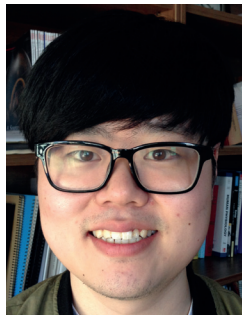
Future MICROBIOLOGY

EDITORIAL

Violacein and bacterial predation: promising alternatives for priority multidrug resistant human pathogens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Within our lab, we often joke that the true meaning of research is to re-search, or to search again. As scientists, we search out the deeper truth of nature and try to understand it and how it can be applied for the betterment of humankind. This was the basis of Alexander Fleming's observation that a contaminating mold inhibited the growth of a bacterium, which eventually led to the discovery of penicillin and the ushering in of the antibiotic era. Unfortunately, it would seem that era is coming to an end with the recent spread and development of many different multidrug resistant (MDR) pathogens, necessitating once more for scientists to re-search and re-evaluate alternatives to conventional antimicrobials. In this review, we discuss two alternatives, one relatively old and one relatively new, currently being pursued by various groups across the globe.

The need for alternatives to conventional antibiotics is strongly emphasized by two recent reports: the 'Review on Antimicrobial Resistance' [1], which was commissioned by the prime minister of the UK, and a news report from the WHO release 27 February 2017 [2]. In the first, it is predicted that the number of deaths annually attributed to MDR infections in 2050 will reach 50 million world-wide. These also highlighted the impact living in a world where antibiotics no longer work, where common surgeries such as cesarean sections will become high risk due to lack of prophylactic antibiotics. The report from WHO, which was published earlier this year, listed a dozen priority pathogens for which new antibiotics are currently needed. This list includes antibiotic resistant strains of *Acinetobacter baumannii*,

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Staphylococcus aureus and *Shigella* spp. Here, we discuss the strengths and weaknesses of two alternatives being researched by various groups.

Violacein: a long-forgotten candidate that is effective against Gram-positive bacteria

With many antibiotics increasingly becoming obsolete due to widespread resistance, one avenue for researchers is to re-evaluate those candidates that were originally set aside for more promising drugs. One such case is violacein, a bisindole molecule produced by various bacterial species, including *Janthinobacterium* sp., *Chromobacterium* sp. and *Duganella* sp. [3]. This compound and its activity against Gram-positive bacteria were first described in 1945 [4]. In part due to the low-level production by natural strains, however, which typically reaches concentrations of only several mg/l, violacein was not seriously considered at that time.

"Another benefit of predatory bacteria over chemical antibiotics is their activity against biofilms..."

Interest in violacein and its activity against *S. aureus* has grown during the last decade, however. In 2011 it was shown to be effective against 15 strains of *S. aureus*, including numerous penicillin-resistant and MDR *S. aureus* strains, with minimum inhibitory concentrations of 6.25 μM or greater [5]. This was followed by several additional studies, including one in 2015 where violacein was effective against one strain of *S. aureus* that is resistant to rifampin, ciprofloxacin, clindamycin, oxacillin, erythromycin, gentamycin and tobramycin [6]. The sensitivity of this MDR *S. aureus* to violacein (15 μM) was the same as *S. aureus* ATCC 25923, which was not resistant to any antibiotic. This implied the mechanism used by violacein to kill *S. aureus* differs from those used by the other antibiotics, and that resistance to those antibiotics does not extend to this bisindole. Violacein's mechanism was elucidated earlier this year; this antibiotic disrupts the membrane integrity, resulting in an efflux of cellular components, including ATP [7]. When vancomycin and violacein were added at their respective minimum inhibitory concentrations, violacein led to a fourfold greater leakage of cellular ATP from both *S. aureus* ATCC 29213 and a strain of methicillin-resistant *S. aureus*, ATCC 43300. Leakage of the cytoplasmic contents from the cells was further corroborated using SEM imaging, as the violacein-treated *S. aureus* cells appeared deflated.

The activity of violacein against *S. aureus* was also tested with 20 different commercial antibiotics, with most showing additive characteristics. The impact of violacein against *S. aureus* was synergistic when used with four of these antibiotics, including vancomycin, kanamycin, gentamycin and oxytetracycline [8]. The only antibiotic that they tested showing an antagonistic nature was cefdinir.

Bacterial predation: using bacteria as therapeutic agents against MDR pathogens

Bdellovibrio bacteriovorus is a predatory bacterium that actively attacks and consumes many other Gram-negative bacterial strains. To date, the activity of this bacterium has been demonstrated with well over a 100 different human pathogens, including strains of *Acinetobacter*, *Klebsiella*, *Salmonella* and *Yersinia* [9] and even those that are MDR [10]. As such, this bacterium has been labeled as a potential living antibiotic.

Laboratory tests show that when a prey culture is attacked by a predatory bacterium, its population typically drops by 2–7 log after 24 h [10,11], a decrease that is comparable with antibiotics [12]. By adjusting the predator-to-prey ratio, similar decreases were achieved within only 1 or 2 h [13], illustrating the rapid killing possible with *B. bacteriovorus*. In contrast with conventional chemical antibiotics, *B. bacteriovorus* not only kills the pathogens but also degrades and consumes the cellular DNA, including any antibiotic genes and recombinant DNA present within the prey [11]. Although not clearly demonstrated, this activity may reduce the chance for downstream horizontal gene transfer and the spread of antibiotic resistance markers to other bacterial strains.

Another benefit of predatory bacteria over chemical antibiotics is their activity against biofilms, a form of bacterial existence that contributes to chronic diseases and higher drug-resistance phenotypes [14]. Biofilms form when bacterial cells adhere to and propagate on a surface, forming a mass of cells that is held together by extracellular polymeric substances, that is, extracellular proteins, DNA and polysaccharides. Owing to their nature, biofilms can be as much as a 1000-fold more resistant to antibiotics, making their treatment and removal much harder [14]. Several studies have shown, however, bacterial predators can effectively remove bacterial biofilms from various surfaces, including polyvinyl chloride [15] and even human cells [16]. Given the results of the studies varied somewhat based upon the predatory and prey strains

employed, which included *E. coli*, *Pseudomonas* and *Shigella*, the biofilm populations were typically reduced 1- to 4-log after 24 h. Although *B. bacteriovorus* is limited to Gram-negative prey, one study showed proteolytic enzymes secreted by a host-independent variant of this strain can also disperse *S. aureus* biofilms [17]. As *S. aureus* is a Gram-positive bacterium, it is not predated upon and its viability did not decrease but the extracellular proteases hydrolyzed the extracellular polymeric substances, leading to a release of the *S. aureus* from the surface. Moreover, the proteases degraded many of the *S. aureus* surface proteins, which reduced its virulence. Not only were the biofilm populations in all of the above studies reduced but the physical presence of the biofilm on the surface was likewise mitigated after predation, as illustrated via crystal violet staining, fluorescence microscopy and scanning electron microscopy [15–17].

Although *B. bacteriovorus* and *M. aeruginosavorus* are both bacteria, recent studies have shown that they are not harmful to human cells [18,19]. Together, both studies evaluated the cytokine responses from nine different human cell lines and six different bacterial predators. For each combination of human cell and predatory bacteria tested, the cytokine responses were negative or, at most, mild when compared with the results with *P. aeruginosa* PA14 and *E. coli* MG1655, which were employed as positive controls. The viability tests corroborated the cytokine results as only one of the predatory strains led to a mild (around 8%) yet significant loss in the human cell viability. Two recent studies took this one step further and studied the *in vivo* impact of predatory bacteria within Zebrafish embryos and rats [20,21]. In both cases, the pathogen populations were attenuated by the activity of the predators, with no clear adverse effects on the host organisms, results that hint at the potential downstream application of predatory strains to treat MDR infections.

Future perspective

In this review, two alternatives to conventional antibiotics currently being researched and studied by various groups were discussed: violacein and predatory bacteria. Violacein, which is primarily active against Gram-positive bacteria, shows promising activity against *S. aureus*, including MDR strains. The drug-resistant natures of the *S. aureus* strains provided no obvious benefit or protection against violacein. Likewise, bacterial predators only attack Gram-negative bacteria, such as *A. baumannii* and *Shigella* spp., and research from Kadouri's group also suggests their activity is not hindered by the drug-resistant nature of their prey [8]. Given the clear activity of violacein and predatory bacterial strains against priority organisms, both of these antimicrobials represent potential alternatives to and supplements for conventional antibiotics.

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References

1 Wellcome Trust. Review on antimicrobial resistance. http://amr-review.org

2 World Health Organization. www.who.int/mediacentre/news

3 Choi SY, Yoon KH, Lee JI, Mitchell RJ. Violacein: properties and production of a versatile bacterial pigment. *Biomed. Res. Int.* 2015, 465056 (2015).

4 Lichstein HC, Vandesand VF. Violacein, an antibiotic pigment produced by *Chromobacterium violaceum*. *J. Infect. Dis.* 76(1), 47–51 (1945).

5 Cazoto LL, Martins D, Ribeiro MG, Duran N, Nakazato G. Antibacterial activity of violacein against *Staphylococcus aureus* isolated from bovine mastitis. *J. Antibiot. (Tokyo)* 64(5), 395–397 (2011).

6 Choi SY, Kim S, Lyuck S, Kim SB, Mitchell RJ. High-level production of violacein by the newly isolated *Duganella violaceinigra* str. NI28 and its impact on *Staphylococcus aureus*. *Sci. Rep.* 5, 15598 (2015).

7 Aruldass CA, Masalamany SR, Venil CK, Ahmad WA. Antibacterial mode of action of violacein from *Chromobacterium violaceum* UTM5 against *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA). *Environ. Sci. Pollut. Res. Int.* 10.1007/s11356-017-8855-2 (2017) (Epub ahead of print).

8 Subramaniam S, Ravi V, Sivasubramanian A. Synergistic antimicrobial profiling of violacein with commercial antibiotics against pathogenic micro-organisms. *Pharm. Biol.* 52(1), 86–90 (2014).

9 Dashiff A, Junka RA, Libera M, Kadouri DE. Predation of human pathogens by the predatory bacteria *Micavibrio aeruginosavorus* and *Bdellovibrio bacteriovorus*. *J. Appl. Microbiol.* 110(2), 431–444 (2011).

10 Kadouri DE, To K, Shanks RMQ, Doi Y. Predatory bacteria: a potential ally against

multidrug-resistant Gram-negative pathogens. *Plos ONE* 8(5), e63397 (2013).

11 Monnappa AK, Dwidar M, Mitchell RJ. Application of bacterial predation to mitigate recombinant bacterial populations and their DNA. *Soil Biol. Biochem.* 57, 427–435 (2013).

12 Silva F, Lourenco O, Queiroz JA, Domingues FC. Bacteriostatic versus bactericidal activity of ciprofloxacin in *Escherichia coli* assessed by flow cytometry using a novel far-red dye. *J. Antibiot.* 64(4), 321–325 (2011).

13 Im H, Kim D, Ghim CM, Mitchell RJ. Shedding light on microbial predator-prey population dynamics using a quantitative bioluminescence assay. *Microb. Ecol.* 67(1), 167–176 (2014).

14 Bjarnsholt T. The role of bacterial biofilms in chronic infections. *APMIS Suppl.* 136, 1–51 (2013).

15 Kadouri D, Venzon NC, O'toole GA. Vulnerability of pathogenic biofilms to *Micavibrio aeruginosavorus*. *Appl. Environ. Microbiol.* 73(2), 605–614 (2007).

16 Dwidar M, Leung BM, Yaguchi T, Takayama S, Mitchell RJ. Patterning bacterial communities on epithelial cells. *PLoS ONE* 8(6), e67165 (2013).

17 Monnappa AK, Dwidar M, Seo JK, Hur JH, Mitchell RJ. *Bdellovibrio bacteriovorus* inhibits *Staphylococcus aureus* biofilm formation and invasion into human epithelial cells. *Sci. Rep.* 4, 3811 (2014).

18 Monnappa AK, Bari W, Choi SY, Mitchell RJ. Investigating the responses of human epithelial cells to predatory bacteria. *Sci. Rep.* 6, 33485 (2016).

19 Gupta S, Tang C, Tran M, Kadouri DE. Effect of predatory bacteria on human cell lines. *PLoS ONE* 11(8), e0161242 (2016).

20 Shatzkes K, Singleton E, Tang C *et al.* Predatory bacteria attenuate *Klebsiella pneumoniae* burden in rat lungs. *mBio* 7(6), e01847-16 (2016).

21 Willis AR, Moore C, Mazon-Moya M *et al.* Injections of predatory bacteria work alongside host immune cells to treat *Shigella* infection in zebrafish larvae. *Curr. Biol.* 26(24), 3343–3351 (2016).