PREDATORY BACTERIA OR "LIVING ANTIBIOTICS" – A NOVEL WAY TO COMBAT INFECTION

Viktoria Gerasimova[1], Rozalina Yordanova[2]
[1,2,3]Medical College, Trakia University, Stara Zagora, Bulgaria
6000 Stara Zagora, 9 Armeyska Street, Bulgaria
[1]Email: *wiki_iwanowa@abv.bg*
[2]Email: *rozalina_@abv.bg*

Introduction:

Antibiotic resistance has become one of the biggest threats to global health and is rising to dangerously high levels in all around the world. New resistance mechanisms are emerging and spreading globally leading to the emergence of untreatable superbugs. In the 1960s, an alternative approach to traditional antibiotics was discovered - predatory bacteria, a new group of bacterial pathogens "enemies".

The purpose of this review is to provide up-to-date information about Bdellovibrio-and-like organisms (BALOs).

Materials and methods: review of scientific literature, studies, and international experience that provide information on predatory bacteria.

Conclusion: The antibiotic-resistance crisis has inspired researchers in recent years to look for new approaches to treat life-threatening bacterial infections. To date, the ability of predatory bacteria to prey efficiently on Gram-negative bacteria suggests a promising, novel way to combat infection. Future studies should focus on assessing the efficacy of predatory bacteria to prey on Gram-negative pathogens in vivo.

Key words: *predatory bacteria, Bdellovibrio bacteriovorus, Micavibrio aeruginosavorus, antibiotic resistance.*

For seven decades, humankind has benefited from the availability of antibiotics to treat bacterial infections [4]. However, the overuse of antibiotics has put pressure on bacteria to evolve resistance against these drugs, leading to the emergence of untreatable superbugs [29]. Antibiotic resistance has become one of the biggest threats to global health and is rising to dangerously high levels in all around the world. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases [31]. Diseases due to antibiotic-resistant pathogens require longer hospital stays treatments tend to higher costs, high economic burden on health care system and more toxic side effects.

In 2016 The United Nations General Assembly met to discuss the seriousness and scope of antimicrobial resistance, in the face of predictions that if antimicrobial resistance continues to spread at current rates, there would be an estimated 10 million deaths globally by 2050 [15]. In 2018, the World Health Organization (WHO) released surveillance data confirmed that around the world, some of the most common infections are becoming drug resistant, resulting in longer illnesses and tens of thousands deaths each year [30]. At the same time, not enough new antimicrobial drugs, especially antibiotics, are being developed to replace older and increasingly ineffective ones, which means that the types and number of new drugs in the pipeline will not be sufficient to overcome the threat. While the creation or discovery of new antibiotics with novel mechanisms of action, changes in treatment modalities, the prudent use of antibiotics are one solution, alternative approaches are also required for reducing the threat from multi-drug resistant bacterial infections[18].

In the 1960s, a new group of "enemies" of bacterial pathogens was discovered - predatory bacteria. Predatory bacteria represent an alternative approach to traditional antibiotics that target essential cellular functions such as protein, DNA, RNA and cell wall synthesis[18]. The group of

Gram-negative proteobacteria including the species *Bdellovibrio bacteriovorus* and *Micavibrio aeruginosavorus* have been hypothesized to be useful "living antibiotics" [16,22,23].

The initial discovery of *Bdellovibrio* happens accidentally while phage is searched in soil samples [24]. In 1982, while searching for *Bdellovibrio* samples in wastewater, researchers isolated a new species of exoparasitic Bdellovibrio-like bacteria that they called *Micavibrio*[13].

B. bacteriovorus have been found in soil samples, rhizosphere of plant roots, rivers, oceans, sewage, intestines and feces of birds and mammals, and even in oyster shells and the gills of crabs [28]. *B. bacteriovorus* are able to thrive in almost any habitat. They need only oxygen and some other Gram-negative bacteria present in its environment.

Bdellovibrio bacteriovorus and *Micavibrio aeruginosavorus* are small, highly motile, uniflagellate Gram-negative bacteria that prey naturally on other Gram-negative bacteria[14,24]. *Bdellovibrio bacteriovorus* is a deltaproteobacterium which invades the periplasm of other Gram-negative bacteria, replicates, and finally lyses the host cell [21,25]. *Micavibrio aeruginosavorus* does not invade its prey like *Bdellovibrio* but rather feasts upon it by attaching themselves to the outer membrane [13,27]. *Micavibrio* unlike *Bdellovibrio*, cannot be cultured without prey and it is an exceptional predator of *Pseudomonas aeruginosa* and *Escherichia coli* [5,11,12]. From literature it was reported that *B. bacteriovorus* is capable to attack different Gram-negative bacterial genera (*Escherichia, Salmonella, Legionella, Pseudomonas, Acinetobacter baumannii and Klebsiella pneumoniae, Proteus* species) and their pre-formed biofilms [11,12,22] and *A. hydrophila* infection in fish [2,3]. It shown to be a useful biocontrol agent against *Salmonella Enterica* in young chickens[1].

Bdellovibrio-and-like organisms (BALOs) are a promising potential novel agent against bacterial pathogens and present several advantages when considering their use for controlling infection [7]. Previous studies have confirmed the ability of predatory bacteria to control a broad range of important human pathogens in vitro, including multidrug resistant bacteria [16], grown both planktonically and in biofilms [5,10,11]. Many authors reported the predatory efficiency of BALOs against *Escherichia coli* in urinary tract infections, enterohemorrhagic *Escherichia coli* in gastrointestinal infections and wound, burn and respiratory infections caused by *Pseudomonas aeruginosa* [7]. In addition, BALOs appear to have no negative effect on human cells when challenged in vitro [20]. Bdellovibrio organisms also have the ability to survive in the absence of prey species in a growth phase known as the preyor host-independent (HI) state, and these cells are isolated from prey- or host-dependent (HD) cells [17]. Due to its selective preying capacity on Gram-negative bacterial species [9,17] *B. bacteriovorus* is probably involved in keeping the balance between the different bacterial species living together in a community, acting as an ecological balancer species" [7,26,28]. It was recently found in the human gut of all healthy individuals examined and might be native commensals of the human gut and might even play a role in maintaining healthy gut flora [8]. *Bdellovibrio* species are reported to be unable to prey on eukaryotic cells, and as such, they pose no direct risk to human or animal health [1]. BALOs also have wide applications in food industry, agricultural field, animal husbandry. As *M. aeruginosavorus* reduce bacteria that form biofilms it also might have industrial uses, such as reducing biofilms in piping, and for medical devices, such as implants that are susceptible to the formation of biofilms.

Such studies have now shown that *B. bacteriovorus* has real potential in a therapeutic setting. Safety profiling in animals has revealed that they show generally low immunogenicity compared with bacterial pathogens, do not damage eukaryotic cells, and are well-tolerated in vivo [31]. When delivered to a bodily site of infection, *B. bacteriovorus* persists long enough to be predatorily effective against its bacterial prey, but the predator population is ultimately transient

and cleared by the host immune system [31]. Finally, its broad prey range and recently reported therapeutic efficacy, in a range of discrete physiological locations in live animals, suggest *B. bacteriovorus* could be a safe treatment option to tackle a variety of serious gram-negative infections [31].

Bdellovibrio strains have long been proposed as a future alternative for antimicrobial therapy, and it has been suggested that they would be suitable for external use (such as in infected skin wounds [22]. Predatory bacteria attack and destroy Gram-negative bacteria irrespective of growth state or antibiotic resistance status.

The antibiotic-resistance crisis has inspired researchers in recent years to look for new approaches to treat life-threatening bacterial infections. One biologically-based microbial control strategy is the use of predatory bacteria [7]. To date, the ability of predatory bacteria to prey efficiently on Gram-negative bacteria suggests a promising, novel way to combat infection. However, while efficacy of predatory bacteria has been shown in vitro, before they can be used clinically, their safety in a mammalian host must be confirmed. Future studies should focus on assessing the efficacy of predatory bacteria to prey on Gram-negative pathogens in vivo and giving insights into the predatory spectrum of BALOs.

References

1. Atterbury, R. J., L. Hobley, R. Till, C. Lambert, M. J. Capeness, T. R. Lerner, A. K. Fenton, P. Barrow, and R. E. Sockett. Effects of orally administered Bdellovibrio bacteriovorus on the wellbeing and Salmonella colonization of young chicks. Appl Environ Microbiol 77:5794-803, 2011.
2. Cao, H., S. He, H. Wang, S. Hou, L. Lu, and X. Yang. Bdellovibrios, potential biocontrol bacteria against pathogenic Aeromonas hydrophila. Vet Microbiol 154:413-8, 2011.
3. Chu, W. H., and W. Zhu. Isolation of Bdellovibrio as biological therapeutic agents used for the treatment of Aeromonas hydrophila infection in fish. Zoonoses Public Health 57:258-64, 2010.
4. Damron F. Heath, Mariette Barbier, Predatory bacteria: Living Antibiotics, Biocontrol Agents, or Probiotics? Journal of Postdoctoral Research Vol. 1, No. 12, December 2013.
5. Dashiff, A., R. A. Junka, M. Libera, and D. E. Kadouri. Predation of human pathogens by the predatory bacteria Micavibrio aeruginosavorus and Bdellovibrio bacteriovorus. J Appl Microbiol 110:431-44, 2011.
6. David Negus, Chris Moore, Michelle Baker, Dhaarini Raghunathan, Jess Tyson, and R. Elizabeth Sockett Annu. Predator Versus Pathogen: How Does Predatory Bdellovibrio bacteriovorus Interface with the Challenges of Killing Gram-Negative Pathogens in a Host Setting? Rev. Microbiol. 71:441-457, 2017.
7. Dwidar, M., Monnappa, A. K., and Mitchell, R. J. (2012). The dual probiotic and antibiotic nature of Bdellovibrio bacteriovorus. BMB Rep. 45, 71–78. doi: 10.5483/BMBRep.2012.45.2.71.
8. Iebba, V., Santangelo, F., Totino, V., Nicoletti, M., Gagliardi, A., de Biase, R. V., et al. (2013). Higher prevalence and abundance of Bdellovibrio bacteriovorus in the human gut of healthy subjects. PLoS ONE 8: e61608. doi: 10.1371/journal.pone.0061608.
9. Jurkevitch E, Minz D, Ramati B, Barel G, Prey range characterization, ribotyping, and diversity of soil and rhizosphere Bdellovibrio spp. isolated on phytopathogenic bacteria. Appl Environ Microbiol 66: 2365–2371, 2000.

10. Kadouri, D., and O'Toole, G. A. (2005). Susceptibility of biofilms to Bdellovibrio bacteriovorus attack. Appl. Environ. Microbiol. 71, 4044–4051. doi: 10.1128/AEM.71.7.4044-4051.2005.
11. Kadouri, D., N. C. Venzon, and G. A. O'Toole. Vulnerability of pathogenic biofilms to Micavibrio aeruginosavorus. Appl Environ Microbiol 73:605-14, 2007.
12. Kadouri, D. E., K. To, R. M. Shanks, and Y. Doi. Predatory bacteria: a potential ally against multidrug-resistant Gram-negative pathogens. PLoS One 8:e63397, 2013.
13. Lambina, V. A., Afinogenova, A. V., Romai Penabad, S., Konovalova, S. M. & Pushkareva, A. P. [Micavibrio admirandus gen. et sp. nov]. Mikrobiologiia 51, 114–117, 1982.
14. Lambina, V. A., Afinogenova, A. V., Romay Penobad, Z., Konovalova, S. M. & Andreev, L. V. [New species of exoparasitic bacteria of the genus Micavibrio infecting gram-positive bacteria]. Mikrobiologiia 52, 777–780, 1983.
15. WHO (World Health Organization). United Nations High-Level Meeting on Antimicrobial Resistance. 2016. Available online: http://www.who.int/antimicrobial-resistance/events/UNGA-meeting-amr-sept2016/en/ (accessed on 20 December 2018).
16. Rendulic, S., Jagtap, P., Rosinus, A., Eppinger, M., Baar, C., Lanz, C., et al. (2004). A predator unmasked: life cycle of Bdellovibrio bacteriovorus from a genomic perspective. Science 303, 689–692. doi: 10.1126/science.1093027.
17. Rogosky AM, Moak PL, Emmert EA Differential predation by Bdellovibrio bacteriovorus 109J. Curr Microbiol 52: 81–85, 2006.
18. Russo Riccardo, Irina Kolesnikova, Thomas Kim, Shilpi Gupta, Androulla Pericleous, Daniel E. Kadouri and Nancy D. Connell; Susceptibility of Virulent Yersinia pestis Bacteria to Predator Bacteria in the Lungs of Mice; *Microorganisms* 2019, *7*(1), 2; https://doi.org/10.3390/microorganisms7010002.
19. Seidler, R. J., and M. P. Starr. Isolation and characterization of prey-independent Bdellovibrios. J. Bacteriol. 100:769–785, 1969.
20. Shanks, R. M. et al. An Eye to a Kill: Using Predatory Bacteria to Control Gram-Negative Pathogens Associated with Ocular Infections. PLoS One 8, e66723, doi: 10.1371/journal.pone.0066723 (2013).
21. Shemesh, Y., Y. Davidov, S.F. Koval, and E. Jurkevitch. Small eats big: ecology and diversity of Bdellovibrio and like organisms, and their dynamics in predator-prey interactions. Agronomie 23:433–439, 2003.
22. Sockett, R. E., and Lambert, C. (2004). Bdellovibrio as therapeutic agents: a predatory renaissance? Nat. Rev. Microbiol. 2, 669–675. doi: 10.1038/nrmicro959.
23. Sockett, R. E. Predatory lifestyle of Bdellovibrio bacteriovorus. Annu Rev Microbiol 63:523-39, 2009.
24. Stolp, H. & Starr, M. P. BDELLOVIBRIO BACTERIOVORUS GEN. ET SP. N., A PREDATORY, ECTOPARASITIC, AND BACTERIOLYTIC MICROORGANISM. Antonie Van Leeuwenhoek 29, 217–248 (1963).
25. Thomashow, M. F., and S. C. Rittenberg. Descriptive biology of the bdellovibrios. In J. H. Parish (ed.), Developmental biology of prokaryotes, 9th ed. University of California Press, Berkeley, CA, 1979.
26. Varon, M., Interaction of Bdellovibrio with Its prey in mixed microbial populations. Microb. Ecol. 7, 97–105. doi: 10.1007/BF02032491, 1981.

27. Wang, Z., D. E. Kadouri, and M. Wu. 2011. Genomic insights into an obligate epibiotic bacterial predator: Micavibrio aeruginosavorus ARL-13. BMC Genomics 12:453.
28. Yair, S., Yaacov, D., Susan, K., and Jurkevitch, E. Small eats big: ecology and diversity of Bdellovibrio and like organisms, and their dynamics in predator-prey interactions. Agronomie 23, 433–439. doi: 10.105, 2003.
29. https://phys.org/news/2017-03-predatory-bacteria-antibiotic.html; Predatory bacteria as a new 'living' antibiotic March 21, 2017.
30. https://www.who.int/news-room/detail/29-01-2018-high-levels-of-antibiotic-resistance-found-worldwide-new-data-shows.
31. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance, 5 February 2018.