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Published in:
Trends in Microbiology

DOI:
10.1016/j.tim.2013.10.006

2014

To harm or not to harm? On the evolution and expression of virulence in group A streptococci

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Keywords: *Streptococcus pyogenes*, virulence, evolution, regulation, epigenetic, bistability.

Group A streptococci (GAS) cause three different types of infection (*sensu lato*) with distinct levels of virulence: asymptomatic colonization, superficial symptomatic infection, and invasive infection. To address why this pattern with several infection types has evolved, we combine mechanistic understanding from infection medicine with recent theory from evolutionary ecology. We propose that asymptomatic colonization and superficial symptomatic infection exploit different states of the host epithelium to maximize transmission between hosts in different epidemiological conditions, whereas the ability of the bacteria to cause invasive infection is a non-adaptive side effect of traits required for superficial symptomatic infection.

Group A streptococci and their types of infection

Humans host a variety of bacteria. Most are commensals, some are pathogens, and some can be either. Pathogenic streptococci are an example of bacteria that can act as both commensals and pathogens, with group A streptococci (GAS; *Streptococcus pyogenes*) as a prime example. A leading cause of death worldwide, GAS give rise to toxic shock syndrome, necrotizing fasciitis, and sepsis, but the majority of clinical infections manifest as superficial and self-limiting pharyngitis and pyoderma [1,2]. In addition, GAS commensally colonize a considerable fraction of the healthy population [3]. The infections (*sensu lato*) caused by these bacteria can therefore be divided into three broad categories: the invasive (e.g., sepsis), the superficial symptomatic (e.g., pharyngitis), and commensal colonization. Infections of different types may occur in sequence or in isolation. The existence of different types of infection with distinct levels of virulence is not unique to GAS and other β-haemolytic streptococci, but is a general phenomenon that applies also to *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* [4–6].

The phenotype of an infection is a result of interactions between the pathogen and its host, and the existence and relative frequency of different types of infection may therefore be explained by variation in either or both of these parties. Hosts differ in their propensity to acquire certain types of infection. For example, invasive GAS infections are associated with old age [7] and with preceding infection with varicella-zoster virus [8]. The relative frequency of different types of infection can therefore change with demography and health care practices, such as varicella vaccination. In addition, there is a host genetic component to the severity of invasive disease [9], and there is some indication that certain individuals are disproportionally likely to become asymptomatic carriers [10]. However, some GAS serotypes are biased towards a given infection type, which shows that the bacterium also plays a role in determining the type of infection. For example, the serotypes M1 and M12 are both able to cause all three types of infection, but M1 is overrepresented in symptomatic infections, whereas M12 is more prone to be asymptomatic [11,12]. Similarly, a large study performed at the Centers for Disease Control and Prevention (CDC) found that the M1 serotype was significantly associated with invasive infection, and M12 with non-invasive infection [13]. The type of infection is thus determined by both the host and the pathogen.

In this opinion article we focus on the contribution to virulence made by the pathogen, and ask: why does GAS have the ability to cause three different types of infection with distinct levels of virulence? To address this question, we integrate mechanistic understanding from infection medicine with recent theory from evolutionary ecology. Both evolutionary theory of virulence and the study of molecular virulence mechanisms are well developed, but the two fields are rarely combined as an integrated whole. Here we provide such a synthesis for an important human pathogen. We consider GAS as a case in point, but the outlined scenario should be applicable to other pathogens as well, given that they have a similar virulence pattern, and are adapted to their host rather than infect it only accidentally.

GAS and virulence evolution theory

A foundation of contemporary virulence evolution theory is the 'trade-off hypothesis'. One version of this may be summarized as follows. The more the bacteria replicate, and the higher densities they attain in the host, the higher the rate of transmission to new hosts (number of daughter infections per unit time). On the other hand, high replication and bacterial load result in virulence, by damaging the host, and limit the time available for transmission, for example by eliciting a protective immune response that clears the infection [14], or by killing the host [15]. From these assumptions, it is concluded that virulence is associated with both positive and negative effects on bacterial transmission. The optimal strategy for the pathogen is therefore an intermediate level of virulence (see Box 1 and [15] for details).

The assumptions behind the trade-off hypothesis are supported for GAS. First, there is evidence for associations between bacterial load and transmission rate [16], and between transmission and symptomatic infection [17]. Second, virulence (symptoms) correlates with bacterial load [18], and third, the duration of

asymptomatic colonization is much longer than that of superficial symptomatic infection [10].

There is, however, a problem. Given that the assumptions of the trade-off hypothesis apply to GAS, the expectation is that GAS evolutionary trajectories should converge on a single optimal level of virulence, but this is not what is observed. GAS cause three distinct types of infection with virulence levels ranging from the entirely innocuous to the highly lethal. This means that the basic trade-off model is unable to account for the virulence pattern seen in GAS.

Which infection types are actually adaptive?

Explanation by optimality models, such as those based on the trade-off hypothesis, requires that the phenomenon to be explained represent adaptation. There are reasons to believe that asymptomatic and superficial symptomatic GAS infections are adaptive, whereas invasive infections are not. First, the two non-invasive types of infection dominate GAS natural history, whereas invasive infections constitute a minute proportion [1]. Second, both asymptomatic and superficial symptomatic infections likely transmit, whereas transmission from the blood stream and deep tissues seems unlikely. If the bacteria do not transmit, the infection type cannot be adaptive. Third, many invasive isolates have mutations in the CovRS regulatory system, but these are most probably a result of *de novo* mutation and evolution within the host individual from which the bacteria were isolated [19,20]. In an animal model of superficial infection, such bacteria had impaired fitness [21]. In other words, these variants represent evolution of virulence through 'short-sighted evolution' [22], not a long-term evolutionary optimum. We hence conclude that the two non-invasive types of infection likely represent two different adaptive strategies, whereas invasive infection does not. Accordingly, asymptomatic colonization and superficial symptomatic infection are both within the scope of optimality models, while invasive infection falls outside.

This does not solve the problem, however, but the question is merely split in two: (i) why are there two adaptive types of infection rather than only one, and (ii) why is there a non-adaptive type of infection? To address these questions, we first describe how the habitat of GAS allows for two different adaptive strategies, and suggest how the bacteria can use them, and what side effects this may have. We then discuss more complex evolutionary models, to explain why GAS has evolved both adaptive strategies, rather than specialized in one of them.

Two adaptive strategies and an unfortunate side effect

Both the potentially adaptive types of infection (asymptomatic and superficial symptomatic) are localized to the host epithelia, specifically those of the skin and upper respiratory tract. When healthy, these epithelia are poor in glucose [23,24], and since glucose is an important nutrient for GAS [25], this makes them poor substrates for GAS proliferation. In contrast, when the epithelia are inflamed, the influx of a plasma exudate supplies glucose and other nutrients, which can be exploited by the bacteria [23,26,27]. However, the function of the inflammatory exudate is hardly to

feed pathogenic bacteria. Rather, it is part of the host's innate immune defence, and besides nutrients it contains a range of factors, which are deleterious to microbes [28]. Thus, the epithelium has two states. When healthy, it is nutrient poor; when inflamed, it is nutrient rich, but also defensive. As nutrients are required for replication, this means that the epithelium may support two different bacterial strategies. In asymptomatic colonization, nutrients are scarce, bacterial replication rate is low, and host defences are not activated. This results in long infections with a low rate of transmission between hosts. In superficial symptomatic infections, there are more nutrients, the replication rate is higher, and the host's defences are activated. This results in short infections with a high rate of transmission (Figure 1a).

The strategy associated with exploitation of the epithelia's nutrient rich state requires at least three bacterial traits. The first is the induction of inflammation, and it is conferred by the proinflammatory virulence factors. Examples include the superantigens, which activate large populations of T cells and thereby elicit inflammation [28]. The resulting inflammatory exudate contains both immune components and nutrients. Accordingly, the second trait required is evasion of immunity. This trait corresponds to virulence factors such as anti-phagocytic proteins and factors inactivating antimicrobial peptides. The third trait is the ability to exploit the supplied resources, which includes both sequestration and metabolism. The system is summarized in Figure 1 b.

An example of a molecular mechanism involved in exploiting the inflamed epithelium is the M-protein, a key virulence factor in GAS [3]. In symptomatic infection (pharyngitis) it is highly expressed, whereas in asymptomatic colonization, it is downregulated [18,29]. It contributes to all three traits discussed previously as required for the exploitation of inflammation: it has proinflammatory activity [30], it protects against phagocytosis [31], and it is involved in nutrient acquisition [26]. Expression of the M-protein is co-regulated with a number of immune evasive and other virulence factors by the multi gene activator Mga (also known as the virulence regulator VirR) [32]. Mga also regulates metabolism [32]. It is associated with the exponential phase of growth [33], and it is probably linked to glucose availability and utilization [34]. In a primate model of superficial infection, the transcription of *mga* and several key virulence genes, including *emm* (encoding the M-protein) and genes for superantigens, were monitored, and found to correlate with symptoms [18]. In summary, the expression of the Mga regulator and the M-protein virulence factor contributes to the induction, evasion, and exploitation of inflammation, and these factors are upregulated in the symptomatic setting, and downregulated in the asymptomatic setting.

Although these traits are adaptations for exploiting the epithelium's nutrient rich state, they may also explain why GAS is capable of producing invasive infections. In a local infection in the pharynx, the induction, evasion, and exploitation of inflammation result in pharyngitis. However, if the bacteria accidentally enter a normally sterile site, the same traits would allow them to grow rapidly, make it hard for the immune system to eradicate them, and induce a systemic immune response. The result would be a systemic inflammatory response syndrome (SIRS) due to

infection, which is the definition of sepsis [35]. On a more general note, the plasma, nutrients, and host defences present at the inflamed epithelium are similar to those in the invasive situation. Therefore, adaptations for the inflamed epithelium can exert their effects also at normally sterile sites, but the effects for the host are then more severe (Figure 1c). In line with this, the M-protein discussed earlier is important in invasive disease [2].

In summary, we have proposed that the epithelium allows for two strategies, corresponding to the adaptive types of infection, and that invasive infection arises as a side effect of adaptations for one of those strategies. We now turn to why GAS would have evolved two adaptive strategies rather than only one.

Why are there two adaptive types of infection?

The trade-off hypothesis, which predicts a single intermediate optimal level of virulence, is very general and simplified, which is both its strength and weakness. More recent models consider more factors, and yield a more complex picture. In particular, there are now models that allow coexistence of two distinct strategies, where one strategy has low virulence and the other has high. Here we discuss three different, but not mutually exclusive scenarios.

Boldin and Kisdi showed that pathogens which transmit both directly between hosts and indirectly via the environment can have two adaptive levels of virulence, where the lower level is optimal under direct transmission and the higher under environmental transmission [36]. GAS transmit directly [3], but are also shed into the environment where they remain viable for months, and shedding of large numbers of bacteria is associated with high nasal bacterial load [37,38]. These fomites do not seem to give rise to pharyngitis, however, at least not when dry, but can result in other infections [39–43].

Another model allowing the coexistence of different levels of virulence was proposed by Brown *et al.* [44]. Here, a pathogenic strain gains a competitive advantage over a commensal by inducing an immune response that the commensal cannot cope with. This strategy requires that the pathogen can both provoke immune responses and protect itself against them, which fits well with the strategies and virulence factors of GAS. The scenario allows the coexistence of pathogenic and commensal strains in the population [44]. The models of Brown *et al.* and of Boldin and Kisdi were not developed specifically for GAS. Nevertheless, they show that when some complexity is allowed for, a pattern with two adaptive levels of virulence can emerge.

GAS epidemiology involves abundant asymptomatic carriage alternating with outbreaks of symptomatic infections. Based on this pattern, we propose a third scenario, where the two adaptive infection types represent adaptation to different epidemiological phases. The trade-off model of virulence evolution reviewed previously is based on the idea that the Darwinian fitness of a pathogen is maximized by maximizing the number of daughter infections to which a primary infection gives rise in a fully susceptible host population (R_0; Box 1). However, this assumes that the population dynamics of both the pathogen and host have reached equilibrium, with a

stable prevalence of the pathogen and a stable density of susceptible hosts, which means that the pathogen is in endemic phase. In contrast, during the epidemic phase, when the prevalence of the pathogen is still increasing, it is more important to maximize the rate of transmission than R_0. This is because, when the prevalence of the pathogen is increasing, the infection that produces the most daughter infections per unit time, rather than total number of daughter infections, will have the highest representation in future generations. During the epidemic phase, therefore, natural selection favours high transmission rates and virulence even if it shortens the transmission period and thereby lowers R_0 [45]. Hence, a pathogen for which outbreaks (epidemic phase) alternate with equilibrium conditions (endemic phase) could experience selection for two different strategies. In outbreaks, selection would favour high transmission rates at a cost of transmission time span and R_0. The equilibrium conditions would instead select for high R_0, favouring longer transmission time span at a cost of transmission rate. Alternation between different epidemiological conditions, such that the bacteria experience repeated cycles with epidemic and endemic phases, could potentially maintain the ability for both strategies, and the associated types of infection.

An epigenetic mechanism for virulence bistability

As reviewed in the introducing paragraph, certain GAS serotypes are biased towards causing certain types of infection relative to other serotypes, indicating genetic variation for infection type. However, variation among serotypes explains only part of the variation in infection type [11,12,17], the residual typically being ascribed to variation among host individuals. Here we explore another factor that may contribute: bacterial epigenetic regulation of virulence.

Phenotypic bistability due to epigenetic mechanisms has been described in a number of bacteria, and has been argued to be ubiquitous [46]. A common epigenetic mechanism is regulatory systems with positive feedback loops [46]. Positive feedback loops perpetuate regulatory states by continuing the production of regulators after the initial stimulus is removed [47], and as bacteria have no specialized germline, but reproduce by division, the regulators and the corresponding regulatory states are inherited by the daughter cells. Depending on the exact characteristics, such systems may result in the occasional switching between alternative heritable phenotypes [46]. A positive feedback loop was recently found to mediate bistability in the expression of a virulence factor in *S. pneumoniae* [48]. A similar phenomenon seems likely in GAS. For example, the Mga regulator (discussed in a previous paragraph) induces itself in a positive feedback loop, and is inhibited by the RofA regulator, which is also regulated by positive feedback [49]. Thus, GAS may have two transcriptional states, one dominated by Mga, with high expression of M-protein and other Mga induced virulence factors, and the other dominated by RofA, with low expression of these factors. These systems may therefore allow GAS to switch between phenotypes appropriate for asymptomatic colonization and superficial symptomatic infection, respectively, without genetic changes. The existence, heritability, and reversibility of such alternative phenotypic states are supported by data from several studies on GAS

[29,50–53]. (In contrast, the CovRS mutations often found in GAS isolated from invasive infections (discussed earlier), in virtue of being mutations are not part of the epigenetic phenotypic switch described here).

If bacteria initiating an infection inherit their transcriptional state from their ancestors in the previous infection, and the expression of virulence factors contributes to the induction of symptoms, then primary infections should tend to give rise to daughter infections of their own type, regardless of bacterial genotype. This would be a form of epigenetic inheritance at the level of infection, and it could contribute to the clustering of symptomatic infections in space and time (as outbreaks).

GAS regulatory systems, including Mga and RofA, respond to environmental cues [49]. It is therefore possible that these regulators increase the probability that the transcriptional states of the bacteria match the conditions favouring the different strategies. This is, however, not required for phenotypic switching to be adaptive, but even random switching can be selected for if conditions vary frequently and unpredictably, a phenomenon known as 'bet hedging' [54].

Conclusion and future directions

We have argued that asymptomatic colonization and superficial symptomatic infection represent two different adaptive strategies. The two strategies utilize different host niches: the healthy and the inflamed epithelium, respectively. We also suggested that natural selection has maintained both strategies, rather than favoured one of them, because GAS have two modes of transmission (direct and via the environment), experience different levels of competition when establishing an infection, and/or alternate between epidemic and endemic phases. Thus, the adaptive significance of GAS virulence factors is to induce, evade and exploit inflammation to maximize Darwinian fitness under certain conditions (environmental transmission, competition, and/or the epidemic phase). These same virulence factors occasionally give rise to invasive disease as a non-adaptive side effect. Infection type is partly determined by bacterial genotype, but may also be due to epigenetic effects, which make the phenotype heritable for a number of generations. In Box 2 we list questions that are important to address in evaluating our proposed scenario.

Understanding the adaptive significance of different types of infection with a given pathogen is crucial to predicting the evolutionary response to interventions. For example, antibiotic treatment of adaptive infections often results in the evolution of resistance in the bacterial population, while treatment of non-adaptive infections should not have this effect. We hope that continued cooperation between evolutionary and medical research will result in formal and empirically tested models that can be used to design treatment and prevention strategies that maximize effect and minimize the evolution of resistance.

Box 1. The trade-off hypothesis of virulence evolution

In this text box we summarize the line of thought associated with the trade-off hypothesis.

The Darwinian fitness of a pathogen is determined by its ability to transmit to new host individuals. For a pathogen to persist in the host population, infections need to produce on average at least one daughter infection, so that $R_0 \geq 1$, where R_0 denotes the number of new infections arising from an infected host in a population where all hosts are susceptible. In contrast, if $R_0 < 1$, the pathogen will eventually become extinct. When there is variation in R_0 among different genotypes of a pathogen, natural selection should favour those with characteristics that result in higher R_0. The question is then, how does virulence relate to R_0, or in other words, how does natural selection act on virulence?

Most models of virulence evolution have focused on virulence in the sense of infection-induced mortality of the host, and assumed that increasing pathogen replication within the host increases virulence. Thus, increased replication comes at a cost for the pathogen because host death reduces the time span available for transmission to other hosts. This led to the 'conventional wisdom' that prevailed until the 1980s, that pathogens should eventually evolve avirulence to maximize their Darwinian fitness [55]. However, increased replication may also benefit the pathogen (i.e., increase R_0) by increasing the rate of transmission to new hosts (i.e., the number of daughter infections per unit time). If replication affects both virulence and transmission rate, fitness (i.e., R_0) may be maximized at some intermediate level of virulence, rather than at infinitesimal virulence as under the 'conventional wisdom' scenario. An intermediate optimum for virulence can also come about in several other ways than through a trade-off between infection-induced mortality and transmission rate. For example, if higher pathogen replication induces a stronger immune response, there may be an intermediate optimal level of virulence as a result of a trade-off between transmission rate and immune clearance [14]. Thus, when virulence has not only costs, for example reduced time span for transmission, but also a benefit in the form of increased transmission rate, natural selection can favour an intermediate level of virulence rather than avirulence, an idea that has become known as the 'trade-off hypothesis'. Assumptions of the trade-off hypothesis have now been addressed and confirmed in several host-pathogen systems. The reader is referred to [15] for a review of the trade-off hypothesis and its empirical support. In the main text we discuss some possibilities that go beyond this basic scheme.

Box 2. Outstanding questions

- What is the role of environmental transmission in GAS, especially for infections other than pharyngitis (Boldin and Kisdi's model [36])?
- What are the roles of inter-strain competition and competition with commensal microbiota in establishment of GAS infection (Brown et al's model [44])?
- How large do GAS epidemics need to be for selection to favour virulence? Would a kindergarten of up to 20 children suffice or is the population of an entire country necessary (our proposal for the existence of two virulence optima)?
- How do the classical virulence factors described for invasive infection increase the Darwinian fitness of the pathogen in superficial symptomatic infections?

Acknowledgements

We would wish to thank the following people for their input on this project: Magnus Rasmussen, Oonagh Shannon, Mattias Collin, Martin Lembke, Lars Björck, Inga-Maria Frick, Samuel Alizon, and two anonymous reviewers. This work was supported by the Swedish Research Council (K2011-56X-07480 and 2011-5680) and the Knut and Alice Wallenberg Foundation (KAW 2011.0037).

Conflicts of interest

The authors declare that they have no conflicts of interest.

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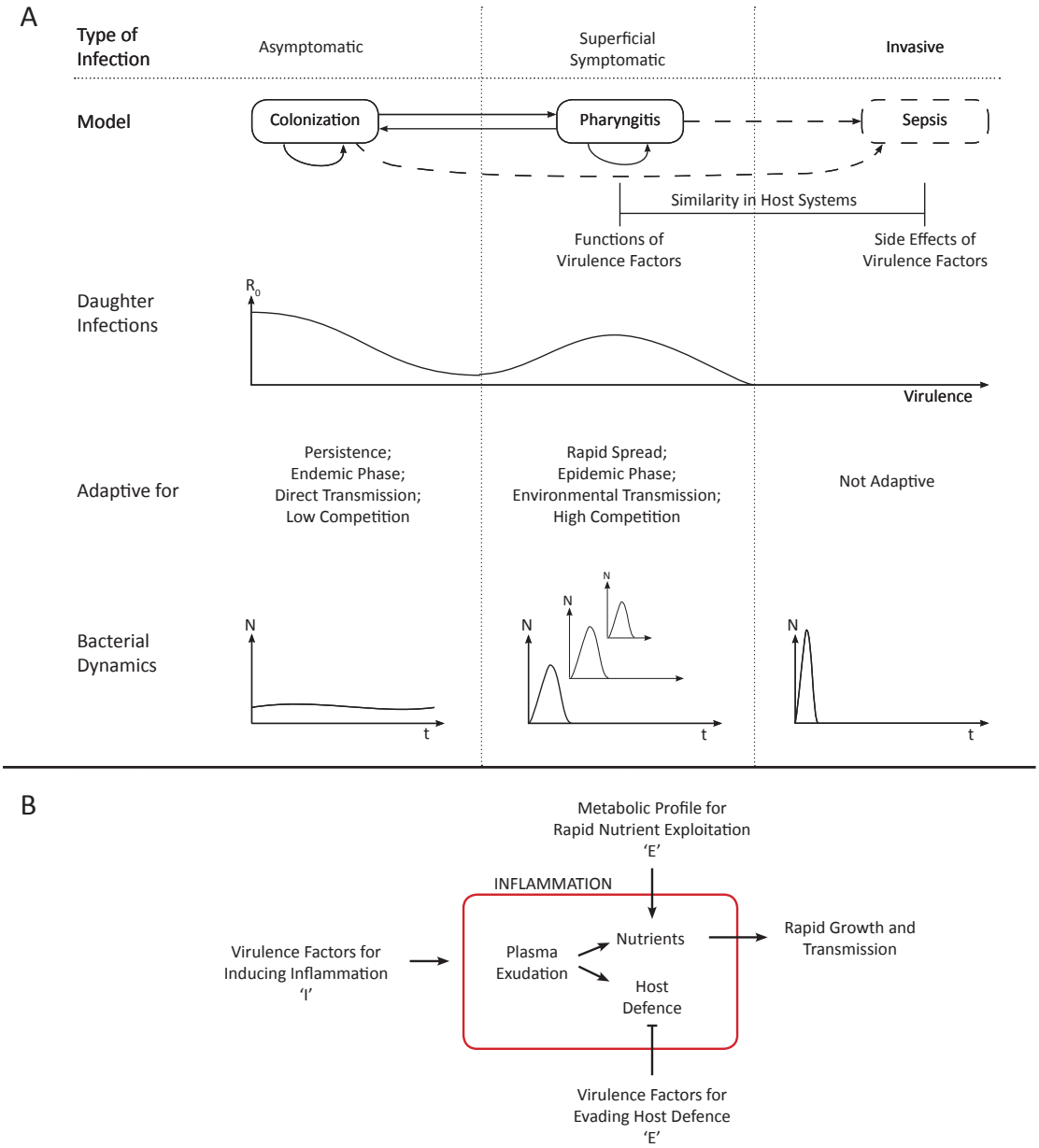
Figure legends

Figure 1. Infection and adaptive strategies of GAS.

A. Types of infection. There are three distinct types of infection (*sensu lato*) with GAS: asymptomatic (colonization), superficial symptomatic (e.g., pharyngitis), and invasive (e.g., sepsis). These are here represented in a model with three compartments. Arrows indicate transmission, that is infections of one type giving rise to new infections of the same or a different type. Solid arrows represent frequent transmission events, and dashed arrows represent infrequent events. Asymptomatic colonization and superficial symptomatic infection are different strategies, and they involve different kinds of interactions with the host, such that symptomatic infection requires virulence factors. They also result in different bacterial dynamics. Superficial symptomatic infections have burst-crash like dynamics with short infections of high intensity as the bacteria spread from host to host (represented as separate coordinate systems), whereas colonization has a lower intensity, but lasts for a longer time. 'N' denotes the number of bacteria in the infection, and 't' denotes the time. Superficial symptomatic infection and asymptomatic colonization may serve different functions for the bacterium, with colonization contributing more to persistence and symptomatic infection to rapid spread, as in outbreaks, and they may be adaptive under different conditions. Invasive infection is depicted as a dead end without function for the bacterium. Virulence is taken in a broad sense.

B. Traits required for exploitation of the epithelium's nutrient rich state. There are virulence factors that induce immune responses, and others that counteract them. In principle, this may be interpreted as representing the bacterium being targeted by host defences and defending itself, or as a way to achieve a fine tuned balance. Here we adopt neither of those perspectives. Instead we propose that some virulence factors ('I' for induction of inflammation) induce a general inflammatory response. This response has a range of components, many of which are deleterious to the bacteria, whereas others (nutrients) are beneficial. The counteracting factors ('E' for evasion of immunity and exploitation of inflammation associated nutrients) more specifically inhibit various deleterious effects, leaving the beneficial ones to be exploited ('E') for growth and transmission.

C. Sepsis as a side effect of the traits described in B. Plausibly, many or most virulence factors are adaptations for the superficial symptomatic type of infection, with its nutritious plasma exudate and activated host defences, and are selected for in that context. Accordingly, the traits 'I' (induction of inflammation) and 'E' (evasion of immunity and exploitation of inflammation associated nutrients) allow the bacteria to induce local inflammation, evade its antibacterial effects, and exploit the nutrients for growth and transmission. Since the host components encountered in the invasive setting are similar to those at the inflamed epithelium, those same traits are functional in the invasive environment as well, but the systemic nature of invasive infection makes the consequences more severe. This has no function for the bacterium, but is a mere side effect.


A
Type of Infection
Asymptomatic
Superficial Symptomatic
Invasive
Model
Colonization
Pharyngitis
Sepsis
Similarity in Host Systems
Functions of Virulence Factors
Side Effects of Virulence Factors
Daughter Infections
R_0
Virulence
Adaptive for
Persistence; Endemic Phase; Direct Transmission; Low Competition
Rapid Spread; Epidemic Phase; Environmental Transmission; High Competition
Not Adaptive
Bacterial Dynamics
N
t
B
Metabolic Profile for Rapid Nutrient Exploitation 'E'
INFLAMMATION
Virulence Factors for Inducing Inflammation 'I'
Plasma Exudation
Nutrients
Host Defence
Rapid Growth and Transmission
Virulence Factors for Evading Host Defence 'E'
C
LOCAL
INVASIVE
P
'I'
'E'
IMMUNE SYSTEM
Local Inflammation
Exploit Inflammation
Growth and Transmission
Systemic Inflammation
Hard to Eradicate
Sepsis