ALTERNATIVE THERAPEUTIC APPROACHES IN THE ERA OF ANTIMICROBIAL RESISTANCE: AN OVERVIEW[†]

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The problem of antimicrobial resistance (AMR) becomes pandemic and losses associated with it globally are enormous. Although discovery of new antimicrobials and making it available in the arsenal of clinicians is the best possible solution in tackling the current scenario of AMR, it is not happening for last two decades due to many factors. Focus of research is now diverted in development of non-antibiotic antimicrobials as an alternative therapeutic option. The major research activities and their potential application as alternative therapeutic agents are tried to narrate in the present article. The traditional approaches like antibiotic stewardship, increase of awareness, strengthen of diagnostic capabilities, improvement of vaccination schedules etc are not included in the article. With the advancement of new technologies including microbiology, molecular biology, biotechnology, genetic engineering, synthetic chemistry the scientists throughout the world could develop various natural as well as synthetic products as potential therapeutic candidates, which can replace the antibiotics in future. The article is focused on the major breakthrough on new or renewed technologies published in various parts of the world on non-antibiotic antimicrobial agents as potential alternative therapeutic approaches.

Key Words: Alternative approaches, AMR, Non-antibiotic antimicrobials

Antimicrobial resistance (AMR) is posing a serious threat globally and every year 7,00,000 patients die due to. It is also estimated that by 2050, death toll may reach up to 10 million leading to reduction of GDP by minimum 2.5% (Ghosh *et al.*, 2019). Tackling AMR amicably becomes a serious challenge and discovery of new antibiotics are not offering any positive directions. Due to this ambiguous situation, scientists have started thinking about alternatives to existing and conventional antibiotics as therapeutic options. As indicated in various existing literatures, various approaches were tried to find out possible

alternatives to replace antibiotics as therapeutic options. Few of the major approaches with encouraging results are described here.

Phage therapy

Use of bacteriophages to treat infected animal and human subjects is much older than the antibiotic era. Bacteriophages propagate in bacteria through taking over the host cell machinery resulting in the synthesis of phage components and assembly of new phages within the infected bacteria. This method eventually causes bacterial lysis followed by release of phage progeny, which can initiate a

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second infection cycle (Abedon *et al.*, 2011). As bacteriophages are known to select between mixed populations of bacteria, this could be exploited to use them as an alternative but selective approach to target pathogenic bacteria over commensal bacteria. So far, many bacteriophages were approved by the FDA for treatment of different bacterial illness in human and animals (Ghosh *et al.*, 2019). But certain issues related to bacteriophage therapy such as their high specificity towards a particular type of bacteria, development of immunity in the host body, pharmacokinetics and accumulation of endotoxins are major concern to be addressed (Kutter *et al.*, 2010; Pelfrene *et al.*, 2016).

Majority of the disadvantages with the natural bacteriophages were addressed with the advent of genetically modified/engineered phages. The engineered bacteriophages are capable to produce bacterial-biofilm-degrading enzymes upon infection (Lu and Collins, 2009). Therefore, both the biofilm and the bacteria that were embedded within the biofilm can be lysed. Engineered phages are also designed to overexpress *lexA3* that represses the bacterial SOS DNA repair system and make them more susceptible to bactericidal antibiotics (Chan *et al.*, 2016). Phagemids are also tried to deliver small regulatory RNAs inside drug-resistant bacteria, which converts the resistant bacteria such as *P. aeruginosa* into susceptible one for conventional antibiotics (Edgar *et al.*, 2012; Libis *et al.*, 2014). Numerous engineered bacteriophages are currently used clinically or under clinical trials (Ghosh *et al.*, 2019).

Antimicrobial peptides (AMPs)

AMPs and host defense peptides (HDPs) are the first line of defense produced by many multicellular organisms against invading pathogens (de Castro and Franco, 2015). Many AMPs/HDPs have been discovered and tried to be used as antibacterial agents, so far, with variable rate of success. In addition to that, they have diverse activities like anti-viral, anti-fungal, anti-plasmodial, anti-cancer, anti-protista, insecticidal and spermicidal including immunomodulation. AMPs are being used to

selectively kill the bacteria without any activities against host cells. Although several AMPs are undergoing clinical trials, *in vivo* toxicity and difficult industrial scalability have hindered clinical translation of this class of alternative antimicrobial agents. In addition, reports of bacteria developing resistance against AMPs also do exist (Fosgerau and Hoffmann, 2015; Andersson *et al.*, 2016; Mahlapuu, 2016; Zhang and Gallo, 2016; Greber and Dawgul, 2017).

Synthetic mimics of antimicrobial peptides (SMAMPs)

SMAMPs are genetically modified products developed to overcome the inherent disadvantages of AMPs (Engler *et al.*, 2012). SMAMPs are modified and developed in such a way that they maintain all the basic properties of AMPs as well as can withstand protease activities, increasing penetration power and act as broadspectrum agents. Use of SMAMPs could also modify the MDR bacteria in such a way that they become re-sensitized to existing conventional antibiotics. Few of the SMAMPs are in advanced stage of clinical trials and are expected to be approved by the FDA as therapeutic agent against MDR bacteria. These compounds are synthetically simple, rapidly bactericidal and also effective against fungi, the malaria parasite and Ebola virus (Uppu *et al.*, 2015; Czyzewski *et al.*, 2016; Teyssières *et al.*, 2016; Uppu and Halder, 2016; Sang *et al.*, 2017; Konai *et al.*, 2018).

Antibodies

Antibodies are very useful alternatives for the treatment of bacterial infections due to its direct recognition and neutralization capacity of specific components of the pathogens. Numerous antibodies against various bacterial pathogens of human and animals are in various stages of clinical development and, in fact, some of them have already been approved by the FDA. But the major drawback of using antibodies for antibacterial therapy is the cost of the production and poor shelf life (Bebbington and Yarranton, 2008; Saylor *et al.*, 2009).

Bacteriocins

As self defense mechanism and in order to prevent competition and enhance survival, many bacterial species produce small AMPs termed as bacteriocins, which act against other bacteria within the population. These ribosomally synthesized peptides are classified into two groups; class I and class II. Their mechanism of action is similar to AMPs. So far, few of the potential bacteriocins are undergoing clinical trials at various stages. In fact, bacteriocins also suffer from the same problems as AMPs but their use in certain cases can offer more advantages like conventional antibiotics. Like AMPs, resistance against bacteriocins is slow but imminent (Cotter *et al.*, 2013; Yang *et al.*, 2014).

Probiotics

The gut microbiota of mammals comprises over 1000 microbial species, including bacteria and yeast, which play a crucial role in energy metabolism and immune function (Hamilton-Miller, 2003). Treatment of patient with antibiotics make serious disbalance in gut microbiota leads to secondary infections, like *C. difficile* induced colitis (CDI). Therefore, probiotics and prebiotics are indicated in the treatment of various gastrointestinal infections (Jia *et al.*, 2008). Treatment with probiotics and prebiotics is advantageous over antibiotics as these agents are safe for long term consumption and do not cause side effects or allergies. There are many probiotics under clinical trials for the treatment of infections (Stier and Bischoff, 2016; Lewis and Pamer, 2017).

Fecal transplant therapy (FTT)

FTT is an attractive and safe alternative strategy, in which the microbiome from a healthy donor is being introduced into the diseased gut leading to curing bacterial infections or other cases involving gastrointestinal dysbiosis. The cure rate for patients undergoing colonoscopic FTT for recurrent CDI was recorded 90% and accordingly the FDA has approved the use of FTT for the treatment of patients suffering from CDI that is unresponsive to standard therapy. So far, it has shown highly promising

result against MDR *Enterobacteriaceae* including *E. coli*, *Salmonella*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, MRSA and VRE (Aroniadis and Brandt, 2014).

Predatory bacteria

With the advent of science of microbiology, the use of predatory bacteria such as *Bdellovibrio* and like organisms (BALOs) has been considered as a highly promising alternative to antibiotics (Atterbury *et al.*, 2011). These organisms multiply only upon entering their prey such as *E. coli*, *Salmonella*, *Legionella*, *Pseudomonas* as well as other pathogenic bacteria of human and animals without affecting the host cells. In addition to degradation of prey cells by lysing, BALOs can also penetrate into bacterial biofilm, giving them an advantage over conventional antibiotics (Kadouri *et al.*, 2013). The lipopolysaccharide (LPS) of BALOs lacks the negatively charged phosphate group, resulting in reduced binding affinity to the LPS receptors in human immune cells and accordingly they exhibit minimum or no inflammatory response. The activities against wide range of bacteria, low immunogenicity, low toxicity, and negligible propensity to induce resistance make this bacterium a promising candidate for the treatment of infections (Sokkett and Lambert, 2004).

Innate defence regulatory peptides (IDRPs)

IDRPs are peptides with no antibacterial activity but with anti-endotoxin and immunomodulatory activities are proven to protect mice from succumbing to severe bacterial and malarial infections, without having any direct antimicrobial activity. They are a promising alternative to conventional antibiotics, and one of them has completed Phase I clinical trials for the treatment of bacterial infection (Achtman *et al.*, 2012; Mansour *et al.*, 2015; Hancock *et al.*, 2016).

Antibacterial oligonucleotides

Gene silencing therapy has become popular for the treatment of numerous diseases including infectious diseases, where silencing of essential

and resistance causing genes by the use of antisense oligonucleotides with sequences complimentary to the target mRNA was used. The oligonucleotides are modified to confer resistance to nuclease degradation as well as improved transport across the negatively charged bacterial membrane (Ayhan *et al.*, 2016). They have shown potential antibacterial activity against a variety of bacteria such as *E. coli*, *S. enterica* and *A. baumannii* both *in vitro* and *in vivo* (Howard *et al.*, 2017). The agents have also exhibited growth inhibition in the presence of sub-lethal concentrations of colistin and polymyxin B as well as restoration of the susceptibility of MRSA to oxacillin both *in vitro* and *in vivo*. This could also sensitize resistant staphylococci to other existing β -lactam antibiotics (cefatoxin, cephalothin, cefoperazone, cefoxitin and oxacillin) (Meng *et al.*, 2015; Sully *et al.*, 2017)

CRISPR-Cas 9

The CRISPR (clustered, regularly interspaced, short palindromic repeats)-Cas9 systems are key components of a bacterial immune system, in which 20 nt small RNA acts as a guide for Cas9 to remove foreign genetic elements like in plasmids and phages at specific sites (Bikard and Barrangou, 2017; de la Fuente-Nunez and Lu, 2017). Like in many other fields, the CRISPR-Cas9 system has been used in the field of antimicrobial therapeutics with a great level of success. Bacteriophages were used to deliver the

CRISPR-Cas9 system to target multidrug-resistant bacteria to make them susceptible (Doudna and Charpentier, 2014; Goren *et al.*, 2017). The CRISPR-Cas9 system was also successfully tried to selectively kill virulent, but not avirulent, strains of *S. aureus*. Overall, CRISPR-Cas9 based systems offer an excellent alternative to antibiotics but their industrial scalability remains a question (Yosef *et al.*, 2015; Wang *et al.*, 2016).

In conclusion, it may be mentioned that numerous alternatives to conventional antibiotics have been developed so far to combat antimicrobial resistance and treat bacterial infections. Some of the approaches have shown encouraging level of progress, whereas others are still at the laboratory stage. Majority of the approaches are strain or species specific in contrast to the broad spectrum activities of conventional antibiotics and therefore, multiple therapeutics require to be practised for the treatment of different infections. Although some progresses have been made but the current status indicates that alternative approaches can only partially replace antibiotics. Recent progress in genetic engineering offers significant possibilities for further improvement. Future studies must investigate the efficacy of combining conventional antibiotics with one of these alternative therapies to fully assess their potential. The field is in its nascent stage and further research must be focused on development of next generation therapeutic agents.

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