analysis of the full human model in order to capture uncertainty around model outcomes and to refine plausible ranges for model...
parameters. All parameters were included except the urethral carrying capacity \( k_1 \) and the total PMN count \( N_{\text{max}} \) as these parameters define more global constraints on model behaviour and interact strongly with the NG growth and survival parameters. For most parameters a factor of 2 above and below the point estimate (4-fold range) was used (shown in Table 2). Using Latin Hypercube Sampling (LHS) (Blower and Dowlatabadi 1994), 100,000 parameter samples were generated assuming uniform distributions for all parameters within the defined ranges. The software package SaSAT was used to generate the LHS samples and carry out the multivariate sensitivity analysis (Hoare, et al. 2008). When the model was run using these parameter sets, only those samples that met the desired broad criteria around the peak NG load, peak time and the infection duration (described below) were retained for the subsequent analysis. These retained parameter sets were then analysed for pairwise correlations, with correlations between the parameters \( d \) and \( c \) used to inform a revised LHS analysis. The parameter ranges associated with this revised analysis are reported in Table 2. More details on the multivariate sensitivity analysis are provided on the Supplementary File Section 2.1.

**Outcome criteria:** We retained only the parameter sets which were consistent with peak NG load of \( 10^6 - 10^8 \) bacteria occurring 1-7 days after infection (Ramsey, et al. 1995; Schmidt, et al. 2001; Schneider, et al. 1995; Schneider, et al. 1991; Schneider, et al. 1996), with clearance of infection (<10 NG, as in Schneider, et al. 1995) between 1 and 6 months (Based on pre-antibiotic era empirical studies (Hill 1943; Pelouze 1939) and theoretical estimates (Johnson, et al. 2010; Korenromp, et al. 2002)). We also conducted a sub-analysis of the samples that met the above criteria and cleared infection in the more restricted window of 2-6 months.
**Fitting to mouse model data**

As an exercise in validation of the model structure and qualitative behaviour, and due to the unavailability of human data on prolonged untreated NG infection, we fitted our model to time course data from a mouse model of NG infection. The main purpose of this fitting exercise was to assess whether the model could describe the quantitative time course data in a related animal model. In addition, we were interested in what features of the human model were required to describe mouse infection and to obtain a comparison of parameters between the two hosts.

NG load data on eight mice were obtained from the study by Jerse (1999) where mice were treated with estradiol to facilitate prolonged infection. As the effects of estradiol declined after around 9 days, we included only the first 9 days of NG data for each mouse in this fitting exercise. The total NG load \((B + B_a + B_i + B_s)\) was fitted to the data for the full model described above and then model fitting was repeated for 3 progressively simpler models where first the epithelial internalisation state \((B_i)\) was removed, then both attachment \((B_a)\) and epithelial internalisation states were removed, and finally a model where in addition neutrophil internalisation \((B_s)\) was removed. The parameters that were estimated and kept fixed at each stage of the fitting are summarised in Table S3 in the Supplementary File Section 5.3.

As there were only 9 NG data points per mouse, we had to limit the number of parameters estimated in the fitting process. We kept several parameters \((a_1, a_2, d_3, d_2, e, r_2\) and \(k_2\)) at the same values as assigned for the human model point estimates (Table 2) as discussed in the Supplementary File Section 5.3. The capacity related parameters \(k_1\) and \(N_{max}\) were adjusted to take into account the smaller relevant cell counts for mice. Hence, for mice, \(N_{max}\) was taken as \(8.32 \times 10^6\) cells and the carrying capacity \((k_1)\) was taken as \(3 \times 10^6\) bacteria.
This left us with the need to estimate the parameters \( d, c, \mu, p, \eta, r_3 \) and \( r_1 \) through the fitting process. After experimenting with individual model fits (see Supplementary File, Section 5.2 for details), we were able to fix the values of \( p \) and \( \eta \) at \( 5.4 \times 10^{-5} \) and \( 5 \times 10^{-6} \) hour\(^{-1}\), respectively, across all mice. Finally, the five parameters that govern the growth \( (r_3 \) and \( r_1 \)) and decline \( (d, c, \mu) \) of the NG load were estimated per mouse using least squares optimisation (in the model where neutrophil internalisation was removed, \( r_3 \) is not relevant). For the optimisation procedure, the MATLAB (MathWorks, Natick, MA) function ‘fmincon’ was used with the potential for multiple local minima investigated through the use of the ‘multistart’ function by using 1000 different initial starting points for the parameter values for each mouse. Initial conditions for unattached bacteria and PMN were based on the first data point for each mouse.

Results

Parameter estimation for the human model

The parameter estimates obtained by fitting the sub-models to respective in vitro data are presented in this section and summarized in Table 2. The credible intervals around estimates of respective parameters derived from the in vitro sensitivity analyses described in Supplementary File, Section 2.2 are shown in the shaded region in Fig. 3(b) and 3(c). Fits to data on attachment of piliated and non piliated NG to HeLa cells (Gubish, et al. 1979), are shown in Fig. 3(a) and were used to estimate the attachment rate \( (a_1) \) and the maximal NG attachment capacity of an epithelial cell \( (a_2) \). Estimates of the replication rate of internalized NG within epithelial cells \( (r_2) \) and rate of internalization \( (\eta) \) are shown in Fig. 3(b) (estimated values and credible intervals around in vitro estimates are presented in Supplementary Table S1). Fig. 3(c) shows the best fit to in vitro data in the study by Rest, et al. (1982) compared to
the curve based on estimates obtained by fitting to the simulated data based on the qualitative features of the untreated human infection. A comparison of estimates of $d$, $p$ and $c$ from the optimal fits to in vitro data as opposed to the simulated data informed by qualitative features, is provided in Supplementary Table S2 along with the 95% credible intervals around the in vitro estimates.

**Human infection model results based on point estimates.**

**Time course of urethral infection in symptomatic men**

The simulated cell populations for the first 40 days of infection are shown in Fig. 4(a) and for the full time-course of infection in Fig. 5(a). The total NG load, consisting of NG in all four states ($B$, $B_a$, $B_s$ and $B_i$), reached a peak of $4.27 \times 10^6$ bacteria by 3.6 days. The PMN response followed a qualitatively similar pattern to the NG load. Peaks in PMN and NG curves were reached around the same time point with the peak PMN cell count reaching $2.8 \times 10^5$ cells. The NG load remained above $10^6$ bacteria from days 2.3 to 7.5, after which it declined due to PMN killing. By 75 days, the NG load declined to <10 bacteria, which was our condition for clearance (Fig. 5(a)).

We also analysed the proportion of NG in the various states of attachment and internalisation over time (Fig. 4(b) and (c)). The initial NG load consisted of only unattached bacteria. However, two hours into infection, the modelled NG population had started to colonise the host and become attached to or internalized within epithelial cells (Fig. 4(c)). The two intracellular NG populations (NG surviving within PMN ($B_s$) and NG internalized within epithelial cells ($B_i$)) showed similar dynamics. The NG populations occupying these two states increased with continued entry and replication and showed a small decline as NG exited from these compartments. In the later stages of infection, the non-internalized population (NG attached ($B_a$) and unattached ($B$) to epithelial cells) reached a stable value of...
~20% of the total NG population. In the long term, NG that survived within PMN and NG internalized within epithelial cells comprised 56% and 24% of the total NG population, respectively (Fig. 4(b)).

**Multivariate sensitivity analysis**

**Human infection model results based on simulations from LHS samples**

The 95% credible intervals of model parameters, derived from model simulations that met the outcome criteria, are given in Table 2. The point-estimates for $d, p, c, \mu$ and $r_1$ were broadly consistent with the refined ranges derived from the multivariate sensitivity analysis as shown in Table 2.

The corresponding 95% range for total NG load obtained is shown in Fig. 5(b), while the distributions of these simulations in terms of the peak time, peak load, and infection duration are shown in Fig. S4 (in the Supplementary File, Section 2.1.2) and the distribution of proportions of NG by state is shown in Fig. S6 (in the Supplementary File, Section 2.1.2).

Partial rank correlation coefficients (PRCC) were calculated to assess the relative contribution of each parameter’s associated uncertainty to variability in the model outcomes. This analysis identified PMN activation rate ($\mu$), replication rate of non-internalized NG ($r_1$) and internalisation rate into epithelial cells ($\eta$) as important contributors to variability in the peak time and the peak load. Parameters associated with NG killing (ratio dependent constant ($c$) and $\mu$) as well as the capacity constraint on NG surviving within PMN ($k_2$) were identified as the most important contributors to variability in the infection duration. These results are presented in Fig. S5 in the Supplementary File, Section 2.1.2.

**Validation against mouse model data**

The results of the model fit to bacterial load data of the eight mice obtained from the study by Jerse (1999) are presented in this section. Fits of different versions of the re-
parameterised model to mouse NG load data are shown in the panels of Fig. 6. The mouse data are broadly grouped to reflect similar load patterns over the 9 days. In general, based on the sums of squared errors (SSE) shown in the Supplementary File, Table S4, the simplified models without the \( B_i \) state (internalisation within epithelial cells) or the \( B_i \) and \( B_a \) states (internalisation within and attachment to epithelial cells) fitted data in 7 of the 8 mice just as well as when using the full human infection model. However, further removal of the state where NG survive within PMN (\( B_s \)) led to poor fits, both in terms of qualitative behaviour and the SSE.

The estimated values for \( d, c, \mu, r_3 \) and \( r_1 \) for the four model variants are summarized in Table S4 and Fig. 7. In general, mouse-derived estimates of \( d, r_3 \) and \( p \) were substantially lower than those estimated for the human model (Fig. 7). However, the 95% range of the estimates of the ratio of \( d/c \) and \( r_1 \) were similar in both mouse and human models. Although the PMN activation rate (\( \mu \)) was substantially lower in the human model, when multiplied by the total PMN, the neutrophils recruited per unit time (\( \mu N \)) were consistent across human and mouse models. The high variation in the NG time course between mice is reflected in large ranges for resulting parameter estimates when summarised across the 8 mice (Fig. 7).

**Discussion**

In this study, we developed a within-host model to describe untreated symptomatic urethral NG infection in men. Assuming that the peak NG load is reached around the time period of symptom expression, the model using our default parameters produces a bacterial load and time course consistent with the known features from experimental infection (Schmidt, et al. 2001; Schneider, et al. 1995; Schneider, et al. 1996; Stupiansky, et al. 2011) and pre-antibiotic era studies of natural infection (Hill 1943; Pelouze 1939). In addition, when the model was validated using time course data on bacterial load from a study in mice
by Jerse (1999), the model was able to closely match the course of infection in seven out of eight mice.

Model simulations produced NG load > $10^6$ bacteria from days 2.3 to 7.5, with a similar plateau seen in the human experimental studies by Schneider, et al. (1995), Ramsey, et al. (1995) and Schmidt, et al. (2001), where the bacterial load remained high (NG > $10^6$ bacteria) from around day 1-2 to the initiation of treatment at day 5-7. However, in these studies, as treatment was initiated shortly after reaching the peak load, it is not possible to know the exact duration of this high NG load around the peak.

In our model, the growth phase for NG is monotonic, without an “eclipse” period as has been observed in some human experimental studies. The “eclipse” period is the time period in which few NG could be recovered in the exudate. In the experimental studies this was observed 2 - 4 hours after inoculation (Cohen, et al. 1994; Hobbs, et al. 2011; Schmidt, et al. 2001; Schneider, et al. 1995). At the end of the eclipse period, the bacterial population was seen to expand exponentially from the few NG that were present in the inoculum (Hobbs, et al. 2011). While the underlying reason for this eclipse period has not been established (Cohen, et al. 1994; Schneider, et al. 1995), it is plausible that it relates to the initial period of NG attachment and internalisation. Our aim, however, was to capture bacterial dynamics over a time-scale of days to weeks rather than hours.

To the best of our knowledge, Craig, et al. (2015) is the only previous mathematical modelling study that has described the NG load of an infected individual. In that study, the NG load was primarily used to determine the infectiousness of an individual and the potential impact of a vaccine. However, they describe the NG load through a parametric function without explicit consideration of the mechanisms of infection or the interaction of NG with host cells, in contrast to our explicit description of these processes. Comparisons between our
predicted total NG curve and that from Craig, et al. (2015) show agreement in terms of the qualitative features of the time-course of infection (see Fig. S9 in the Supplementary File, Section 4). However, in addition our model facilitates predictions related to intracellular NG growth and decline, including relative proportions over time of the bacterial population that are unattached, attached and internalised and the effectiveness of PMN in infection clearance.

Based on urethral exudates from 33 male patients, the in vivo study by Veale, et al. (1979) reported the relative extracellular, epithelial internalised and PMN internalised NG proportions as 35.1 ± 3.6%, 15.7 ± 3.1% and 49.2 ± 4.4%, respectively. Precise timing of these measurements is not reported but was likely during the incubation period 2-6 days after inoculation. By comparison, at 2-6 days post infection, our model produced relative bacterial proportions of 15% - 44% (extracellular), 19% - 36% (epithelial internalisation) and 20% - 65% (PMN internalisation). In the later stages of infection, our model indicated that the intracellular populations consisting of $B_g$ and $B_i$ comprised 80% of the total NG population, with the bacterial population within PMN ($B_e$) stabilising at 56% of the total NG population. This suggests that the model infection dynamics are mainly driven by the intracellular NG populations and these cell populations are mainly responsible for prolonging the duration of infection. This finding is consistent with observations that in addition to killing a portion of NG, PMN provide a reservoir for bacterial survival and replication that prolongs infection (Criss and Seifert 2012; Quillin and Seifert 2018).

A full multivariate sensitivity analysis was also conducted, with parameters relating to PMN availability and PMN engulfment of NG (through the ratio dependent constant) being most influential in terms of clearance time, further supporting the importance of PMNs in the infection dynamics. In addition, after filtering the parameter sets generated through the analysis according to qualitative outcome criteria around peak load, timing and clearance, the
retained parameter sets showed good agreement with our default parameters but departures from values estimated purely from in vitro data.

The absence of human bacterial load data over longer timeframes led us to attempt further validation of the model against mouse model data (Jerse (1999)). The single mouse (mouse 2) for which the model could not describe the data well showed weak correlation between NG load and measured PMN levels found in Jerse (1999) whereas the other mice showed stronger association as the bacterial load declined at high PMN levels. We note that internalisation within mouse epithelial cells and survival within mouse PMN are impaired as some of the mechanisms that NG uses for internalization and survival are explicitly human host restricted (Edwards, et al. 2016; Jerse, et al. 2011; Sadarangani, et al. 2011). In line with these observations, our estimates for the proportion of NG surviving within PMN (p) was 3-5 orders of magnitude lower than estimated for the human model. The ineffectiveness of NG internalisation and attachment to mouse epithelial cells was made evident by equivalent fits to the mouse data of simplified models that excluded attachment and epithelial internalisation. This supports the observed differences in infection durations between human and mouse infection, as in humans the intracellular compartments were vital in prolonging infection durations. However, when internalisation within PMN was also excluded it resulted in poor fits to mouse data, suggesting that although NG survival within PMN is limited in mice, it is still needed to explain the dynamics of mouse infection.

Limitations of our model design mainly arise from simplifications in the representation of the immune response. We only considered the immune response mediated by PMN and did not consider the contribution from macrophages or the possibility of an adaptive immune response. During infection with NG, the PMN response is considered to be the primary immune response that is initiated, evidenced by the fact that purulent discharge in
symptomatic infection occurs as a result of PMN influx (Edwards and Apicella 2004; Handsfield, et al. 1974). However, it is believed that macrophages also play a role by phagocytosing NG and recruiting PMN to the infection site (Chateau and Seifert 2016). Furthermore, as with PMN, NG has acquired mechanisms to replicate and survive within macrophages providing NG with an additional reservoir to facilitate prolonged infection (Chateau and Seifert 2016). Therefore, macrophages may play a role in defining infection dynamics. We also did not include immune responses mediated by the adaptive immune response as while its role in controlling and eliminating infection is not clearly understood, it is generally considered to be only weakly effective (Schmidt, et al. 2001; Stupiansky, et al. 2011). Furthermore, the characteristic antigenic and phase variations exhibited by surface proteins (e.g., Opacity associated proteins (Opa) and pili (Alcorn and Cohen 1994; Dehio, et al. 1998; Virji 2009)) were not considered in the model and these may be important in determining within-host behaviour.

Parameter estimation and model validation were limited by the paucity of data on human infection, and the limited applicability of mouse model data and in vitro studies to in vivo infection. In particular, the absence of more sophisticated in vitro experiments capturing the simultaneous interaction of NG, epithelial cells and PMN made it difficult to further constrain model parameters. This was addressed in our study to some extent through the multivariate sensitivity analysis, which provided ranges for parameter values and intervals around outcomes that might potentially be tested through future experiments. Improved data on within-host NG interactions would likely reduce parameter uncertainty and facilitate refinements to model structure and assumptions.

Conclusion

In this study, we developed a mathematical model of in-host gonorrhoea infection that broadly reproduces features of untreated symptomatic male infection as described in
experimental, pre-antibiotic and in vitro studies. Untreated NG infection dynamics are poorly understood as it is not possible to obtain experimental human infection data over prolonged time periods, and in vitro experiments involving multiple cell types (e.g., both PMN and epithelial cells) are very difficult to conduct. Our model goes some way to filling this knowledge gap by providing a means of understanding how NG interacts with and occupies host cells in the later stages of the infection and points to the importance of the intracellular compartments (NG surviving within PMN and NG internalised within epithelial cells) in determining the course of human infection. Validation of the mathematical model on human infection using mouse model data demonstrated that the model can closely replicate the course of untreated infection in a related animal model using differing parameter values that account for biological differences between the two species.

This model should provide a foundation for extensions in several directions, including the interaction of multiple NG strains and emergence of resistance under selective pressure from antibiotics. Other potential developments include extension to asymptomatic infection and infection at different anatomical sites, and consideration of potential vaccine conferred immunity such as recently reported for meningococcal vaccines (Petousis-Harris, et al. 2017; Seib 2017).

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**Conflicts of interest**

No conflicts to declare.
References


Liu Y, Russell MW. Diversion of the immune response to Neisseria gonorrhoeae from Th17 to Th1/Th2 by treatment with anti-transforming growth factor beta antibody generates immunological memory and protective immunity. MBio 2011;2: e00095-11.


Figure 1: Schematic illustration of the within-host model of NG infection. The arrows indicate transitions between model states: unattached NG (B), attached NG (B_a), bacteria internalized within epithelial cells (B_i), NG surviving within PMN (B_s) and activated PMN (N). Model parameters and their assigned values are given in Table 2 (d_c refers to the engulfment rate of NG by PMN subject to the ratio dependent constant. When N is relatively low, d_c \rightarrow d while when N is relatively high d_c \rightarrow \frac{d}{c}).
Data: NG that were internalised over 6-12 hours in the *in vitro* study Shaw and Falkow (1988).

**States omitted during fitting**: Unattached NG, NG within PMN and PMN response.

Sub model: \[
\frac{dn}{dt} = -\eta n R_n, \quad \frac{dn}{dt} = (\eta n R_n + r_2 B_1) \left(1 - \frac{R_n}{k_n a_2}\right)
\]

Data: NG attachment to epithelial cells over 4 hours measured in the *in vitro* study Gubish, et al. (1979)

Fitted function: \[a_2(1 - e^{-a_1 t})\]

Data: PMN phagocytosis of NG over 135 minutes measured in the *in vitro* study Rest, et al. (1982).

**States omitted during fitting**: NG internalised within epithelial cells, attached NG. Assumed constant PMN level.

Sub model: \[
\frac{dn}{dt} = r_1 B - \frac{N d N}{c N + d}, \quad \frac{dn}{dt} = \left(p d - \frac{N d N}{c N + d} + r_3 B_1\right) \left(1 - \frac{R_n}{k_n a_2}\right)
\]

**Parameter estimates**: \(r_2\) and \(\eta\)

**Parameter estimates of** \(a_1\) and \(a_2\)

**Parameter estimates of** \(d, c\) and \(p\) did not meet qualitative features

**Data**: 1000 data sets generated based on known qualitative features of the time-course of infection.

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>~ Uni (1, 2)</th>
<th>~ Uni (2,5)</th>
<th>~ Uni (5,7)</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG load</td>
<td>1000 (fixed)</td>
<td>(Y_1) ~ Uni (10^6, 5 \times 10^6)</td>
<td>~ Uni (Y_2, 2 \times 10^7)</td>
<td>(Y_2)</td>
<td>10 (fixed)</td>
</tr>
</tbody>
</table>

**Method**: Optimised the total NG load obtained from the full human infection model by fitting to the generated data.

**Parameter estimates of** \(d, c, p, \mu\) and \(r_1\)

**Human infection model point estimates**

---

Figure 2: Flow diagram of the sub-models and data used to estimate the human infection model point estimate parameters indicating how the estimates feed into the final model.
Figure 3: Model fit to *in vitro* studies to estimate parameters. (a) The *in vitro* data on NG attachment to HeLa cells by two types of NG (piliated and non piliated) from the study by Gubish, et al. (1979) is shown with the fitted function described in Supplementary File, Section 1.2.1. (b) Data on NG internalization over the period of 6-12 hours as observed in the study by Shaw and Falkow (1988) is shown with the best fit curve obtained by fitting the sub-model on NG internalization to these data (sub-model explained in Supplementary File, Section 1.2.2). The optimal fit is shown by the solid line and the credible intervals obtained around the *in vitro* point estimates are shown by the shaded region. (c) NG killing by PMN as measured by the study by Rest, et al. (1982). The solid line represents the curve obtained from least squares minimization by fitting to the sub model explained in Supplementary File, Section 1.2.3, while the dashed line is the equivalent curve for the base-case parameters, determined through fitting the full model to simulated data based on the qualitative features of the time course of infection. The credible intervals obtained around the *in vitro* point estimates are shown by the shaded region.
Figure 4: (a) Changes in the four bacterial populations as well as the neutrophil population are shown along with the total bacterial load ($B + B_a + B_s + B_t$) over the first 40 days of infection. (b) Proportions of NG across the bacterial states over 40 days. Colours in each panel relate to bacterial populations as defined in the panel (a) legend. (c) Changes in the relative proportions of NG in each bacterial state during the first 2 days of infection.
Figure 5: (a) Log-scale time course for all model states using the point estimates in Table 2 as parameter values, with shaded regions representing realistic intervals for incubation and clearance periods and the peak load. (b) Comparison of log-scale infection time course for overall bacteria obtained using point estimate values in Table 2, along with 95% credible interval from multivariate sensitivity analysis (clearance at 1-6 months) and bacterial curve when using in-vitro parameter estimates without adjustment.
Figure 6: Model fits to mouse NG data. (a)-(c) NG data from mouse model described in Jerse (1999) and fits to each mouse for the 5-state model developed for human infection. (d)-(f) As above but using a 3-state model where epithelial internalisation ($B_i$) and attachment ($B_a$) states have been removed. Mice are labelled m1 to m8, with data represented by markers and fits by lines of the same colour.

Figure 7: Estimated parameter values of the ratio $\frac{d}{c}$, $r_1$, $\mu \times N_{max}$, $d$, $r_3$, and $p$ obtained across the mouse models (labelled A to D) compared with the human estimates (labelled h) are shown in panels (a to f) respectively. Here, A represents the full 5-state model, B the model without an epithelial internalisation state ($B_i$), C the model without attachment ($B_a$) or epithelial internalisation states ($B_i$) and D the model without attachment ($B_a$), epithelial ($B_i$) and neutrophil ($B_s$) internalisation states. Ranges for mice are minimum and maximum values across the 8 mice while for the human estimates, the 95% credible intervals shown in Table 2 are displayed along
with the median value. Note that in panel (f) no range is displayed because we fix the neutrophil internalisation proportion \( p \) in the relevant mouse models.
Table 1: Human infection model initial conditions

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Parameter</th>
<th>Value</th>
<th>Reference/ Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Initial Conditions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$B_a$</td>
<td>Attached bacteria</td>
<td>0 \textit{bacteria}</td>
<td>Assumption</td>
</tr>
<tr>
<td>$B_s$</td>
<td>Bacteria surviving within PMN</td>
<td>0 \textit{bacteria}</td>
<td>(Criss and Seifert 2012; Ramsey, et al. 1995)</td>
</tr>
<tr>
<td>$B_i$</td>
<td>Bacteria internalised within epithelial</td>
<td>0 \textit{bacteria}</td>
<td>(Shaw and Falkow 1988)</td>
</tr>
<tr>
<td>$N$</td>
<td>Activated PMN</td>
<td>$10^{-8}$ \textit{cells}</td>
<td>(Criss and Seifert 2012; Ramsey, et al. 1995)</td>
</tr>
</tbody>
</table>
Table 2: Model parameter values and the parameter ranges based on the sensitivity analysis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Point estimate</th>
<th>Parameter range used to generate LHS samples</th>
<th>Reference / Comments</th>
<th>95 % credible interval after outcome filtering</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_1 ) Replication rate of non-internalized bacteria</td>
<td>0.489 ( \text{hour}^{-1} )</td>
<td>0.374 – 0.53 ( \text{hour}^{-1} )</td>
<td>Point estimate was obtained by fitting the model to total NG load data generated based on qualitative features of the time course of infection. Range based on estimates by Craig, et al. (2015), using the individual variation in human experimental studies Schmidt, et al. (2001) and Schneider, et al. (1996). The study-derived range 0.13 - 0.53 ( \text{hour}^{-1} ) was refined after initial LHS to this range as values outside of this were inconsistent with the outcome ranges.</td>
<td>0.386 – 0.527</td>
</tr>
<tr>
<td>( r_2 ) Replication rate of internalized NG</td>
<td>0.533 ( \text{hour}^{-1} )</td>
<td>0.27 – 1.06 ( \text{hour}^{-1} )</td>
<td>Four-fold range around point estimate from Shaw and Falkow (1988).</td>
<td>0.289 – 1.014</td>
</tr>
<tr>
<td>( r_3 ) Replication rate of NG surviving within PMN</td>
<td>0.340 ( \text{hour}^{-1} )</td>
<td>0.31 – 0.41 ( \text{hour}^{-1} )</td>
<td>Based on the variation of measured values in Simons, et al. (2005).</td>
<td>0.312 – 0.408</td>
</tr>
<tr>
<td>( a_1 ) NG attachment rate to epithelial cells</td>
<td>0.340 ( \text{hour}^{-1} )</td>
<td>0.3 – 0.43 ( \text{hour}^{-1} )</td>
<td>Based on fitting to data regarding piliated and non-piliated NG strains in Gubish, et al. (1979)</td>
<td>0.303 – 0.427</td>
</tr>
<tr>
<td>Parameter</td>
<td>Description</td>
<td>Value</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
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<td></td>
</tr>
<tr>
<td>$a_2$</td>
<td>Maximal NG attachment capacity per epithelial cell</td>
<td>12</td>
<td>6 – 12</td>
<td>Based on fitting to data regarding piliated and non-piliated NG strains in Gubish, et al. (1979)</td>
</tr>
<tr>
<td>$d$</td>
<td>NG engulfment rate by PMN</td>
<td>$2.586 \text{ bacteria hour}^{-1} \text{ cell}^{-1}$</td>
<td>$d \sim b \times c + a \text{ bacteria hour}^{-1} \text{ cell}^{-1}$</td>
<td>Point estimate was obtained by fitting the model to total NG load data generated based on qualitative features of the time course of infection. Initial LHS analysis showed that in samples that met outcome criteria $d$ and $c$ were strongly correlated, satisfying a regression line of the form $d \sim b \times c + a$. For revised LHS samples, using this relationship we generated $d$ from $c$, with $b \in (0.207, 0.497)$ and $a \in (0.816, 1.95)$.</td>
</tr>
<tr>
<td>$d_2$</td>
<td>Wash out rate of unattached bacteria</td>
<td>$10^{-3} \text{ hour}^{-1}$</td>
<td>$5 \times 10^{-4} – 2 \times 10^{-3} \text{ hour}^{-1}$</td>
<td>Assumption, with four-fold range around the point estimate.</td>
</tr>
<tr>
<td>$d_3$</td>
<td>Death rate of activated PMN</td>
<td>$1/24 \text{ hour}^{-1}$</td>
<td>$0.02 – 0.045 \text{ hour}^{-1}$</td>
<td>Point estimate derived from Simons, et al. (2005). Four-fold range around the point estimate reduced through initial LHS comparison to outcome ranges.</td>
</tr>
<tr>
<td>$e$</td>
<td>Exit rate of internalized NG</td>
<td>$0.650 \text{ hour}^{-1}$</td>
<td>$0.55 – 1.3 \text{ hour}^{-1}$</td>
<td>Assumption, with four-fold range around the point estimate reduced through initial LHS comparison to outcome ranges.</td>
</tr>
<tr>
<td>$\mu$</td>
<td>PMN activation rate</td>
<td>$5.72 \times 10^{-13} \text{ hour}^{-1}$</td>
<td>$2.82 \times 10^{-13} – 8.76 \times 10^{-13} \text{ bacteria}^{-1} \text{ hour}^{-1}$</td>
<td>Point estimate was obtained by fitting the model to total NG load data generated based on qualitative features of the time course of infection. For LHS, four-fold range around the point estimate reduced through initial LHS comparison to outcome ranges.</td>
</tr>
<tr>
<td>Parameter</td>
<td>Description</td>
<td>Value</td>
<td>Range</td>
<td>Notes</td>
</tr>
<tr>
<td>-----------</td>
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<tr>
<td>$c$</td>
<td>Ratio dependent constant</td>
<td>3.135</td>
<td>$10^{-3} - 6.27$</td>
<td>$bacteria\ cell^{-1}$</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Rate of internalization into epithelial cells</td>
<td>0.28 hour$^{-1}$</td>
<td>$0.14 - 0.37$ hour$^{-1}$</td>
<td>$\text{Point estimate derived from Shaw and Falkow (1988), with four-fold range. For LHS, four-fold range around the point estimate reduced through initial LHS comparison to outcome ranges.}$</td>
</tr>
<tr>
<td>$p$</td>
<td>Proportion of NG surviving within PMN</td>
<td>0.25</td>
<td>$0.01 - 0.5$</td>
<td>$\text{Point estimate was obtained by fitting the model to total NG load data generated based on qualitative features of the time course of infection. Lower limit set to include range from <em>in vitro</em> estimates derived from Rest, et al. (1982) and upper limit } 2 \times \text{ point estimate.}$</td>
</tr>
<tr>
<td>$k_1$</td>
<td>Urethral carrying capacity</td>
<td>$10^4$ bacteria</td>
<td>Kept fixed during the sensitivity analysis</td>
<td>Kept fixed during the sensitivity analysis explained in text.</td>
</tr>
<tr>
<td>$k_2$</td>
<td>Survival capacity of NG per PMN</td>
<td>8</td>
<td>$4 - 16$</td>
<td>$bacteria\ cell^{-1}$</td>
</tr>
<tr>
<td>$N_{max}$</td>
<td>Total number of PMN in the body</td>
<td>$2.50 \times 10^{10}$ cell</td>
<td>Kept fixed during the sensitivity analysis</td>
<td>Kept fixed during the sensitivity analysis explained in text.</td>
</tr>
<tr>
<td>Study</td>
<td>Related parameters</td>
<td>Study description</td>
<td></td>
<td></td>
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<td>-----------------------</td>
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<tr>
<td>Gubish, et al. (1979)</td>
<td>Bacterial attachment rate ($a_1$) and maximal NG attachment capacity of an epithelial cell ($a_2$).</td>
<td>NG attachment to HeLa cells was measured over a period of 4 hours using two types of NG; piliated and non-piliated (in vitro study).</td>
<td></td>
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</tr>
<tr>
<td>Shaw and Falkow (1988)</td>
<td>Rate of internalization of NG into epithelial cells ($\eta$) and the replication rate of internalized NG ($r_2$).</td>
<td>Number of NG that survived gentamicin exposure and invaded epithelial cells over the period of 12 hours (in vitro study).</td>
<td></td>
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<tr>
<td>Rest, et al. (1982)</td>
<td>Bacterial engulfment rate ($d$), the proportion of NG surviving within PMN ($p$) and the ratio dependent constant ($c$).</td>
<td>PMN phagocytosis of non piliated NG in the absence of serum, measured over a period of 135 minutes (in vitro study).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simons, et al. (2005)</td>
<td>Intracellular growth of NG within PMN ($r_3$).</td>
<td>Number of viable intracellular NG within PMN measured over 6 hours (in vitro study).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jerse (1999)</td>
<td>NG load time-series in 8 mice that was used to as a validation exercise for the human infection model.</td>
<td>NG load recovered in vaginal swabs of 8 mice over the time course of 14 days is reported. However, as mentioned in section ‘Fitting to mouse model data’, only the first 9 days of bacterial data were used for fitting.</td>
<td></td>
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