Evolution of virulence in opportunistic pathogens: generalism, plasticity, and control☆

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Standard virulence evolution theory assumes that virulence factors are maintained because they aid parasitic exploitation, increasing growth within and/or transmission between hosts. An increasing number of studies now demonstrate that many opportunistic pathogens (OPs) do not conform to these assumptions, with virulence factors maintained instead because of advantages in non-parasitic contexts. Here we review virulence evolution theory in the context of OPs and highlight the importance of incorporating environments outside a focal virulence site. We illustrate that virulence selection is constrained by correlations between these external and focal settings and pinpoint drivers of key environmental correlations, with a focus on generalist strategies and phenotypic plasticity. We end with a summary of key theoretical and empirical challenges to be met for a fuller understanding of OPs.

Opportunistic pathogens and a challenge to virulence evolution theory

The study of infectious diseases has become a major focus within evolutionary biology; however, remarkably little attention has been paid to an extremely broad class of pathogens, the opportunists. This oversight stems from the theoretical convenience of treating host–parasite interactions as closed systems in which a single, obligate pathogen specialises on a single host [1,2]. Most pathogens actually fail to meet these assumptions, with many coexisting relatively peacefully with their human host (i.e., they are not obligately pathogenic) or even exploiting an entirely different environment outside of human hosts [3].

Opportunistic pathogens (OPs) are typically characterised in the medical literature as organisms that can become pathogenic following a perturbation to their host (e.g., disease, wound, medication, prior infection, immunodeficiency, and ageing). These opportunists can emerge from among the ranks of normally commensal symbionts (e.g., *Streptococcus pneumoniae* and *Staphylococcus aureus*) or from environmentally acquired microbes (e.g., *Pseudomonas aeruginosa* and *Burkholderia cepacia*). Many more pathogens are recognised as opportunists in the sense that although they regularly cause disease in healthy humans, they are also zoonotic and exploit numerous other hosts (e.g., *Bacillus anthracis* and rabies virus).

We propose a broad and simple definition of OPs: non-obligate and/or non-specialist parasites of a focal host. Thus, if the classic assumptions (obligate parasite and specialist on one host) of virulence evolution theory fail, we have an OP. In Table 1 we outline, with examples, how the combinations of these two conditions give us a classification of OPs into commensal opportunists, environmental opportunists and parasite opportunists (or zoonoses).

Given the failure of the two key assumptions of classical virulence evolution theory (Table 1), what can this body of theory tell us about OPs? For some parasites, the strict failure to meet these assumptions might not be important if the approximation is reasonable in practice, for instance if humans are the major source of parasite transmission (to any host) and the parasite does not routinely enter a commensal stage (e.g., *Salmonella enterica* among humans living in dense and unsanitary conditions). In these cases, standard predictions from virulence evolution theory may still apply, such as a trade-off between transmission and virulence [4,5]. Yet as the biological reality moves further away from these assumptions, we are left only with the prediction that multi-environment opportunists are likely to experience non-optimal virulence in a given host [6,7]. However, ecological and evolutionary theory offers an increasing number of insights into other key features of many opportunists, in particular plasticity and generalism. All human OPs are generalists in the sense that they are able to grow in more than one environment. In addition, many OPs display remarkable phenotypic plasticity, being able to modify phenotypic expression as a function of their changing environmental context.

If we can understand microbial plasticity and generalism, we can understand why opportunistic bugs become pathogens, when they are likely to do this and how we can interfere with their plastic responses to control their virulence in a sustainable manner. In this review, we aim to develop a general and integrative framework for the understanding and management (on ecological and evolutionary time scales) of opportunistic pathogens.

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Table 1. An ecological classification of pathogens with representative examples

	Obligate parasite	Facultative parasite
Specialist on humans	Current virulence evolution paradigm: Plasmodium falciparum , HIV, influenza virus (A, B, C), Mycobacterium tuberculosis	Commensal opportunists: Staphylococcus aureus , Enterococcus faecalis , Haemophillus influenza , Streptococcus pneumoniae
Non-specialist on humans (generalist)	Parasite opportunists (zoonoses): Borrelia burgdorferi , rabies virus, Salmonella spp., Bartonella henselae	Environmental opportunists: Pseudomonas aeruginosa , Burkholderia cepacia , Rhodococcus equi , Mycobacterium marinum , Vibrio vulnificus

What is virulence, and why damage your host?

For population biologists, virulence is typically the increase in host mortality resulting from parasite infection [1]. Although this is an explicit and measurable quantity, it ignores many aspects of parasite biology that cause harm without death [6,8–10]. For medical microbiologists, virulence is understood as harm or morbidity to the host, but the focus is on the mechanistic basis of harm, such as identifying virulence determinants or factors (VFs). VFs are typically defined as pathogen components whose loss specifically impairs virulence but not viability (in rich media); classic bacterial examples include toxins, exoenzymes, adhesins, and secretion systems [11].

VFs can be mechanistically complex and therefore are presumably products of natural selection. However, the nature of selection for maintaining and strengthening VFs remains controversial. Levin and Svanborg Edén made an important distinction between direct and coincidental selection for VFs [12]. Under direct selection (by far the most influential model), expression of the VF is correlated with the ability of the parasite to exploit and/or be transmitted from the host; in other words, parasitic VF expression (and consequent costs in terms of host mortality) is rewarded by some benefit. These benefits can either be gained through transmission [1,2] or through within-host growth [13]. This dichotomy, highlighting the importance of multiple scales in disease processes [14], forms the basis of the standard evolutionary view of virulence.

By contrast, purely coincidental selection argues that VF expression is not positively correlated with any measure of parasitic success within the focal host; in other words, there are no benefits in the parasitic context. VFs are then fascinating spandrels [15,16], byproducts of selection for adaptations not related to disease. The mystery of why a VF exists must then be answered elsewhere in the parasite life history, with VF maintenance caused by some benefit in an extra-host habitat or a within-host habitat in which the organism does not cause disease. In this case, can we still make general statements about the dynamics (ecological and evolutionary) of virulence, or are we relegated to case-by-case considerations [12,17]? To begin to answer this challenge, we develop a descriptive model framework to outline how four key selective pressures (coincidental, colonisation, export, and within-host) can combine to shape the evolutionary dynamics of VFs.

Virulence factor dynamics across multiple environments

A characteristic of all opportunistic pathogens is compartmentalisation into environments where they cause disease (e.g., burn wounds for *P. aeurignosa* and the circulatory system for *S. pneumoniae*) and environments where they do not (soil and nasopharynx, respectively). This compartmentalisation can be within a focal host (in particular, either side of mucosal barriers) or between a focal host and another environment (e.g., animal reservoirs vs human hosts). To conceptualise this split, we divide the world of a microbe into two compartments: the virulence compartment V, the sensitive parts of a focal host where microbial VFs result in disease symptoms; and the asymptomatic compartment A, which is everywhere else it can grow (Figure 1a, schema inspired by [18]). In Figure 1b–d, we illustrate how four selective pressures (coincidental, colonisation, export and within-host; the four arrows in Figure 1a) can combine to recover existing theories on the evolution of virulence (Box 1).

The analysis in Box 1 illustrates that if the within-host and transmission (colonisation and export) pressures

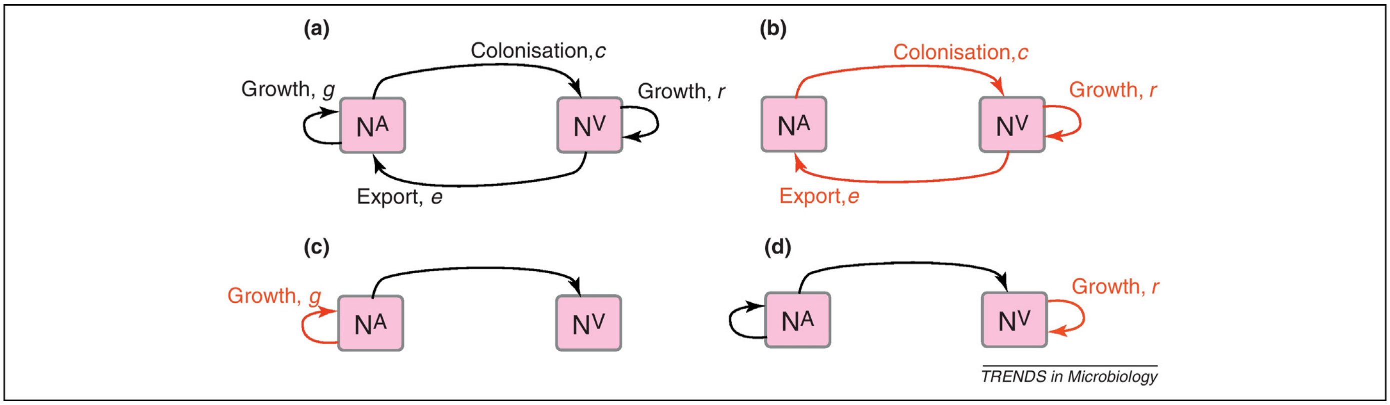

Figure 1. Ecological and evolutionary dynamics of virulence factors across two growth environments. N^A and N^V represent bacterial densities in the asymptomatic and virulence sites, respectively. Arrows represent demographic processes of growth (g, r) and transmission (colonisation c, export e). Red arrows represent a positive selective impact of VF expression. **(a)** Our basic ecological model (see Box 1 for analysis). **(b)** Epidemiological selection [1,2]; the dominant theoretical paradigm for virulence evolution states that virulence factors yield a net benefit during parasitic exploitation because of positive effects on within-host growth and/or transmission. **(c)** Purely coincidental selection [12]. **(d)** Purely within-host selection [13].

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