Alternatives to Antibiotics: Current Trends and Future Perspectives

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ABSTRACT

The serendipitous discovery of antibiotics in the 20th century resulted in a major decline in infection related death rate, but soon after, overexploitation of antibiotics led to the development of antibiotic resistance in microbial populations. The emergence of multidrug resistance and extreme drug resistance in pathogens further intensified the whole situation. Around 100000-2000000 tons of antibiotics are being used in agriculture, horticulture, and medicine annually at a global level. The death rate due to antibiotic-resistant bacteria has risen far greater than the death rate due to HIV/AIDS and Tuberculosis. Modification of the site of action, efflux of antibiotics and degradation of drugs are the main mechanisms bacteria use as a defense strategy against antibiotics. Finding better drugs and methods to combat antibiotic resistance has become a need of the hour. This increase in antibiotic resistance has forced researchers to go back to the pre-antibiotic era in order to develop alternative drugs and processes to combat drug resistance. This review emphasizes the urgency of tackling this situation and deploy all promising alternatives of antibiotics like phage therapy, use of bacteriocins, organic acids, nanotechnology and CRISPR-Cas technique to address the antibiotic resistance challenge.

KEYWORDS: Antibiotic, Drugs Resistance, Phage Therapy, Bacteriocins, Nanotechnology.

INTRODUCTION

Discovery of "Antibiotics"-a class of drugs naturally produced by microorganisms in the 20th century was a life-changing discovery in the field of medical sciences. They played a highly significant role in controlling infectious diseases caused due to bacteria. Production of penicillin in 1940s laid a foundation stone for the drug discovery against several types of infections. The era from 1950s to 1970s is considered as a golden era of discovery of numerous antibiotics [Aminov, 2010]. At that time, it was believed that an end of infectious diseases has been achieved as there was a major decline in mortality and morbidity due to various infections. But to ensure their survival, bacteria very soon developed counter strategies-kind of defense mechanisms in response to antibiotics and became antibiotic resistant. This condition is getting worse day by day as many pathogens are developing multiple resistance against different drugs [Kapil, 2005].

The over-exploitation of antibiotic drugs in medical, agriculture, horticulture, animal sciences and other sectors contribute largely to the emergence of antibiotic resistant microorganisms (also called superbugs) [Pattanayak,

2017]. As soon as the first antibiotic, penicillin was produced and came to be widely used for clinical applications,many bacterial pathogens like the deadly *Staphylococcus aureus* started producing penicillinase enzyme to develop penicillin resistance [Davies and Davies, 2010]. After that, cloxacillin was produced to overcome the problem of resistance against penicillin, but then again bacteria, changed the target site of cloxacillin and developed resistance against cloxacillin, too. With the discovery of new antibiotics, newer strains of resistant bacteria kept emerging. By this time, *S. aureus* showed resistance to numerous antibiotics such as methicillin (first semisynthetic antibiotic against penicillin-resistant bacteria), chloramphenicol and macrolides. In 2002, vancomycin resistant *S. aureus* (VRSA) were also detected after 44 years of vancomycin introduction to the market [Appelbaum, 2006]. be it community or hospital-acquired infections due to, Vancomycin Resistant Enterococci (VRE), Vancomycin-Intermediate Staphylococcus aureus (VISA), ESBL (extended spectrum beta-lactamase) or Methicillin-Resistant S. aureus (MRSA) enzyme producing Gram-negative bacteria [Kumar and Singh, 2013], effective antibiotics are no longer available to cure the bacterial infections. The multidrug-resistant strains of *Mycobacterium tuberculosis, Pseudomonas aeruginosa, Streptococcus pneumonia, Entrococcus faecium* were also reported [D'souza, *et al* 2009] and [Pendleton, *et al*, 2013].

The widespread use of the same antibiotic in almost all sectors has also led to a rapid development of resistance [McEwen *et al*, 2002]. The food chain is one of the main paths for transmitting resistant bacteria from animals to humans [Witte, 1998)], since animals getting antibiotics in their feed and water can become carriers of resistant bacteria for that specific antibiotic [McEwen *et al*, 2002]. The ease of fast travel to different parts of the world also contributes significantly to the spread of drug-resistant microorganisms in different regions [Chadwick and Goode, 2008]. The destructive effects of antimicrobial resistance have already been observed as these infections are claiming more than fifty thousand lives in a year across Europe and the US. The death rate due to antibiotic-resistant bacteria is found to be far greater than the death rate due to HIV/AIDS and Tuberculosis. Modification in the site of action, efflux of antibiotics and degradation of drugs are the main mechanisms that bacteria use as a defense mechanism against antibiotic. It is estimated that if there is a continued rise in resistance levels, by 2050 it would lead to 10 million deaths annually [Mendelson, 2015]. Antimicrobial resistance also causes high treatment expenses because of longer and high cost of hospitalization. Latest findings on plasmid-transferable genes that help in the development of carbapenems [Rolain *et al*, 2010] and colistin [Liu *et al*, 2016] resistance show that the last protective wall against MDR bacteria has already been breached. World Health Organization (WHO) in Fact Sheet 2016 on Antibiotic Resistance, has warned that a post-antibiotic era will result in frequent, life-threatening infections and even small injuries may be fatal if we fail to act against antibiotic resistance now.

Due to the availability of less efficient antibiotics against drug resistant microbial pathogens, developments of alternatives have become the need of the hour. This increases the pressure on a researcher to go back to the pre-antibiotic era in order to develop alternative drugs and therapies, which should be economical, target specific and of low toxicity to combat drug resistance, in the post-antibiotic era. This review, emphasizes all the possible alternatives including the application of bacteriocins, antimicrobial peptides, antibodies, organic acids and some newly used therapies,which include use of phages, nanoparticles and of late use of CRISPR-Cas to combat the problem of antibiotic resistance.

ALTERNATIVES TO ANTIBIOTICS – FUTURE PERSPECTIVES

To tackle the issue of antibiotic resistance, studies on different alternative approaches are being actively pursued throughout the world. These approaches are: i) Modification in the already available antimicrobial compounds to restore their activity/potency. ii) Searching new ways of attacking pathogens by using microorganisms and their products. iii) Using different compounds and methods against pathogens which have not yet been tried.

i. **Modification in the already available antimicrobial compounds to restore their activity/potency.**
Addition of chemicals like clavulanic acids, tazobactam, and sulbactam to penicillin in order to overcome microbial resistance [Pattanayak, 2017]. Bacterial efflux inhibitors and antibiotics analogs were also used for the same purpose [Pattanayak, 2011]. But the efficacy of these methods, also decreased after some years, as pathogens bring about an alteration in their resistance mechanisms.

ii. **Searching newer ways of attacking pathogens by using microorganisms and their products.**

BACTERIOCINS

Bacteriocins are the antimicrobial peptides (30-60 amino acids) produced by some bacteria as primary metabolites or against closely related bacteria in the competition of food. [Swiatkiewicz and Arczewska, 2012]. They are also synthesized by the normal gut microflora in order to prevent infection [Kelley *et al*, 1998]. They are amphipathic molecules having a positive charge, diverse in structure and function. They are usually used in food industry for preservation and hold a very promising role in treating infectious diseases, in place of antibiotics, due to its antimicrobial property. Numerous bacteriocins with possible industrial applications have been isolated even though the Food Drug Administration (FDA) only approves the use of nisin and pediocin [Van der *et al*, 2011]. Colicin- like bacteriocins, microcins and tailocins are examples of bacteriocins produced by Gram-negative bacteria [Van der *et al*, 2011].

They are even active at a very minute concentration (nM). They either act on the plasma membrane by binding to negatively charged phospholipids or recognize a specific surface receptor on the plasma membrane, interrupting electron transportation which ultimately causes cell lysis. Chances of development of resistance against bacteriocins are very infrequent because of the two different target sites and nonspecific modification of the cell envelope. However, they are generally active against bacteria closely related to the producer. They even affect spore germination [Lee *et al*, 2017]. Broad spectrum bacteriocins can be effective in the treatment of unidentified infection or infection due to numerous bacteria whereas narrow spectrum bacteriocins are helpful in targeting pathogens only, without killing the helpful microorganisms.

Several studies show that the bacteriocins produced by *Lactobacillus fermmentum* isolated from colostrum show inhibitory action against several pathogenic bacteria [Park *et al*, 2009]. They also have the ability to degrade mycotoxins. [Partanen, *et al* 1999]. In a study, *Lactobacillus acidophilus* also showed noteworthy action against *Pseudomonas aeruginosa* resistant to multi drugs [Abudabos *et al*, 2014]. Another study showed the effectiveness of Lacticin 3147 in the treatment of systemic infection due to S. aureus Xen 29. It has also been tested against *Mycobacterium tuberculosis* and other nontuberculous mycobacteria (*M. kansasii & M. avium*) [Carroll *et al*, 2010]. Application of bacteriocins (nisin, pediocinO2, leucocin k, BH5 etc) against gastrointestinal ulcers due to

Helicobacter pylori are under investigation. Some healthcare items such as anti-infectious cream and toothpaste having bacteriocins are also commercially available (Biosynexus Inc, MA, USA).

ORGANIC ACIDS

Organic acids such as formic, citric, propionic, malonic, fumaric, citric, sorbic, acetic and short chain fatty acids can also be used in feedstuff of animals instead of antibiotics [Swiatkiewicz *et al*, 2012], as antibiotics may contribute to reservoirs of antibiotic-resistant bacteria and may transfer its effects on animal products [Kelley *et al*,1998]. They inhibit growth of pathogens and promote the growth of beneficial microorganisms by altering the pH of the gastrointestinal tract and respiratory tract and improve immune response [Yesilbag and Colpan, 2006].

They are well accepted as an alternative to antibiotics as growth promoters in pigs [Partanen and Mroz, 1999] and boilers [Abudabos *et al* 2014]. In recent studies, it has been reported that microencapsulated organic acid blend (17% fumaric acid, 13% citric acid, 10% malic acid) with 1.2% Medium chain fatty acids (MCFAs) showed positive results on the production and strength of hen's eggs and improve the level of calcium in their blood hence the use of organic acids with MCFs could be a potential candidate in place of antibiotics in laying hens. [Lee *et al*, 2015]. Another experiment was conducted to check the effect of butyric acid instead of antibiotics on physiological performance of broilers Ross. This study shows that butyric acids in sufficient amount could replace antibiotics in chicken's diet [Ali *et al*, 2018].

PHAGE THERAPY

The concept of using bacteriophages (viruses having DNA or RNA specifically infecting and lysing bacteria) in the treatment of bacterial infection is not something new. It has been there for a century. It has been used for the treatment of dysentery due to *Shigella* in 1919 [Chanishvili, 2012], and still in practice in several parts of Eastern Europe and Georgia [Wittebole *et al*, 2014]. The global decline in the effectiveness of antibiotics, has regenerated the interest of researchers in "pre-antibiotic era" where phage therapy was a common practice. Phages enter inside a bacterium through a specific receptor present on the bacterial cell surface and replicates inside, and finally causing lysis of the bacterial cell. Two major lytic enzymes encoded by phage genes are holin, an integral membrane protein and lysin also called endolysin, present in peptidoglycan cell wall, initiate the lysis of bacterial plasma membrane. When a phage completes its lytic cycle holin helps in opening of cytoplasmic side of plasma membrane, meanwhile, lysin hydrolyzes the cell wall [Roach and Donovan, 2015]. The major advantage of phage therapy over antibiotics includes i) less toxicity to humans and generally considered as safe, when administered orally, and on the other hand, the adverse effect of antibiotics such as anaphylaxis, neurotoxicity, liver toxicity, gastrointestinal complication and allergy responses, are also not reported [Shehab *et al*, 2015 and Abedon, 2015]. Phage translocation also lowers the immune reactions against normal gut microbiota by inhibiting the synthesis of IL-2, TNF, and interferon gamma [Górski *et al*, 2006]. ii). Because of its high specificity, it affects only harmful microorganisms, and beneficial microorganisms are left unharmed. iii). Phage contains EPS depolymerase in its capsid that degrades EPS and allows the phage to make a connection with all the pathogens present in the extracellular matrix [Abedon, 2015]. A high amount of antibiotics is needed to degrade the intense biofilm, yet there are fewer chances of complete eradication of the pathogen [Anwar *et al*, 1992]. Phage therapy also degrades the biofilms from medical devices like catheter, lenses, etc [Motlagh *et al*, 2016] and therefore, a better option.

Instead of having many theoretical benefits of phage treatment, there are various disadvantages and some practical challenges exist. One of the major challenges, lies in its specificity, phage infects only specific pathogen, so it is very challenging to treat multi-drug resistant microorganisms and an infection or wound which have occurred due to multiple microbes. Making a cocktail for phage therapy and maintaining it is a very time consuming and expensive process. Further studies on phage therapy and genetically engineered bacteriophages to combat resistance are ongoing [Czaplewski *et al.* 2016].

PHYTOCHEMICALS

The emergence of antibiotic-resistance have forced the researchers into going back to the pre-antibiotic era, where plants products were used to cure infectious diseases. Phytochemicals can be a solution to combat antibiotic resistance since half of the medicines are nowadays extracted from micro-propogated medicinal plants which can yield tons of secondary metabolites. Antibiotics may be substituted with juice and parts of the therapeutic plant for partial treatment of herbivorous animals. (Pattanayak, *et al.* 2016). Antimicrobial properties in the solvent extract of many plants have also been reported. Since many plants produce antimicrobial agents as a response to stress, to evolve and survive, it can be assumed that mode of action of those agents is different than presently used antibiotics and chances of development of resistance are less. A recent study shows that isothiocyanates – natural phytochemicals present in *Nasturtium* and horseradish plant might be used as a promising treatment instead of antibiotics against urinary tract infections due to MDR *E. coli* [Mutters *et al*. 2018].

Use of novel compounds and modern methods against disease pathogens

ANTIMICROBIAL PEPTIDES

Antimicrobial peptides are small proteins which have the potential to kill bacteria. They are produced by almost all organisms from fungi to higher animals and plants. Many peptides produced by some reptiles and amphibian species are under investigation for their antimicrobial property, for medical use [Readon 2015]. They generally increase the expression of some anti-inflammatory chemokines, cytokines and decrease pro-inflammatory cytokine expression.

The anti-biofilm peptides for inhibiting biofilm formation by bacteria have been identified and in are in preclinical stage [Czaplewski *et al.* 2016].

NANO PARTICLES

Nanoparticles are an extremely promising class of candidates, which generate an effective and potent antimicrobial activity, complementary to antibiotics [Seil and Webster, 2012]. Because of their extremely large surface and highly dynamic nature, only a small dose of nanoparticles is found to be highly effective [Beyth *et al*, 2015]. Since they act directly on the cell wall, without penetrating the cell, there are very fewer chances of resistance development against nanoparticles. They are also highly effective in treating multidrug resistance and

bring about biofilm degradation [Pelgrift *et al*, 2013]. Currently antimicrobial nanoparticles,in use, are metals, metal oxides and organic nanoparticles that show considerable diversity in their mode of action. The damaging effect of nanoparticles depend on environmental factors (pH, temperature and aeration) and physiological factors including their shape, size, chemical modification, the solvent used, coating and amount of nanoparticles in mixture with others [Gatoo *et al*, 2014]. Generally, nanoparticles follow two deadly pathways, first causing damage to the plasma membrane through disrupting membrane potential and integrity, which in turn, results in a disturbance in respiration and energy production and eventually cell lysis and death [Pelgrift, *et al*, 2013]. The second pathway involves the formation of O_2 free radicals also called reactive oxygen species (ROS), which are highly reactive and result in lipid peroxidation, protein modification, and damage to DNA, RNA and enzymes leading to cell death [Pan, *et al*, 2010]. Apart from the above two, nanoparticle's modes of action, inhibition of the particular important enzyme, apoptosis [Beyth *et al*, 2015] and induction of nitrogen reactive species. In a study, TiO_2 nanoparticle was used under UV light resulting in lipid peroxidation and impaired respiration leading to the death of *E.coli* cells. [Matějka and Tokarský, 2014]. Another study shows the role of silver (Ag) ions having a high affinity for sulfur and nitrogen in inhibiting and damaging the structure of a protein by binding to its amino and thiol group [Choi, *et al*, 2008]. Ag nanoparticle, when combined with an antibiotic, shows a synergistic killing effect on bacteria [Shahverdi, *et al* 2007].

Table 1: Metallic Nanoparticles(NPs) and their possible action on targeted Bacteria [Hemeg, 2017].

NPs	Target Pathogens	Mode of Action and Antimicrobial Effect
Ag (Silver)	*Acinetobacter baumannii, Salmonella typhi, Vibrio cholerae, Bacillus subtilis, Staphylococcus aureus,* MDR *Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa,* coagulase-negative *Staphylococcus epidermidis, Enterococcus faecalis, Klebsiella pneumoniae, Listeria monocytogenes, Proteus mirabilis, Micrococcus luteus*	ROS generation, lipid peroxidation, inhibition of cytochromes of ETC, bacterial membrane disintegration, inhibition of cell wall synthesis, increase in membrane permeability, dissipation of proton gradient resulting in lysis, adhesion to cell surface causing lipid and protein damage, ribosome destabilization, intercalation between DNA bases and disruption of biofilms.
Au (Gold)	*E. coli, S. aureus, B. subtilis, K. pneumoniae*	Loss of membrane potential, disruption of the respiratory chain, reduced ATPase activity, decline in subunit of ribosome for tRNA binding bacterial and bacterial membrane disruption.
ZnO (Zinc oxide)	*S. aureus, E. coli, P. aeruginosa, B. subtilis,*	ROS generation, inhibition of biofilm, ZnO release and enhanced membrane permeability.

	Stenotrophomonas acidaminiphila	
	Methicillin-resistant *Streptococcus agalactiae*, methicillin-resistant *Staphylococcus aureus*.	ROS production, disruption of membrane, adsorption to cell surface, lipids and protein damage, inhibition of microbial biofilm formation
	Enterobacter aerogenes, *E. coli*, *Klebsiella oxytoca*, *S. aureus*, *S. pyogenes*	Cell membrane interaction
Cu (Copper)	*B. subtilis*	Formation of ROS, disorganization of membrane, inhibition of DNA replication
	E. coli	Dissipation of cell membrane potential, ROS generation, lipid peroxidation, protein oxidation, DNA degradation
Se (Selenium)	*S. aureus*, *E. coli*	Biofilm inhibition
TiO_2 (Titanium dioxide)	*E. coli*, *P. aeruginosa*, *S. aureus*, *Enterococcus faecium*	ROS generation, adsorption to cell surface and inhibition of biofilm
NiO (Nickel oxide)	*S. aureus*, *Streptococcus pneumoniae*	Increase in bacterial cell wall permeability
CdS (Cadmium sulfide)	*E. coli*	Antibiofilm activity
YF_2 (Histone acetyltransferase activator)	*E. coli*, *S. aureus*	Antibiofilm properties
MgF_2(Magnesium fluoride)	*E. coli*, *S. aureus*	ROS generation, penetration of cell envelope, lipid peroxidation and biofilm inhibition.
MgO (Magnesium oxide) NPs with Cl_2 and Br_2	*E. coli*, *Bacillus megaterium*, *B. subtilis*	Adsorption on cell membrane.
Bi(Bismuth) NPs	*Streptococcus mutans*	Inhibition of biofilm.
Bi NPs with X-ray treatment	MDR *P. aeruginosa*	Free radical generation that damages bacterial DNA.
Al_2O_3(Aluminum oxide) NPs	*E. coli*	Cell wall damage, enters cytoplasm
Ag/Cu bimetal NPs	*E. coli*	Synergistic effect
Cu/Zn bimetal NPs	*E. coli*, *S. aureus*, MRSA	Antioxidant activity
Ce(Cerium) doped TiO_2 NPs	*E. coli*	Membrane damage, penetration of cell envelope
Superparamagnetic iron oxide NPs coated with Ag or Au	*E. coli*, *S. aureus*, *P. aeruginosa*, *E. faecalis*,	Inhibition of bacterial biofilms

	S. epidermidis	

IMMUNOTHERAPY

Use of antibodies to treat infection is quite promising but innovation lies in the production of high-affinity monoclonal and polyclonal antibodies against a number of molecular targets. Use of multi-epitope vaccines, use of monoclonal and polyclonal antibodies for passive immunization, immune-stimulant therapy for sepsis have been under investigation for a long time and are currently in product development as prophylactic and therapeutic agents [Opal, 2016]. Antibodies have made a major clinical impact because of its highly specific and potent mode of action that brings about inactivation of the pathogen by binding to its epitopes, toxins & virulence factors. They are safe, show no toxicity and have demonstrated a high degree of practical feasibility [Czaplewski *et al*. 2016].

USE OF NEWLY EMERGING TECHNOLOGIES- CRISPR- CaS

CRISPR (Clustered regularly interspaced short palindromic repeats)-Cas technique is a gene editing method, recently developed on the basis of a defense mechanism that some bacteria use against phages. Researchers are using the same mechanism to kill bacteria themselves. Generally, when bacteriophage invades the bacteria, bacteria produce a short RNA sequence similar to the sequence of phage genome. This RNA guides an enzyme known as Cas9 to target and chop the phage DNA [Pattanayak, 2017]. Currently, with the help of CRISPR-Cas9 defense system, scientists are designing CRISPR sequences to target particular pathogens and genes responsible for antibiotic resistance [Readon 2015].

REGULATORY CONCERNS OF ANTIBIOTIC RESISTANCE

Similar guidelines for managing the overexploitation of antibiotics at the international level are not available and different countries follow different regulatory guidelines. Maintaining such guidelines should be a priority. In some developed countries, WHO recommend using antibiotics only in severe case of cholera and bloody diarrhea. Insufficient guidelines for evaluation of home and industry hygienic condition also increase the risk of resistance development Easy and cheaply availability of antibiotics also increases the risk of resistance development. A study in the UK revealed that around 11.3% of participants had not even completed the last prescribed antibiotic course. Strictest rule and regulations and social awareness can be a key in controlling overuse of drugs.

CONCLUSION

Treatment of infectious diseases is getting extremely challenging due to antibiotic resistance mechanism which outpaces the development of new antibiotics, which haven't shown any progress in recent times. The challenges of Antibiotic Resistance are not only grave but highly complex. There is a dire need to reconsider our treatment mechanisms and find viable solutions to fight the spiraling menace of Antimicrobial Resistance. Thus, there is a need to multiply our efforts to ensure the safety and efficacy of the antibiotic drugs already available and at the same time to expedite the discovery and development of new drugs & therapies against pathogens and ever evolving drug-resistant microorganisms. Several domestic and global policies should be revised to stop the over-exploitation and injudicious use of antibiotics so as to control this humanitarian crisis and further prevent the emergence and re-emergence of superbugs. Furthermore, deployment of the alternative methods discussed in the present paper, antimicrobial resistance stewardship and proper implementation of pragmatic and effective government policies in this regard are the ways forward. Control of antibiotic resistance should be considered as a "global priority", to be taken on a war footing by one and all, before it becomes too late and the very existence of humanity comes under threat.

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