Predatory Bacteria: A Possible Key for the Lock of Antibiotic Resistance

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ABSTRACT Antimicrobials that previously brought the medical sciences to a new strata are in grave danger due to the spread of antimicrobial resistance (AMR). Predatory bacteria that were discovered in the 1960s offer a glimmer of hope as they demonstrate the capacity to predate upon pathogenic Gram-negative bacteria. The current review attempts to describe the therapeutic potential of bacterial predators in multiple medical conditions that have bacteria as the aetiologic agent. In the same vein, the review provides accounts in which the safety of bacterial predators are substantiated in addition to providing a discourse which evaluates their usage method(s) that would offer the highest degree of benefit in clinical settings.

KEYWORDS: Antimicrobial Resistance (AMR), BALO, *Bdellovibrio*, *Micavibrio,* Predatory bacteria.
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Review Article

INTRODUCTION

As soon as one hears the word 'antibiotic', a significant portion of individuals would associate its significance with Alexander Fleming and Paul Ehrlich for their work in developing penicillin and arsphenamine (Salvarsan) respectively (Aminov, 2010). Interestingly, this would not be the first instance in which humans were exposed to antibiotics, as researchers have identified the presence of tetracycline in human skeletons from ancient Sudanese Nubia dating back to 350-550 AD (Bassett *et al*., 1980; Nelson *et al*., 2010). Similarly, herbs used in traditional Chinese medicine (TCM) have also been noted to possess antimicrobial activity (Wong *et al*., 2010). The discovery of antibiotics has allowed mankind to benefit greatly in a myriad of aspects over the past seven decades. All good things come to an end, and this saying could apply to antibiotics as well in the near future due to the worsening problem of antimicrobial resistance (AMR).

AMR is a rather archaic problem as evidenced by the analysis of ancient DNA from 30,000 year old Beringian permafrost sediments which revealed the presence of an array of genes that encode resistance towards β-lactam, glycopeptide and tetracycline antibiotics (Dcosta *et al*., 2011). AMR is a pressing matter as the negative impact affects the society on multiple areas ranging from increased healthcare costs up until elevated morbidity and mortality (Friedman *et al*., 2016). The AMR predicament shows no sign of waning and may even cripple healthcare systems in the future as bacteria have demonstrated resistance towards the aminoglycoside, penicillin, quinolone, sulfonamide and tetracycline classes of antibiotics which are all commonly employed to ameliorate bacterial infections (Zaman *et al*. 2017). In the midst of this global crisis, a potential solution is around the horizon if one were to explore one of the fundamental laws of nature, prey-predator interaction. Predator-prey relationships are present everywhere and the predatory techniques employed by organisms differ greatly. The microbial world is no stranger to these interactions and it has even been postulated that bacterial predation is one of the forces that influences bacterial shape and size (Young, 2006).

The discovery of predatory bacteria was a fortuitous one as the initial member, *Bdellovibrio* was discovered in the 1960s by researchers that were hunting for bacteriophages in soil samples (Stolp

and Starr, 1963). Since then, multiple *Bdellovibrio* and like organisms (BALOs) have been identified and these can be classified under the α-proteobacteria and δ-proteobacteria classes respectively (Rotem *et al*., 2014). The BALOs in the α-proteobacteria class are members of the *Micavibrio* genus while the BALOs in the δ-proteobacteria class are from three distinct families which would be *Bacteriovoracaceae, Bdellovibrionaceae* and *Peridibacteriaceae* (Rotem *et al*., 2014). The genus *Bdellovibrio* belongs to the *Bdellovibrionaceae* family and consists of two members which would be *Bdellovibrio bacteriovorus (B. bacteriovorus)*, a periplasmic predator and *Bdellovibrio exovorus (B. exovorus)*, an epibiotic predator. The genus *Micavibrio* also has two members, *Micavibrio admirantus (M. admirantus)* and *Micavibrio aeruginosavorus (M. aeruginosavorus)* which are both epibiotic predators (Lambina *et al*., 1982; Wang *et al*., 2011). In regard to the context of this review, only members of the genus *Micavibrio* and *Bdellovibrio* would be discussed moving forward as medical applications have only been described for these organisms.

The discovery of *Bdellovibrio* and other members resulted in the birth of a new research area as the academic community became interested in identifying their potential value in clinical settings with particular interest being placed in exploiting them as "living antibiotics" (Dwidar *et al*., 2012; Gupta *et al*., 2016). However, there is a dearth in literature as there has not been a thorough discussion pertaining the use of obligate predatory bacteria in healthcare settings. Within this context, the review attempts to describe the potential for their usage as treatment options for ailments in addition to discussing administration methods for their optimal therapeutic effect.

POTENTIAL MEDICAL AREAS OF APPLICATION

The key healthcare application of predatory bacteria would be to treat ailments in which multi drug resistant (MDR) organisms are the aetiologic agents. Dharani and co-workers lucidly illustrated this avenue in their study where they compared the susceptibility of multiple pathogens harbouring the *mcr-1* gene that encodes resistance to colistin and their *mcr-1* negative counterparts towards predation by *B. bacteriovorus* and *M. aeruginosavorus,* in which they identified that there was no alteration in predation between the two variants (Dharani *et al*., 2018). Similarly, another group reported that MDR strains of *Acinetobacter baumannii (A. baumannii), Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae)* and *Pseudomonas* spp. were highly susceptible to predation by *B. bacteriovorus* 109J and *B. bacteriovorus* HD100 and to a lesser degree by *M. aeruginosavorus* strain ARL-13 (*A. baumannii* and *E. coli* were not assessed for susceptibility to predation by *M. aeruginosavorus*) (Kadouri *et al*., 2013). These findings are in line with reports from other groups that mention the capability of bacterial predators to prey upon MDR strains of pathogens as well, albeit at varying levels of efficacy (Shanks *et al*., 2013; Sun *et al*., 2017; Willis *et al*., 2016). It is worth noting that in most of the studies, bacterial predators demonstrated excellent activity against *A. baumannii, K. pneumoniae* and *Pseudomonas aeruginosa (P. aeruginosa)*, that are all members of the "ESKAPE" class of pathogens which cause the lion's share of nosocomial infections (Navidinia, 2016).

There exists a considerable body of literature pertaining the use of bacterial predators for ameliorating periodontitis and this could potentially be due to the fact that the disease is associated with Gram-negative organisms that form polymicrobial biofilms (Berezow and Darveau, 2011). Interestingly, previous research illustrates that only *B. bacteriovorus* exhibits significant potential as an addition to the armamentarium against periodontitis, as predation upon both aerobic and anaerobic pathogens has been observed. Indeed, an investigation which evaluated multiple BALOs for their predation capacity on a panel of periodontitis pathogens revealed that *B. bacteriovorus* HD100 had the highest versatility as it was capable of preying upon four of the six pathogens evaluated which would be *Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans), Eikenella*

corrodens (E. corrodens), Fusobacterium nucleatum (F. nucleatum) and *Prevotella intermedia (P. intermedia)* (Van Essche *et al*., 2011). Prior research corroborates these findings (Loozen *et al*., 2015; Patini *et al*., 2019), and increased the predation spectrum of *B. bacteriovorus* HD 100 as predation upon *Porphyromonas gingivalis (P. gingivalis)* was also documented (Loozen *et al*., 2015). *B. bacteriovorus* 109J has also been explored as a treatment option but the predation spectrum is narrower as accounts of predation are only present against *A. actinomycetemcomitans, E. corrodens* and *P. intermedia* (Dashiff and Kadouri, 2011; Patini *et al*., 2019). The oral cavity is a well aerated environment yet fascinatingly a large portion of the organisms are facultative or obligate anaerobes (Faran Ali and Tanwir, 2012). The usage of bacterial predators as anti periodontitis agents *in vivo* is a grey area as of now because their aerobic lifestyle (Rotem *et al*., 2014), dictates that they would not be able to survive the conditions yet the survival of halotolerant *Bdellovibrio* AP cells in anoxic conditions for nine days suggests otherwise (Schoeffield *et al*., 1996).

The prevalence of biofilms in acute and chronic wounds is about 6% and >90% respectively (Attinger and Wolcott, 2012). Biofilms impede wound healing severely and addressing it proves difficult due to its adherence to surrounding tissue, resistance to antimicrobials and evasion of the immune system (Attinger and Wolcott, 2012). Predatory bacteria demonstrate immense potential for usage within this context as prior research illustrates that biofilms offer no protection against predation (Kadouri and O'Toole, 2005). *In vivo* usage of predators as topical applications to clear biofilms has not been attempted hitherto, yet their spectrum of activity suggests that this could be possible. *B. bacteriovorus* HD 100 (Im *et al*., 2018; Kadouri *et al*., 2013; Monnappa *et al*., 2014; Pantanella *et al*., 2018), *B. bacteriovorus* 109J (Dashiff *et al*., 2011; Kadouri *et al*., 2013; Sun *et al*., 2017), and *M. aeruginosavorus* strain ARL-13 (Dashiff *et al*., 2011; Kadouri *et al*., 2007, 2013), have all been identified to be effective against a range of pathogens that are notorious biofilm producers such as *A. baumannii, E.coli, Enterobacter* spp., *K. pneumoniae, Proteus* spp., *P. aeruginosa* and *Staphylococcus aureus (S. aureus)*. The activity demonstrated against the biofilms of *S. aureus* is interesting as it has been previously believed that bacterial predators can only prey against Gram-negative organisms. A previous study revealed that *B. bacteriovorus* HD 100 was able to secrete two different proteases (Bd2269 and Bd2692) to hydrolyse *S. aureus* biofilms which in turn released amino acids that benefited the predators energetically (Im *et al*., 2018). A similar finding was reported as culture supernatant from a host-independent mutant of *B. bacteriovorus* HD100 effectively dispersed *S. aureus* biofilms and attenuated virulence without affecting the viability of the pathogen (Monnappa *et al*., 2014). The wide spectrum of activity demonstrated by bacterial predators coupled with their non-toxic nature and their secreted proteases against human cells (Gupta *et al*., 2016; Pantanella *et al*., 2018), suggest that direct topical application on wounds is a direction worth exploring.

Despite the extensive array of diagnostic tools available, it is a paradox that the aetiologic agents for pneumonia are not discovered in most cases (Rames, 2019). However in cases that do have a causative agent identified, bacteria are the most common culprit and they pose the problem of AMR (Rames, 2019; Sattar and Sharma, 2018). *In vitro* experimentation has revealed that *B. bacteriovorus* HD 100 and *B. bacteriovorus* 109J are able to prey upon common causative agents of pneumonia such as *A. baumannii, K. pneumoniae* and *P. aeruginosa* regardless of their drug resistance status (Dharani *et al*., 2018; Kadouri *et al*., 2013). Similar results have been reported for *M. aeruginosavorus* strain ARL-13 albeit at lower levels of efficacy (Dharani *et al*., 2018; Kadouri *et al*., 2007, 2013). Interestingly, bacterial predators demonstrate predation upon *Yersinia pestis (Y. pestis)* which is the aetiologic agent of plague (Russo *et al*., 2019, 2015). The activity against *Y. pestis* has also been observed *in vivo* as administration of *B. bacteriovorus* 109J in mice challenged with *Y. pestis* CO92 decreased colony forming units (CFUs) in the lung by a median of 86% within 24 h of inoculation (Russo *et al*., 2019). Another *in vivo* study demonstrated greater than 3.0 $\log_{10}$ reductions in copy numbers of *K.*

pneumoniae in 83.3% of *B. bacteriovorus* 109J-treated rats and 66.6% of *M. aeruginosavorus*-treated rats (Shatzkes *et al*., 2016). Bacterial predators also provide hope for cystic fibrosis (CF) patients. *B. bacteriovorus* HD100 was able to prey on *P. aeruginosa* and *S. aureus* isolated from the sputa of CF patients and interestingly, the predator demonstrated a dual foraging strategy whereby the former was preyed upon periplasmically while the latter was preyed upon epibiotically (Iebba *et al*., 2014). Previous studies suggest that bacterial predators are not pathogenic to mammalian hosts even when administered intranasally. A prior investigation illustrated that intranasal administration of *B. bacteriovorus* 109J, *B. bacteriovorus* HD100 and *M. aeruginosavorus* strain ARL-13 to mice did not result in a sustained immune response or a decrease in viability (Shatzkes *et al*., 2015). The results align well with findings from another experiment (Shatzkes *et al*., 2016). Collectively, the literature shows that bacterial predators could be effective antibacterial agents and fears pertaining safety can be cast aside as experimental results suggest that predators do not induce a sustained immune response and are rapidly cleared from mammalian hosts.

Globally, bacterial keratitis is one of the most common causes for irreversible blindness (Al-Mujaini *et al*., 2009). The usage of contact lens predisposes individuals to bacterial infection, particularly by Gram-negative organisms from the genus of *Haemophilus, Moraxella, Serratia* and *Pseudomonas* (Al-Mujaini *et al*., 2009). A prior investigation probed the efficacy of bacterial predators to kill keratitis isolates of *P. aeruginosa* and *Serratia marcescens (S. marcescens)* (Shanks *et al*., 2013). *B. bacteriovorus* HD100 proved to be the most effective as 100% of isolates were susceptible while *B. bacteriovorus* 109J and *M. aeruginosavorus* strain ARL-13, were able to prey upon 70% and 86% of the isolates respectively. The authors did not evaluate the susceptibility of *S. marcescens* to *M. aeruginosavorus* in the previous study as existing literature demonstrates that the predator does not possess the capacity to prey upon the said pathogen (Dashiff *et al*., 2011; Kadouri *et al*., 2007). This inability could potentially be ascribed to the PrtS (serralysin) metalloprotease secreted by *S. marcescens* but results illustrate that the metalloprotease does not affect predation by *B. bacteriovorus* (Garcia *et al*., 2018). The mechanism by which protection is conferred remains unclear but it has been identified that the metalloprotease does not affect the pathogen's viability (Garcia *et al*., 2018). Experimentation using an *in vitro* model of infectious bovine keratoconjunctivitis (IBK), a disease caused by *Moraxella bovis (M. bovis)* demonstrated *B. bacteriovorus* 109J is an effective antibacterial agent (Boileau *et al*., 2011), while an *in vivo* study for the same pathogen suggested otherwise (Boileau *et al*., 2016). The literature pertaining the safety of bacterial predators as antibacterial agents on the corneal surface provides assurance. Indeed, challenging human corneal-limbal epithelial (HCLE) cells and human stromal keratocytes with *B. bacteriovorus* 109J, *B. bacteriovorus* HD100 and *M. aeruginosavorus* strain ARL-13 revealed that cell viability was unaffected (Romanowski *et al*., 2016; Shanks *et al*., 2013). *In vivo* safety assessments in rabbits substantiate the safe status of predators even further (Romanowski *et al*., 2016). An overview of literature suggests that not much effort has been placed in exploring bacterial predators as a treatment option, as evidenced by the lack of studies evaluating their predation capability on ocular pathogens. However, it is proffered that this avenue should be researched further given that bacterial predators appear to be harmless to the corneal surface.

Ten to twenty percent of acute gastroenteritis cases have bacteria as the aetiologic agent with the most common organisms hailing from the genus *Campylobacter, Salmonella, Shigella* and *Yersinia* (Chiriac *et al*., 2017). Bacterial predators illustrate potential for usage within this avenue as *B. bacteriovorus* HD100 has been identified to prey upon *Campylobacter jejuni (C. jejuni), E. coli, Helicobacter pylori (H. pylori), Salmonella typhimurium (S. typhimurium), Shigella dysenteriae (S. dysenteriae)* and *Vibrio cholerae (V. cholerae)* (Markelova, 2010). The data is corroborated by other research groups as activity against other gastrointestinal pathogens from the genus of *Salmonella*

(Fratamico and Cooke, 1996), *Shigella* (Gillis and Nakamura, 1970), and *Yersinia* (Dashiff *et al*., 2011), has been documented. The activity of *B. bacteriovorus* on *C. jejuni* is an area of ambiguity as there are conflicting reports pertaining efficacy (Dashiff *et al*., 2011; Markelova, 2010). Bacterial predators could potentially be used to address infections due to *Helicobacter pylori (H. pylori),* a group 1 carcinogen because multiple strains of *Bdellovibrio* demonstrate predation capacity against the said pathogen in both viable and viable but nonculturable (VBNC) states (Markelova, 2010; Parikh and Ahlawat, 2020). In the same vein, *in vivo* evaluation of *Salmonella enterica (S. enterica)* clearance by *B. bacteriovorus* HD100 in chicks revealed that only a modest reduction by 1.0 $\log_{10}$ occurred hence suggesting that further work is needed to optimize the dosing regiments and conditions (Atterbury *et al*., 2011). The safety data pertaining use of bacterial predators for gastrointestinal ailments is limited. Indeed, hitherto there is only one study evaluating this aspect and the results obtained *in vivo* suggest that bacterial predators do not cause negative ramifications (Atterbury *et al*., 2011). However, the predators did induce some changes in cecal bacterial populations but the consequences of these changes are unclear. Cumulatively, studies suggest that employing predatory bacteria in ameliorating bacterial gastroenteritis is a promising avenue, yet further studies pertaining *in vivo* safety and efficacy are warranted.

ADMINISTERING PREDATORS: MONOTHERAPY OR COMBINATORIAL THERAPY?

Granted that bacterial predators hold the keys to be potential vanguards against AMR in bacteria, it is imperative that efforts are made to discern the manner of administration that would bring maximal benefit. Taking into account the myriad of avenues in which bacterial predators can be employed, it is convenient to assume that they could be a panacea for Gram-negative infections but this would be erroneous. Despite prior research showing very promising results, it is worth noting that only pathogen reduction was reported and no complete elimination of pathogens by bacterial predators has been observed. The prey-predator relationship of *B. bacteriovorus* (and possibly other BALOs) are governed by the Lotka-Volterra prey-predator oscillation (Varon and Zeigler, 1978), and as per theory persistent predator-prey systems should demonstrate cyclic oscillations in the absence of additional stabilizing mechanisms (Tahara *et al*., 2018), hence suggesting that prey would adapt to the challenge posed by predators thus preventing complete wipeout. Indeed, a prior investigation highlighted that cocultivating *B. bacteriovorus* and *Erwinia carotovora* ssp. *carotovora* (a plant pathogen), led to an increase in prey cells that demonstrated resistance to the predator (Shemesh and Jurkevitch, 2004). In the same study, the authors re-attempted the investigation with other predators and prey, which yielded similar findings. However, it should be noted that this resistance was not due to a mutation and instead was a phenotypic plastic response as predator removal restored susceptibility (Shemesh and Jurkevitch, 2004). This demonstrates an advantage over antibiotics, as permanent resistance towards them are generally acquired via mutational resistance or horizontal gene transfer (Hoffman 2001). Nevertheless, this does not imply that permanent resistance against predatory bacteria is not possible. Reports are somewhat conflicting, but literature states that the type IV pili is employed by *Bdellovibrio* to irreversibly attach to prey cells (Mahmoud & Koval 2010; Avidan *et al*. 2017; Duncan *et al*. 2019). However, the exact structure to which the pili attach and gain entry into prey cells is an enigma, not just for *Bdellovibrio* but for all other BALOs as well. Therefore, it is not impossible that in any given prey population mutants may evolve to obviate this hypothetical structure.

Considering the variation of the *in vivo* activity of bacterial predators for different ailments, it is postulated that combinatorial therapy would be the ideal course of action. A study reported that combined usage of *B. bacteriovorus* HD100 and phage on *E. coli* S17-1 caused eradication of prey after 14 h by decreasing their numbers to below detectable levels (<10 CFU/mL) (Hobley *et al*., 2020).

Similarly, another combination that promises usage in polymicrobial infections would be the coadministration of predators and violacein where the former would act on Gram-negative organisms while the latter would act on Gram-positive organisms. It was identified that dual treatment of violacein and *B. bacteriovorus* HD100 on a coculture of *S. aureus* and *K. pneumoniae* caused a 99.999% reduction in pathogen viability, which was higher than individual treatments with violacein and *B. bacteriovorus* HD100 that caused pathogen viability reduction by 41% and 81% respectively (Im *et al*., 2017). *B. bacteriovorus* 109 is resistant towards penicillin as no variation in attachment or invasion of *E. coli* was observed post-treatment (Varon and Shil, 1968). Genomic analysis of multiple predators also revealed that both epibiotic and periplasmic predators have an array of antibiotic resistance genes that mostly encode for efflux pumps (Pasternak *et al*., 2014). Given the ability of predators to resist antibiotics, dual therapy employing antibiotics and predatory bacteria appears to be a promising avenue.

Monotherapy with bacterial predators could also be an attractive avenue in specific scenarios. An *in vivo* experiment in zebrafish revealed that *B. bacteriovorus* worked in tandem with eukaryotic leukocytes to clear an AMR variant of *Shigella flexneri (S. flexneri)* M90T (Willis *et al*., 2016). The authors proposed that the predatory bacteria decreased pathogen levels to a manageable degree which were then cleared by the vertebrate innate immune system. The observation that bacterial predators are unable to completely clear pathogens is analogous with antibiotics (Zaman *et al*. 2017), yet they come with the benefit of not having negative side effects (none have been recorded hitherto) unlike antibiotics that may cause undesirable side effects such as gastrointestinal, haematological and nervous disturbances (Heta & Robo 2018). Despite the potential for monotherapy, it is most likely not possible for *M. aeruginosavorus*. Across the board, the predator appeared to be less effective in comparison with *B. bacteriovorus*. This could potentially be ascribed to the predator's life cycle. Indeed, *Bdellovibrio* spp., undergo replication via filamentous fragmentation in the periplasm of their prey which is followed by the release of multiple progeny (Rotem *et al*., 2014), while *Micavibrio* spp., divide via binary fission which leads to the formation of only a single daughter cell (Afinogenova *et al*., 1987; Lambina *et al*., 1982). Due to this, it is recommended that the usage of *Micavibrio* spp., should be with the co-administration of another agent.

Based on the above points, one would stumble upon the question, "Would a broad spectrum agent be better or would an agent with a limited spectrum suffice?" The answer to this is hard to determine as it is affected by an interplay between healthcare and economics. By using antibiotics as a reference point, broad spectrum agents would be more economically rewarding as they can act on multiple organisms but come at the cost of increased selection for resistance and alterations of the microbiome (Melander *et al*., 2018). Narrow spectrum agents do not promote cross-resistance in non-target organisms and have decreased collateral effects upon the microbiome yet their economic potential is lower as their usability would be possible only in specific conditions (Melander *et al*., 2018). In the context of bacterial predators obtaining broad spectrum agents is possible as repeated passage on a particular pathogen increases the prey range (Boileau *et al*., 2011), and isolation of novel BALOs may yield predators with a wider predation spectrum. Obtaining narrow spectrum agents could be possible by isolating BALOs in particular areas to be suited for specific niches. For instance, the isolation of BALOs that survive and possibly thrive in anoxic conditions (Schoeffield *et al*., 1996), could permit usage against anaerobic pathogens. Similarly, it may be possible to reprogram bacterial predators to seek out specific pathogens as there has been a report of reprogrammed *E. coli* that specifically targets *P. aeruginosa* (Hwang *et al*., 2014).

CONCLUSION

The current global crisis of AMR is an issue that needs to be addressed and bacterial predators offer a potential path forward. The present findings illustrate that predatory bacteria demonstrate impressive activity *in vitro* against multiple pathogens but these results are not necessarily indicative of *in vivo* efficacy. Despite the efficacy of predators, monotherapy does not appear to be the best course of action as development of plastic phenotypic resistance may occur. As such, combinatorial therapy would be the preferred method of use as bacterial predators appear to function better as an ancillary therapy, yet monotherapy could be suitable in specific conditions. Existing literature states that bacterial predators would only be effective against Gram-negative organisms yet the activity against *S. aureus* suggests otherwise. Therefore, efficacy on other Gram-positive organisms and intracellular organisms should be assessed. In the same vein, safety data pertaining usage of predatory bacteria *in vivo* is limited in some areas, hence research to address these gaps is warranted.

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