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Original Research Article

Non-Antibiotic Therapy for Infectious Diseases in the Era of Antibiotics: A Foresee into the Future

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Abstract:

Antibiotic misuse or unregulated use has contributed to the rise and spread of resistant microorganisms. Owing to increase rates of resistance and stagnating rates of the discovery of novel antibiotics, the efficacy of conventional antibiotics in the treatment of bacterial infections has been put under growing strain. As a result, it is widespread to meet bacterial strains that are pan-drug resistant. Fears have been expressed about a "post-antibiotic era" where many bacterial illnesses might not be curable. It is essential to investigate alternative non-antibiotic therapy methods to guarantee that practitioners have access to a wide range of potent treatments. In an era of multidrug-resistant (MDR) bacterial infections, the new therapeutic techniques for bacterial infections (beyond antibiotics) may offer a solution to shorten the utility of present antibiotics. This review focuses on major alternatives to antibiotics on which include: phages, bacteriocins, antibacterial activities of non-antibiotic drugs, quorum sensing inhibitors, probiotics, nanobiotics, faecal microbiota transplant, stem cell derived antimicrobial peptides, immunotherapeutics, and hemofiltration devices.

Keywords: Non-Antibiotics, antibiotic era, microbe, Infectious diseases, future trends.

Introduction

Today, the potential benefit of lowering the number of infections-related deaths has been eclipsed by the problem of antimicrobial resistance (AMR). Antimicrobial resistance (AMR) is a global crisis. Internationally, approximately 1.3 million deaths were estimated to be directly attributable to antimicrobial resistant bacterial pathogens in 2019. In the United States, antimicrobial resistant pathogens caused more than 2.8 million infections and over 35,000 deaths annually from 2012 through 2017, according to the Centers for Disease Control and Prevention (CDC) and Antibiotic Resistance Threats in the United States Report.[1] According to estimates, 4.7 million fatalities in Asia could be directly linked to AMR by the year 2050. In India, extended beta lactamase (ESBL) and carbapenemase (CP) manufacturers account for up to 12–59% and up to 30% of Escherichia coli, respectively, and up to 50% and fast rising polymyxin resistance in *Klebsiella pneumoniae,* respectively. Additionally, up to 30% of *Staphylococcus aureus* isolates nationwide exhibit methicillin resistance (MRSA). Antibiotic abuse is generally known to be one of the main causes of antibiotic resistance, hence it is important to maximise the use of antibiotics. India is the largest consumer of antibiotics in the world i.e., 13 billion standard units in 2010 and from 2000 to 2010 the per capita consumption increased by 66%.[2] Antibiotic resistance has been linked to the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, and Enterobacter* species). This resistance is linked to a high risk of mortality and morbidity, which raises the cost of healthcare, especially in ICU settings in developing nations. (Founou et al., 2017).[3] The world's greatest consumer of antibiotics, according to Laxminarayan et al. (2016), has fostered conditions that encourage drug-resistant diseases. By 2050, it is predicted that the number of human deaths brought on by drug-resistant microbes might

increase from roughly 700,000 to 10 million.[4] The main aggravating factors include poor public health conditions, a lack of public knowledge of drug-resistant bacteria, a high prevalence of infections, easy access to antibiotics, and their careless usage. In order to provide clinicians with a robust pipeline of efficient medicines, it is necessary to investigate alternative non-antibiotic therapeutic methods.[5]

Different therapeutic modalities may be employed in conjunction with antibiotics and could help to prolong the shelf life of important drugs. This study emphasises on some of the most recent developments in this area, such as the use of bacteriophages to eliminate bacteria, focusing on bacterial virulence factors, and meddling with the microbiome to treat infections. Novel therapeutic approaches for bacterial infections (other than antibiotics) may provide a way to prolong the effectiveness of current antibiotics considering the emergence of multidrug-resistant (MDR) bacterial infections. The main antibiotic alternatives discussed in this review are phages, bacteriocins, and hemofiltration devices, non-antibiotic medicines with antibacterial properties, quorum sensing inhibitors, probiotics, nanobiotics, faecal microbiota transplant, and stem cell-derived antimicrobials.

Non-antibiotic drugs:

1. Zinc

The health of our skin, teeth, bones, hair, nails, muscles, nerves, and brain function depends on this naturally occurring element. In humans, more than 200 enzymes and hormones contain zinc. DNA creation would not be possible without zinc. An important public health problem, zinc deficiency was a significant contributing factor to the syndrome of teenage nutritional dwarfism, which affects children and women in India and other countries.

One method for preventing episodes of diarrhoea in children is to promote zinc consumption. Diarrheal illnesses are the second-leading cause of child mortality globally, accounting for nearly 2 million deaths annually in children under the age of 5. Zinc supplementation improves the efficacy of treating diarrheal disease. Pneumonia, the common cold, respiratory infections, better wound healing, fewer paediatric clinical malaria episodes, and immune system maintenance in HIV-positive patients are all treated with zinc. Lower serum zinc levels have been associated with more severe illness and higher death in HIV patients. Zinc is a possible pharmaceutical replacement for the treatment of ailments.[6]

2. Bacteriocins:

Bacteriocins are a diverse group of ribosomally produced proteinaceous compounds that are produced by bacteria and have antibacterial effects on bacteria that are distantly related to the producer strain. As with other bacteriocins, such as pyocins (from *Pseudomonas pyocyanea,* now *P. aeruginosa)* and cloacins (*Enterobacter cloacae),* etc., the suffix "cin" was appended to the generating species in this instance.[7]

There is an appropriate quantity of bacteriocins known to have a strong activity against Gram-negative bacteria, including the pathogenic strains, while bacteriocins produced from Gram-negative bacteria are predominately directed towards Gram-positive bacteria [8]. Take the colicin groups that are regularly reported for *E. coli* or the S-type pyocin group from *P. aeruginosa,* for instance [9]. Infrequently documented bacteriocins include those made by *Serratia marcescens, Citrobacter freundii, Shigella boydii, and K. pneumoniae* [10]. Klebicins have been shown to be effective against MDR and carbapenem-resistant *Klebsiella* species [12], in contrast to entianin, which is effective against MRSA (ATCC 43300) and vancomycin-resistant *Enterococcus faecalis* (ATCC 51299) strains [11]. Novel enterocins DD28 and DD93 additionally demonstrated anti-staphylococcal action against MRSA [13], Carbapenem-resistant *E. coli or K. pneumoniae,* vancomycin-resistant, and MDR *E. faecalis* [14]. In order to address problems brought on by resistant bacteria, Bacteriocins represent a prospective pharmacological replacement for the current antibiotics.

3. Cranberry (Vaccinium macrocarpon):

By primarily altering the molecular structure of the fimbriae on pathogenic strains, pro-anthocyanidins in cranberries prevent bacteria from adhering to the bladder and urinary tract, and indirectly acting on bacteria by lowering intravesical pH, cranberry juice may help prevent and relieve the symptoms of urinary tract infections.[15] Cranberries have the potential to replace current antibiotics in the treatment of diseases caused by resistant bacteria.

4. Faecal microbiota transplant (FMT):

According to a study, FMT is a successful treatment plan for persons with CDI (Clostridioides difficile infection) and other gastrointestinal conditions as IBS (Irritable bowel syndrome), colitis, constipation, diarrhea, etc.[16] Clinical studies have shown that autologous FMT (aFMT) is superior to probiotic therapy and induces a quick and nearly full recovery of Gastrointestinal microbiota in human patients who have been exposed to antibiotics. [17]

5. Probiotics/ Prebiotics:

At this moment, the most encouraging application of microbiome in medicine is in the sphere of treating recurrent infections caused by *C. difficile,* an anaerobic, a sporulating, toxin former, gram-positive bacilli which represents the leading cause of healthcare and antibiotics-associated diarrhoea and *pseudomembranous colitis.*[18]

A. Prebiotics: They are non-absorbable polysaccharides (like inulin and fructo-oligosaccharides) that promote the variety of the human gut microbiota and have a favourable impact on host health. Prebiotics, mostly dietary fibre, give microorganisms the right metabolic substrates. Short chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, which are the most common byproducts of probiotics metabolism, are produced by microorganisms from these polysaccharides.[19]

B. Probiotics: They are living microorganisms that, when utilised in the right amounts, promote the health of the host by preserving the balance of the microbiome and halting the spread of pathogenic bacteria. *Saccharomyces boulardii* and Lactobacillus species, for instance, may lessen the frequency of *C. difficile* infection (CDI); *Lactobacillus salivarius* may prevent *Listeria monocytogenes* from growing; *Streptococcus mutans* may offer protection against the development of dental caries; and vaginal applications of *Lactobacillus jensenii* may protect women from infection caused by *Gardnerella vaginalis, Candida albicans, and Escherichia coli.[*20] Bifidobacteria and lactic acid bacteria (LAB), which include species of *Enterococcus, Lactobacillus, Lactococcus, Pediococcus, Vagococcus, Aerococcus, Carnobacterium, Streptococcus,* and *Weissella,* make up the majority of probiotics, the live microorganisms or microbial feed supplements. Since most LAB are thought to be safe, they are seen as alternative health-promoting measures, as are the abundance of some genera in the female genitourinary tract, mammary gland, and digestive tract.[21] Patients with inflammatory bowel disease (IBD) have different bacterial microbiota than healthy individuals.[22]In this group of patients, notably the anaerobes, there is a noticeable drop in microbiome diversity, with higher levels of *Bacteroides, Escherichia,* and *Enterococci* spp. and lower levels of *Bifidobacterium* spp. and *Lactobacillus* spp.[23]According to their definition, paraprobiotics are "inactivated microbial cells (non-viable), that confer a health benefit to the consumer," and they have the power to control both the innate and adaptive immune systems as well as to have anti-inflammatory, anti-proliferative, and antioxidant properties, and to exert antagonistic effect against pathogens as well.[24] Synbiotics are combined probiotics and prebiotics which can show a synergistic beneficial effect on host health and there is currently a novel approach to evaluate their efficacy while using in treatment of IBD like ulcerative colitis and Chron's disease in humans.[25] So these agents represent a potential drug alternative not only for IBD but also we can use it as a potential adjuvants in infectious diarrheal diseases as well.

6. Gooseberry and wild apple

The juices of Indian gooseberry *(Emblica officinalis),* also known as "Amla," and wild apple *(Docynia indica),* also known as "crab apple," showed broad spectrum antibacterial activity, particularly against methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE), and Gram-negative bacteria that produce extended spectrum -lactamases (ESBL).[26] According to reports, gooseberry fruits contain a significant amount of hydrolysable tannins, alkaloids, phytochemicals, and phenolic substances, primarily gallic acid and rutin-like flavonoids. (Shende K et al., 2016).[27]

The phenolic and flavonoid content of gooseberry and wild apple may be responsible for their antibacterial properties.[28] Phenols alter the protein-to-lipid ratio, the functionality of membranes, and ion channels, whereas catechins are known to compromise the integrity of lipid bilayers in membranes. Rutin, a flavonoid found in wild apple, has been shown to inhibit the bacterial topoisomerase type II.[29] Antibacterial activity of alkaloids against multidrug-resistant organisms such as MRSA and VRE. Alkaloids' antibacterial activity has been linked to the inhibition of protein kinase C. In addition, alkaloids are thought to affect the genetic material in microorganisms, contributing to their antimicrobial activity.[30]

7. Phage therapy in humans:

Bacteriophages are viruses that only infect bacterial cells, referred to as phages. They are biological entities known for over a century, thus are obligate intracellular parasites that require a bacterium to replicate themselves, through their genetic material, by taking over the biochemical machinery of the bacterial cells. Bacteriophage therapy, although not new, makes use of strictly lytic phage particles as an alternative, or a complement, in the antimicrobial treatment of bacterial infections. [31]

Recent scientific developments in this area allow for the possibility of tailoring bacteriophages and improving their properties, specifically: (i) increasing their capacity to penetrate bacterial biofilms; (ii) increasing their potency and effectiveness; (iii) adjusting their range of activities to infections caused by various bacterial species and strains; and (iv) making them more stable and specific. Phage therapy has been improved by purifying specific phage components to target bacteria and modifying phages to expand their host range and infectivity. [32]

The bacteriophage therapy has the potential to replace antimicrobial agents against drug-resistant bacteria in the future, notwithstanding the difficulties. Because phages are common, host-specific, safe, and may be given orally along with

food, the procedure is becoming more and more popular nowadays [33]. Their research as antibacterial agents were based on the highly specialised peptidoglycan hydrolases known as bacteriophage lysins, sometimes known as "enzybiotics."[34] The creation and application of bioengineered lysins with desired features, such as greater lytic activity or broader spectrum bacteriophages lysins, are attractive prospects due to their modular nature. Gram-negative bacteria and other pathogens can all be killed by lysins. These enzymes' appealing qualities include the fact that they do not trigger a negative immunological response or increase resistance. As multidrug-resistant organisms have grown to be a serious and pervasive issue, engineered lysins offer a more modern therapeutic approach that is effective and simple to use to combat antimicrobial resistance.[35]

Whilst animal models have been used for the majority of phage research, some of the studies have included human participants. Studies by Bruttin and Brüssow and Sarker et al. and Sarker et al. indicated that consumption of T4 phage had just a few minor side effects and no major detrimental effects on health. (Nausea, stomach pain, and sore throat).[36,37] Phage treatment was applied to treat *E.coli* and *Pseudomonas aeruginosa* infections in burn patients in a recent study known as "Phagoburn." The two goals of the Phagoburn are lower bacterial load and sepsis in patients with severe burns and infections. Despite the fact that this experiment represents a significant advancement in the study of phage treatment, more in vitro and clinical trials are still required to acquire universal support for the use of phage therapy to treat pathogenic or MDR bacterial infections in humans.[38]

8. Racecadotril (Cap AD 100)

The cornerstone of paediatric acute diarrhoea treatment is oral rehydration solution (ORS). Racecadotril is an anti-secretory drug that, without changing intestinal motility, can stop the loss of fluid/electrolytes from the colon as a result of acute diarrhoea. Racecadotril works differently from other anti-diarrheal medications already on the market and can be taken with ORS to avoid fluid and electrolyte loss. Adding racecadotril to ORS may reduce both the quantity and size of stools passed during the diarrhoeal illness, as well as the length of time that symptoms last, according to the data gathered. In addition to being a different medicine for the treatment of infectious diarrhea, racecadotril appears to be well-tolerated in children with acute diarrhea, allowing us to prevent the development of resistance to empirical antibiotics used in the treatment of infectious diarrhea. [39]

9. Spirulina extracts:

Three Spirulina extracts (methanol, acetone, and hexane) and biological selenium nanoparticles (SeNPs) produced by *Bacillus subtilis* AL43 were tested for antimicrobial and antioxidant activity. SeNPs have been shown to have significant antimicrobial activity against six different strains of bacteria, including three gram-positive *(B.cereus, S.aureus, and L.monocytogenes),* three gram-negative (*Salmonella Typhi, Escherichia coli, and Klebsiella pneumonia),* and three strains from both *Candida sp.(Candida tropicalis, Candida albicans, Candida glabrata)* and *Aspergillus sp.(Aspergillus niger, Aspergillus flavus, and Aspergillus fumigatus)* respectively. Spirulina's bioactive substances have the potential to weaken bacterial cell walls and enhance cell permeability, which can cause cytoplasmic content leakage. Thus, Spirulina and SeNPs may act as promising antimicrobial agents as well as natural antioxidant substitutes. Therefore, they can be utilized as alternatives to antibiotics and traditional chemical drugs.[40]

10. Nanobiotics:

In addition to serving as vehicles for the delivery of targeted drugs, nanoparticles can also exert their own antibacterial effects through a variety of mechanisms, including bacterial cell wall disruption, biofilm inhibition, host immune response modulation, production of reactive oxygen species, and damage to important DNA and protein molecules. [41] Recent studies have shown that bismuth nanoparticles have wide anti-candidal efficacy and inhibit the spread of *Candida auris* strains that are resistant to a variety of drugs in hospital settings. (Vazquez-Munoz et al., 2020).[42]

11. Green tea extracts for Human papilloma virus (HPV):

Green tea is rich in substances referred to as polyphenols and is also known by the Latin name *Camellia sinensis.* Polyphenols are substances that occur naturally and have favourable effects on health. People with HPV infection can benefit notably from the polyphenols in green tea since they strengthen their immune systems. Epigallocatechin gallate (EGCG) and polyphenol E are efficient in treating cervical lesions and warts brought on by HPV. Most significantly, it has been shown that green tea extract has a part to play in controlling immunological molecules called T cells, which are crucial immune system modulators. It has been discovered that EGCG from green tea extract increases the generation of T regulatory cells, assisting in the regulation and balancing of the immune response. They can therefore be used as adjuvant therapy or be used with podophilin resins.[43]

12. CRISPR (Clustered regularly interspaced short palindromic repeats) cas system:

There are two categories for CRISPR-Cas systems: class 1 includes types I, III, and IV, while class 2 includes types II, V, and VI. While a single multi-domain Cas protein recognises and cleaves the DNA in class II, numerous Cas proteins participate in DNA recognition and cleavage in class I [44]. The Class II CRISPR-Cas9 system is used to modify the genomes of prokaryotes and eukaryotes, revolutionising molecular biology in the past ten years. CRISPR-Cas9 has been demonstrated by several groups to be effective in the targeted elimination of antimicrobial resistance (AMR) genes from bacterial populations. It has been demonstrated that phagemids and conjugative plasmids can transport CRISPR-Cas to specifically target AMR genes in plasmids and chromosomes, respectively.[45]

When AMR genes are removed, the bacteria become more susceptible to antibiotics. Specific bacteria can be eliminated in vivo by CRISPR-Cas9 phagemids.[46] The mecA gene involved in methicillin resistance can be successfully targeted by nanosized CRISPR complexes inside S. aureus. The CRISPR-Cas9 system's usage against AMR genes faces several significant obstacles despite its potential. These include changes in the microbial ecology following the elimination of AMR bacteria, the CRISPR Cas vectors' limited host range, resistance brought on by anti-CRISPR genes, and laws. (Pursey et al., 2018).[44] In order to combat diseases that are resistant in the future, the CRISPR-Cas system can be deployed.

13. Papaya leaf extracts:

It has been demonstrated that papaya leaf saponins can stimulate antibody production in animal models, hence enhancing both humoral and cell-mediated immunity.[47] Papaya leaf ethanolic extract was found to dramatically reduce the amount of TNF-induced by isopentenyl pyrophosphate in LPS-induced dendritic cells.[48] The methanol extract of papaya leaves has also been seen to decrease the release of pro-inflammatory TNF alpha, IL-1a, IL-1b, IL-6, and IL-8.[49] There are a number of theories put up to explain why papaya leaves may increase platelet counts. A protease involved in viral assembly has been discovered to be inhibited by flavonoids extracted from papaya leaves.[50] It has been proposed that dengue virus serotype 2 can bind to platelets directly to destroy them or can cause their peripheral destruction indirectly by promoting the production of anti-platelet or cross reaction antibodies.[51]

According to reports, papaya leaf extract can stabilise membranes, which may make it easier to stop dengue virus from destroying peripheral platelets.[52] It has also been proposed that papaya leaf extract's antioxidant and free radical-scavenging abilities may help in the prevention of hemolysis and bleeding. [53] However, dengue virus inhibits megakaryocytopoiesis or prevents stem cells from developing into megakaryocyte precursor cells, which reduces platelet proliferation.[51] It has been discovered to 15-fold boost the expression of the ALOX 12 gene (arachidonate 12-lipoxygenase), increasing platelet synthesis through the 12-HETE (12-Hydroxy eicosa tetraenoic acid) driven pathway as well as megakaryocyte and platelet conversion.[54,55] Additionally, it has been discovered that papain, a substance extracted from papaya leaves, can stop immune-mediated platelet destruction.[56] Therefore, they can be utilized as supportive therapy in patient having dengue with severe bleeding manifestations.

14. Stem cell derived peptides:

Mesenchymal stem cells (MSCs) from humans have recently been found to produce substances that serve as antimicrobial peptides (AMPs), which are used to kill bacteria through a variety of methods, including the suppression of the production of bacterial cell walls.[57,58] Secretome from MSCs is a promising strategy or supportive treatment in the future against many associated illnesses because it dramatically lowers bacterial infections, including the antibiotic-resistant MRSA. Human bone marrow (BM) and human Umbilical Cord Mesenchymal Stem Cells (hUCMSCs)-derived AMPs termed lipocal, hepcidin, and LL-37 were discovered to have antibacterial activities against drug-resistant clinical pathogens like *E. coli, S. aureus, and K. pneumonia.*[59] Imipenem-resistant *P. aeruginosa* isolated from human babies was directly hostile to hUMSCs. This therapy can therefore be employed as a substitute against infections that are resistant. [60]

15. Vitamin C:

Vitamin C is a reliable and effective antibacterial agent. Vitamin C significantly inhibited the growth of bacterial strains that were both Gram-positive and Gram-negative like *K. pneumoniae, E. coli, P. aeruginosa, P. mirabilis, S.aureus, B. subtilis, and B. licheniformis.* It was discovered that vitamin C is more stable over a range of temperatures and pH levels. All bacterial strains showed the greatest growth inhibition at an acidic pH. Therefore, vitamin C can be employed in the future as an adjunct therapy option to treat illnesses in people brought on by bacteria that are multidrug resistant. In addition to its antimicrobial actions, vitamin C has the potential to be even more effective than antibiotics because it directly boosts the immune system. Both zinc and vitamin C are used as adjuvant therapy in treatment of COVID19, which is recommended by Ministry of Health and Family welfare.[61]

16. Hemofiltration devices:

Devices are utilised in intense clinical care settings for hemofiltration or renal replacement therapy to bind to and remove circulating bacterial products, inflammatory mediators, and cytokines.[62] Hemofiltration can help patients who are elderly or new-borns lessen the negative effects of infections. In patients with bacterial sepsis and hepatic failure, it was discovered to lower the serum levels of bile acid microbial metabolites including total bilirubin, direct bilirubin, total bile acids, lactate, and IL-6.[63] Hemofiltration devices come in a variety of styles.

17. Quorum sensing inhibitors:

Biofilms are microbial sessile communities characterized by cells that are attached to a substratum or interface or to each other, are embedded in a self-produced matrix of extracellular polymeric substances and exhibit an altered phenotype.[64] Biofilms are estimated to be associated with 80% of microbial infections.[65]

Bacteria employs a technique called quorum sensing (QS) to generate and detect signal molecules and, as a result, coordinate their behavior in a cell-density-dependent way. The acyl-homoserine lactone (AHL) QS system in Gram-negative bacteria, the autoinducing peptide (AIP) QS system in Gram-positive bacteria, and the autoinducer-2 (AI-2) QS system in both Gram-negative and -positive bacteria are the three main QS systems that may be identified. [66] Several bacterial species, including *Bacillus spp., P. aeruginosa, Acinetobacter* spp., *Delftia acidovorans, Sphingomonas spp., Agrobacterium tumefaciens,* and *Klebsiella pneumonia* produce enzymes capable of degrading AHLs. [67] In addition, several human cell lines show AHL degrading activity and these enzymes can be used to interfere with biofilm formation of pathogenic bacteria. [68] Halogenated furanones have been shown to affect biofilm formation in *P. aeruginosa, E.coli, B. subtilis, S. epidermidis, Phorphyromonas gingivalis, S. enterica serovar Typhimurium, Streptococcus spp. and Vibrio spp,. Cinnamaldehyde* was shown to affect biofilm formation of *B. cenocepacia, B. multivorans, P. aeruginosa, E. coli* and *Vibrio* spp. Both compounds were also shown to increase biofilm susceptibility towards antibiotic treatment. [69]

Numerous organic and synthetic compounds that are being investigated in experimental models with intriguing outcomes can interfere with quorum sensing. Several classes of compounds with potential quorum-sensing inhibitions are reported like gallic acid, capsaicin, furanones, curcumin, phloridizin, and resveratrol.[70] Despite this, QSI have been shown to be promising antibiofilm agents and can be of great value in the future treatment of bacterial infections.

18. Immunotherapeutic agents:

Biomolecules known as immunotherapeutic are those that strengthen the host's immune system and provide immunity from infectious pathogens. One of the most widely used immunotherapeutic agents is Peg-filgrastim, a granulocyte colony-stimulating factor (G-CSF). It is used to increase the severely decreased neutrophil count in patients undergoing chemotherapy.[71] It is also recommended to increase the survival rate of patients who have been acutely exposed to myelosuppressive doses of radiation (acute radiation syndrome), and to reduce the occurrence of infections, as shown by febrile neutropenia, in people with non-myeloid malignancies taking myelosuppressive anti-cancer medications.[72]

19. Turmeric:

Turmeric *(Curcuma longa),* a plant native to India and Southeast Asia, contains the yellow pigment Curcumin, which is derived from the naturally occurring polyphenol diferuloylmethane.[73]

Curcumin has been shown to have antiviral effect against both enveloped and non-enveloped DNA and RNA viruses, including those that cause the flu, hepatitis, respiratory syncytial viruses, herpes, HPV, arboviruses, and noroviruses. Inhibiting viral attachment and penetration into the host cell as well as interfering with viral replication machinery and host cell signaling pathways are all part of curcumin's modus operandi. Furthermore, curcumin acts on the viral envelope or proteins to act as a virucidal agent.[74]

Additionally, curcumin prevents bacterial biofilms. Damage to the cell wall or membrane, interference with cellular functions through targeting proteins & DNA, and disruption of bacterial quorum sensing are all components of curcumin's antibacterial mode of action. Additionally, CUR caused lipid peroxidation, increased DNA breakage, and has an impact on the L-tryptophan metabolism in Gram-positive *(Staphylococcus aureus),* but not in Gram-negative *(Escherichia coli). S. aureus* that is resistant to methicillin can be killed with curcumin.[75]

A colorless curcumin derivative called tetrahydrocurcumin (THC) was studied as a potential vaginal anti-HIV medication. Curcumin and THC both have similar inhibitory gp120-CD4 binding activity, according to an in-silico investigation, raising the possibility of an anti-HIV impact. Therefore, Curcumin is a compelling choice for antibiotic-resistant strains as a result of its numerous targets.[76]

20. Honey:

Honey contains a variety of enzymes, including glucose oxidase, which breaks down sucrose into

simple glucose and fructose and creates gluconic acid, as well as water, sucrose, glucose, fructose, amino acids, beeswax, pollen, colours, and minerals. They all aid in healing. Especially for diabetic foot injuries, decubitus ulcers, venous and arterial ulcers, honey is frequently utilised in the treatment of chronic wounds.[77]

21. Tea Tree Oil (TTO):

Although the Demodex mite, a commensal member of the skin's bacterial flora, often causes no symptoms, its presence in some eyelid tissues may result in an inflammatory response, resulting in development of blepharitis, with a chronic course. Tea tree oil (TTO) is an aromatic essential oil made from the leaves of the Myrtaceae species *(Melaleuca alternifolia).* Terpinen-4-ol (T4O), which is a major component of TTO and is present in concentrations ranging from 30% to 48%, is the principal constituent. This ingredient is particularly effective at eliminating Demodex mites, including eggs and larvae. Additionally, it has acaricidal, anti-inflammatory, anti-fungal, and antibacterial properties.[78]

22. Mastic gum:

Chios mastic gum (CMG), a resin from the mastic tree *(Pistacialentiscus var. chia),* has been used for more than 2500 years to cure a variety of ailments brought on by bacterial infections and digestive problems. It is plausible that CMG controls virus infectivity because host cellular metabolism heavily influences virus replication. The cytopathogenic effect, as well as the synthesis of RNAs, proteins, and infectious particles of Influenza A virus, was significantly reduced by CMG therapy. It's interesting to note that CMG disrupted the first phase of the virus life cycle following viral attachment. Importantly, the administration of CMG significantly reduced mortality and morbidity in mice with Influenza A virus infection. These findings imply that CMG exhibits strong anti-influenza A viral action by preventing the initial phases of viral replication. Considering this, mastic gum may be used as a cutting-edge treatment for Influenza A virus infection in future.[79]

Conclusion:

One of the biggest health issues we'll have to foresee in the future is antibiotic resistance. Since there hasn't been much progress in the discovery of new antibiotics, particularly ones that work against drug-resistant bacteria, using alternate aspects of therapy may be the greatest solution to this issue. Even though, few viable solutions to get around this issue have been presented in this review, it is the responsibility of the relevant authorities to promote and encourage additional research as well as release the necessary funding to ensure that these strategies are successfully put into practice.

Clinicians should refrain from over prescribing or under prescribing antibiotics for common infections. They should also suggest patients to follow proper hygiene, which includes hand washing and infection prevention techniques. The alternative approaches discussed in this review will probably work best when combined with the existing antibiotics rather than taking the place of antibiotics entirely as a treatment option.

Panorama:

There will be numerous obstacles in the coming years to loop in new antibiotics to prevent fatalities due to infections as the resistance rate has soared high enough to cause an apocalypse in this era of antibiotic abuse.

The challenges to alter the view of patients from antibiotic to non- antibiotics for therapy will be cumbersome but by doing so will help us in long term. Thus by broadening our horizon to non-antibiotic therapy intermittently to treat mild to moderate ailments will definitely help us to attain a brief period of cessation of increased frequency of antibiotic resistance.

List of abbreviations:

WHO- World health organization, CDI– *Clostridoides difficile* infection, LAB – Lactic acid bacillus, IBD- Inflammatory bowel disease, VRE – Vancomycin resistant Enterococci, MRSA– Methicillin resistant *Staphylococcus aureus*, ESBL- Extended spectrum beta lactamase, SeNP– Selenium nano-particles, EGCG– Epigallo-cetechingallate, QSI– Quorum sensing inhibitors.

References:

1. Infectious Diseases Society of America 2023. Guidance on the Treatment of Antimicrobial Resistant Gram-Negative Infections. Clin Infect Dis. 2023 Jul 18: ciad428.
2. AMR Research and Surveillance Network, Indian Council of Medical Research, Division of Epidemiology and Communicable Diseases. Annual report: Chapter 1, page 4. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. 2020.
3. Founou, R. C., Founou, L. L., and Essack, S. Y. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. PLoS One 2017;12: e0189621.
4. Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Rottingen, J. A., Klugman, K., et al. Access to effective antimicrobials: a worldwide challenge. Lancet. 2016; 387: 168–175.,.
5. Brooks BD, Brooks AE. Therapeutic strategies to combat antibiotic resistance. Adv Drug Deliv Rev 2014; 78: 14-27.
6. Debjit Bhowmik, Chiranjib, K.P. Sampath Kumar. A potential medicinal importance of

zinc in human health and chronic disease. Int J Pharm Biomed Sci. 2010; 1(1): 05-11.
7. Allison, G. E., Fremaux, C., and Klaenhammer, T. R. Expansion of bacteriocin activity and host range upon complementation of two peptides encoded within the lactacin F operon. J. Bacteriol. 1994;176: 2235–2241
8. Jamali, H., Krylova, K., and Dozois, C. M. The 100 Top-cited scientific papers focused on the topic of bacteriocins. Int. J. Pept. Res. Ther. 2019;25: 933–939.
9. Ghequire, M. G. K., and De Mot, R. Ribosomally encoded antibacterial proteins and peptides from *Pseudomonas.* FEMS Microbiol. Rev. 2014;38: 523–568.
10. Yang, S.C., Lin, C.H., Sung, C. T., and Fang, J.Y. Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. Front. Microbiol. 2014; 5:241.
11. Fuchs, S. W., Jaskolla, T. W., Bochmann, S., Kötter, P., Wichelhaus, T., Karas, M., et al. Entianin, a novel subtilin-like antibiotic from *Bacillus subtilis* subsp. *spizizenii* DSM15029T with high antimicrobial activity. Appl. Environ. Microbiol. 2011;77: 1698–1707.
12. Denkovskien˙e, E., Paškevicius, Š, Misiunas, A., Stockunait˙e, B., Starkevic, U., Vitkauskiene, A., et al. Broad and efficient control of *Klebsiella* pathogens by peptidoglycan-degrading and pore-forming Bacteriocins klebicins. Sci. Rep. 2019; 9:15422.
13. Al Atya, A. K., Belguesmia, Y., Chataigne, G., Ravallec, R., Vachée, A., Szunerits, S., et al. Anti-MRSA activities of enterocins DD28 and DD93 and evidences on their role in the inhibition of biofilm formation. Front. Microbiol. 2016; 7:817.
14. Phumisantiphong, U., Siripanichgon, K., Reamtong, O., and Diraphat, P. A novel bacteriocin from *Enterococcus faecalis* 478 exhibits a potent activity against vancomycin-resistant Enterococci. PLoS One. 2017; 12:e0186415.
15. Tempera G, Corsello S, Genovese C, Caruso FE, Nicolosi D. Inhibitory activity of cranberry extract on the bacterial adhesiveness in the urine of women: an ex-vivo study. Int J Immunopathol Pharmacol. 2010 Apr-Jun; 23(2):611-8.
16. Suez, J., Zmora, N., Zilberman-Schapira, G., Mor, U., Dori-Bachash, M., Bashiardes, S., et al. Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT. Cell. 2018; 174: 1406–1423.
17. Costello, S. P., Hughes, P. A., Waters, O., Bryant, R. V., Vincent, A. D., Blatchford, P., et al. Effect of Fecal Microbiota Transplantation on 8-Week Remission in Patients with Ulcerative Colitis: A Randomized Clinical Trial. JAMA. 2019; 321: 156–164.
18. Crow JR, Davis SL, Chaykosky DM, Smith TT, Smith JM. Probiotics and fecal microbiota transplant for primary and secondary prevention of *Clostridium difficile infection.* Pharmacotherapy. 2015; 35:1016–25.
19. Gill EE, Franco OL, Hancock REW. Antibiotic Adjuvants: Diverse Strategies for Controlling Drug-Resistant Pathogens. ChemBiol Drug Des. 2015; 85: 56–78.
20. Saha S, Tomaro-Duchesneau C, Tabrizian M, Prakash S. Probiotics as oral health biotherapeutics. Expert Opin Biol Ther. 2012; 12: 1207–20.
21. Fijan S. Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Public Health. 2014 May 5;11(5):4745-67.
22. Joossens, M.; Huys, G.; Cnockaert, M.; De Preter, V.; Verbeke, K.; Rutgeerts, P.; Vandamme, P.; Vermeire, S. Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. Gut 2011, 60, 631–637.
23. 2Ott, S.J.; Musfeldt, M.; Wenderoth, D.F.; Hampe, J.; Brant, O.; Fölsch, U.R.; Timmis, K.N.; Schreiber, S. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut. 2004; 53: 685–693.
24. Chassaing, B.; Darfeuille-Michaud, A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. Gastroenterology. 2011; 140: 1720–1728.
25. Frank, D.N.; St Amand, A.L.; Feldman, R.A.; Boedeker, E.C.; Harpaz, N.; Pace, N.R. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc. Natl. Acad. Sci. USA. 2007; 104:13780–13785.
26. Punjabi, K., Chitalia, V. K., Mukadam, T., Sharma, J., Maniar, J., Vasave, S., Vaidya, S., &Dikho Mao, L. Evaluation of the antimicrobial activity of Gooseberry and Wild Apple fruit juices of the northeast region of India. Journal of Medicinal Herbs and Ethnomedicine, 2017;3: 1–7.
27. Shende K, Singh N, Negi P. Phytochemical characterization and biological activities of *Docynia indica (Wall)* fruit extracts. J Mol Genet Med. 2016; 10:1-5.
28. Cowan MM. Plant products as antimicrobial agents. ClinMicrobiol Rev. 1999; 12:564-82.
29. Cushnie TT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26:343-56.

30. Shohaib T, Shafique M, Dhanya N, Divakar M. Importance of flavonoids in therapeutics. Hygeia JD Med. 2011; 3:1-18.
31. Rios AC, Moutinho CG, Pinto FC, Del Fiol FS, Jozala A, Chaud MV, et al. Alternatives to overcoming bacterial resistances: State-of-the-art. Microbiol Res. 2016; 191:51-80.
32. Nobrega FL, Costa AR, Kluskens LD, Azeredo J. Revisiting phage therapy: new applications for old resources. Trends Microbiol. 2015; 23: 185–91.
33. Wittebole, X., De Roock, S., and Opal, S. M. A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. Virulence. 2014; 5: 226–235.
34. Nelson, D., Loomis, L., and Fischetti, V. A. Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme. Proc. Natl. Acad. Sci. U S A. 2001; 98: 4107–4112.
35. Vazquez, R., Garcia, E., and Garcia, P. Phage Lysins for Fighting Bacterial Respiratory Infections: A New Generation of Antimicrobials. Front. Immunol. 2018; 9:2252.
36. Bruttin, A.; Brüssow, H. Human volunteers receiving Escherichia coli phage T4 orally: A safety test of phage therapy. Antimicrob. Agents Chemother. 2005; 49: 2874–2878.
37. Sarker, S.A.; Sultana, S.; Reuteler, G.; Moine, D.; Descombes, P.; Charton, F.; Bourdin, G.; McCallin, S.; Ngom-Bru, C.; Neville, T.; et al. Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: A randomized trial in children from Bangladesh. EbioMedicine 2016; 4:124–137.
38. Servick, K. Beleaguered phage therapy trial presses on. Science. 2016, 352, 1506.
39. Qadir MI, Arshad A, Ahmad B. Zinc: Role in the management of diarrhea and cholera. World J Clin Cases. 2013 Jul 16;1(4):140-2.
40. Abdel-MoneimEid Abdel-Moneim AE, El-Saadony MT, Shehata AM, Saad AM, Aldhumri SA et al., Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. Saudi Journal of Biological Sciences. 2022; 29: 1197–1209.
41. Baptista PV., Mccusker, M. P., Carvalho, A., Ferreira, D. A., Mohan, N. M., Martins, M., et al. Nano-Strategies to Fight Multidrug Resistant Bacteria- A Battle of the Titans". Front. Microbiol. 2018; 9:1441.
42. Vazquez-Munoz, R., Lopez, F. D., and Lopez-Ribot, J. L. Bismuth Nanoantibiotics Display Anticandidal Activity and Disrupt the Biofilm and Cell Morphology of the Emergent Pathogenic Yeast *Candida auris.* Antibiotics. 2020:461.
43. Miyoshi N, Tanabe H, Suzuki T, Saeki K, Hara Y. Applications of a Standardized Green Tea Catechin Preparation for Viral Warts and *Human Papilloma Virus*-Related and Unrelated Cancers. Molecules. 2020 Jun 2;25(11):2588.
44. Pursey, E., Sunderhauf, D., Gaze, W. H., Westra, E. R., and Van Houte, S. CRISPR-Cas antimicrobials: Challenges and future prospects. PLoSPathog. 2018; 14:e1006990.
45. Bikard, D., Euler, C. W., Jiang, W., Nussenzweig, P. M., Goldberg, G. W., Duportet, X., et al. Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. Nat. Biotechnol. 2014;32: 1146–1150.
46. Citorik, R. J., Mimee, M., and Lu, T. K. Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. Nat. Biotechnol. 2014; 32:1141.
47. Gupta, A., Patil, S. S., and Pendharkar, N. Antimicrobial and anti-inflammatory activity of aqueous extract of *Carica papaya.* J. Herbmed. Pharmacol. 2017;6(4): 148–152.
48. Sagnia, B., Fedeli, D., Casetti, R., Montesano, C., Falcioni, G., and Colizzi, V. Antioxidant and anti-inflammatory activities of extracts from *Cassia alata, eleusine indica, eremomastax speciosa, Carica papaya and Polyscias fulva* medicinal plants collected in Cameroon. PloS one. 2014; 9(8): e103999.
49. Salim, E., Kumolosasi, E., and Jantan, I. Inhibitory effect of selected medicinal plants on the release of pro-inflammatory cytokines in lipopolysaccharide-stimulated human peripheral blood mononuclear cells. J. Nat. Med. 2014; 68(3): 647–653.
50. Charan, J., Saxena, D., Goyal, J., and Yasobant, S. Efficacy and safety of Carica papaya leaf extract in the dengue: a systematic review and meta-analysis. Int. J. App. Basic Med. Res. 2016;6(4): 249.
51. da Costa Barros, T. A., and de-Oliveira-Pinto, L. M. A view of platelets in dengue. Thrombocytopenia, Pankaj Abrol, IntechOpen. 2018.
52. Ranasinghe, P., Ranasinghe, P., Sirimal Premakumara, G., Perera, Y., Kaushalya M. Abeysekera, W., Gurugama, P., et al. In vitro erythrocyte membrane stabilization properties of *Carica papaya* L. leaf extracts. Phcog Res. 2012;4(4): 196.
53. Pandita, A., Mishra, N., Gupta, G., and Singh, R. Use of papaya leaf extract in neonatal thrombocytopenia. Clin. Case Rep. 2019;7(3): 497–499.
54. McRedmond, J. P., Park, S. D., Reilly, D. F., Coppinger, J. A., Maguire, P. B., Shields, D. C., et al. Integration of proteomics and genomics in platelets. Mol. Cell Proteomics. 2004; 3(2): 133–144.

55. Sundarmurthy, D., R, J., and C, L. Effect of Carica papaya leaf extract on platelet count in chemotherapy-induced thrombocytopenic patients: a preliminary study. Natl. J. Physiol. Pharm. Pharmacol. 2017;7(7): 1.
56. Lavanya, B., Maheswaran, A., Vimal, N., Vignesh, K., Yuvarani, K., and Varsha, R. Extraction and effects of papain obtained from leaves of *Carica papaya:* a remedy to dengue fever. Extraction. 2018; 3(1): 44–46.
57. Alcayaga-Miranda, F., Cuenca, J., and Khoury, M. Antimicrobial Activity of Mesenchymal Stem Cells: Current Status and New Perspectives of Antimicrobial Peptide-Based Therapies. Front. Immunol. 2017; 8:339.
58. Marx, C., Gardner, S., Harman, R. M., and Van De Walle, G. R. The mesenchymal stromal cell secretome impairs methicillin-resistant *Staphylococcus aureus* biofilms via cysteine protease activity in the equine model. Stem Cells Transl. Med. 2020;9: 746–757.
59. Mccarthy, S. D., Horgan, E., Ali, A., Masterson, C., Laffey, J. G., Macloughlin, R., et al. Nebulized Mesenchymal Stem Cell Derived Conditioned Medium Retains Antibacterial Properties Against Clinical Pathogen Isolates. J. Aerosol. Med. Pulm. Drug. Deliv. 2020;33: 140–152.
60. Ren, Z., Zheng, X., Yang, H., Zhang, Q., Liu, X., Zhang, X., et al. Human umbilical-cord mesenchymal stem cells inhibit bacterial growth and alleviate antibiotic resistance in neonatal imipenem-resistant *Pseudomonas aeruginosa* infection. Innate Immun. 2020;26: 215–221.
61. Mumtaz S, Mumtaz S, Ali S, Tahir HM, Kazmi SAR, Mughal TA, Younas M. Evaluation of antibacterial activity of vitamin C against human bacterial pathogens. Braz J Biol. 2021 Aug 27;83:e247165.
62. Liu, Y., Chen, X., Wang, D., Li, H., Huang, J., Zhang, Z., et al. Hemofiltration Successfully Eliminates Severe Cytokine Release Syndrome Following CD19 CAR-T-Cell Therapy. J. Immunother. 2018;41: 406–410.
63. Cui, Y., Xiong, X., Wang, F., Ren, Y., Wang, C., and Zhang, Y. Continuous hemofiltration improves the prognosis of bacterial sepsis complicated by liver dysfunction in children. BMC Pediatr. 2018; 18:269.
64. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbial Rev. 2002; 15(2): 167-93.
65. NIH "Research on microbial biofilms, National Heart, Lung, and Blood Institute 2002-12-20. Available from: http://grants.nih.gov/grants/guide/pa-files/PA-03-047.html.
66. Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. Ann Rev Cell Developmental Biol. 2005; 21: 319-46.
67. Maddela NR, Sheng B, Yuan S, et al. Roles of quorum sensing in biological wastewater treatment: a critical review. Chemosphere. 2019; 221:616–629.
68. Mangwani N, Dash HR, Chauhan A, et al. Bacterial quorum sensing, functional features and potential applications in biotechnology. J Mol Microbiol Biotechnol. 2012;22(4):215–227.
69. Pihl M, Davies JR, Chávez de Paz LE, Svensäter G. Differential effects of *Pseudomonas aeruginosa* on biofilm formation by different strains of *Staphylococcus epidermidis.* FEMS Immunol Med Microbiol. 2010 Aug;59(3):439-46.
70. Saeki, E. K., Kobayashi, R. K. T., and Nakazato, G. Quorum sensing system: Target to control the spread of bacterial infections. Microb. Pathog. 142:104068.
71. Molineux, G. (2004). The design and development of pegfilgrastim (PEGrmetHuG-CSF, Neulasta). Curr. Pharmaceut. Design. 2004; 10:1235–1244.
72. Cerchione C, Catalano L, Peluso I, Nappi D, Di Perna M, Salvatore D, et al. Managing neutropenia by pegfilgrastim in patients affected by relapsed/refractory multiple myeloma treated with bendamustine bortezomib dexamethasone. Supportive Care in Cancer. December 2016; 24 (12):4835–4837.
73. Trigo-Gutierrez JK, Vega-Chacón Y, Soares AB, Mima EGO. Antimicrobial Activity of Curcumin in Nanoformulations: A Comprehensive Review. Int J Mol Sci. 2021 Jul 1;22(13):7130.
74. Jennings, M.; Parks, R. Curcumin as an antiviral agent. Viruses. 2020; 12: 1242.
75. Adeyemi, O.S.; Obeme-Imom, J.I.; Akpor, B.O.; Rotimi, D.; Batiha, G.E.-S.; Owolabi, A. Altered redox status, DNA damage and modulation of L-tryptophan metabolism contribute to antimicrobial action of curcumin. Heliyon. 2020; 6: e03495.
76. Mirani, A.; Kundaikar, H.; Velhal, S.; Patel, V.; Bandivdekar, A.; Degani, M.; Patravale, V. Tetrahydrocurcumin-loaded vaginal nanomicrobicide for prophylaxis of HIV/AIDS: In silico study, formulation development, and in vitro evaluation. Drug Deliv. Transl. Res. 2019; 9: 828–847.
77. Yilmaz, A.C., Aygin, D. Sakarya University, Faculty of Health Sciences, Nursing Department Serdivan, Sakarya, TR 54055, Turkey. Honey dressing in wound treatment: a systematic review. Complementary Therapies in Medicine, June 2020; 51.

78. Capasso L, Abbinante G, Coppola A, Salerno G, De Bernardo M. Recent Evidence of Tea Tree Oil Effectiveness in Blepharitis Treatment. Biomed Res Int. 2022 Jul 30; 2022:9204251.
79. 79. Dong-In Kim, Yong-Bin Cho, Younghyun Lim, So-Hee Hong, BumsukHahm, Sang-Myeong Lee, Se Chan Kang, Young-JinSeo. Chios mastic gum inhibits influenza A virus replication and viral pathogenicity. Journal of General Virology, vol 102, issue 3. Published: 08 January 2021.