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Review Article

Role of alternatives to antibiotics in mitigating the antimicrobial resistance crisis

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Antimicrobial resistance (AMR) is a burgeoning challenge of global priority, warranting immediate action to prevent the explosion of multidrug-resistant (MDR) pathogens. Indiscriminate antimicrobial use is the most important driver for AMR. AMR has led to depletion of the antibiotic pipeline and developing new antibiotics is extremely challenging due to technical and financial issues and also resistance emerges as soon any new antibiotic is introduced. At present, preserving the power of existing antibiotics by prudent use and curtailing spread of pathogens by infection prevention and control (biosecurity) in both humans and animals are the best available options to defer AMR crisis. Meanwhile, to reduce dependence on antibiotics, other alternatives such as vaccines, antibodies, pattern recognition receptors, probiotics, bacteriophages, peptides, phytochemicals, metals, and antimicrobial enzymes are being explored. This review provides an overview of various promising, potential and under investigative strategies as alternatives to antibiotics.

Key words Antibiotics - antimicrobial resistance - antibiotic alternatives - bacteriophages - multidrug resistance - probiotics - vaccine

Introduction

Antimicrobial resistance (AMR) is a burgeoning challenge of global priority[1]. It is estimated that by 2050, approximately 10 million deaths will occur annually with a cumulative loss of US$ 100 trillion to the global economy, if concrete actions are not taken to slow down the current trend of AMR progression[1]. Antibiotic use is the most important driver for AMR, and more than 50 per cent of antibiotic use is unjustified with irresponsible use occurring in humans, veterinary practice and animal husbandry for therapeutic and non-therapeutic indications (growth promotion)[1,2]. Antimicrobials are used indiscriminately as a substitute for asepsis, cleanliness and infection prevention and control (IPC) across community, hospitals and farms, leading to survival, selection and spread of multidrug-resistant (MDR) pathogens. The magnitude and extent of antibiotic abuse are alike in both human and animals[1,2]. Antibiotics, antibiotic residues and antibiotic-resistant pathogens/genes from humans and animals enter the environment through excreta/sewage and get incorporated in soil and water sources ultimately risking the ecosystem.

A holistic, multidimensional, multisectoral, multidisciplinary 'one-health' approach has

been advocated globally to combat AMR across human–animal–environment interface[3]. The possible solution to combat this escalating threat of AMR is to reduce unjustified antibiotic use or develop new effective antibiotics[2,4]. Preventing infections is the most promising and long-term sustainable solution; several initiatives have been taken by the Government of India in the last decade to tackle the problem of AMR such as *Swachh Bharat Abhiyan* (2014) and *Kayakalp* Award Scheme (2015) which helped in raising awareness about sanitation and hygiene in the community and hospitals, but the impact of these initiatives on reduction in antimicrobial use yet needs to be consolidated[5,6]. Developing new antibiotics is a challenging task in view of various hurdles from discovering a new molecule to several commercial and regulatory issues[7]. Current pipeline for developing new antibiotics has only 27 drug candidates targeting the WHO priority pathogens, and majority are the modifications of previously known classes with already existing cross-resistance[4]. Discovery of new antibiotics is technically demanding and financially non-viable as it requires considerable time, effort and expenses with poor returns on investment[4,5]. Reducing and rationalizing antibiotic use are the next best sustainable solutions to address this crisis[2].

There is an urgent need to explore innovative alternatives to antibiotics acting differently by preventing infections, reducing the emergence of resistance by targeting different mechanisms of action (MOAs) or increasing the effectiveness of existing antibiotics[7-9]. Use of these alternatives for community-acquired infections would ultimately reduce the dependency on antibiotics[9,10].

Alternative to antibiotics refers to products such as vaccines, antibodies, pattern recognition receptors (PRRs), probiotics, bacteriophages, peptides, phytochemicals, metals and antimicrobial enzymes[7,8]. The available literature on alternatives to antibiotics is scattered in terms of different focus areas, different development stages and restricted to either human or animal use[6,8,9]. This review is aimed to provide a holistic overview of various promising, potential or under-investigation alternatives to antibiotics with their MOA, current status, challenges associated in commercialization and future scope. The Table gives an overview of status of the uses of alternatives and the Figure summarizes the major mechanism of action of antibiotic alternatives.

Immunomodulation

Modulating immune response has proved to be a promising approach in various autoimmune, inflammatory, anticancer therapies, with considerable potential in reducing the incidence of infections[12]. Immune response to any foreign antigen is brought by a complex-interdependent interaction of innate and adaptive immunity ultimately mediated by effectors such as cytokines, chemokines, inflammatory cells and their products[12]. Pathogenic microbes downregulate the innate immunity, thereby allowing pathogen survival and multiplication within host[12]. Immunomodulators have the potential to counteract this downregulation and induce protective immunity[13]. Immune modulators enhance the efficacy of antibiotics, augment host-specific immunity or limit pathogen-specific toxic effects[13-15]. A variety of biological or synthetic substances (such as cytokines, interleukins, chemokines, synthetic cytosine phosphate-guanosine, oligodeoxynucleotides, glucans, granulocyte colony-stimulating factor, interferons, imiquimod and cellular membrane fractions) can modulate immune system to boost immunity and indirectly fight infections[12]. The immunomodulators with potential to reduce antibiotic use are briefly discussed below.

Vaccines: Vaccines generate a highly specific and efficient immune response with rapid and robust response in case of re-infection[13,15]. These prevent the establishment of infection or reduce the disease severity, if infection sets in[13-16]. Vaccines also provide herd immunity and protect unvaccinated individuals from infections[15]. Antibiotics are often misused for viral illness due to diagnostic uncertainty. Further, antibiotics are often prescribed to prevent secondary bacterial infections in these patients[8,17].

Applications: Introduction of vaccines for diphtheria, pertussis, tetanus, meningococcal meningitis, tuberculosis, typhoid fever, pneumococcus and *Haemophilus influenzae* in human beings; vaccines for parvovirus, rabies, distemper and viral hepatitis in pets and vaccines for necrotic enteritis, coccidiosis, infectious bronchitis, *Escherichia coli*, rotavirus, pink eye and brucellosis in livestock have met with considerable success in reducing the antibiotic prescriptions[8,12,13,16]. In addition, vaccines bring indirect health benefits and growth promotion by preventing diseases, thereby reducing the need of antibiotics[8].

Challenges: Advancement in vaccinology has led to licensing of many novel vaccines in the last 40 yr, but

Table. Uses of alternatives to antibiotics for health promotion, prophylactic and therapeutic purposes in humans, animals and food preservation*

Substitute	Human use			Animal use			Food	Major strength	Major limitations
	Promotion$	Prophylactic	Therapeutic	Promotion	Prophylactic	Therapeutic	Preservation/ disinfection		
Vaccines	X	●	X	X	●	X	X	Specific, efficient immune response with memory	Reversion to virulence, no/ limited cross-protection, parenteral route
Polyclonal antibody	X	●	●	X	●	●	X	Immediate response for fatal infections	Supply chain issues
Monoclonal antibody	X	●	●	X	?	?	X	-do-	Need for diagnosis/ pathogen escape
Pattern recognition receptors	X	●	X	X	?	X	X	Increase immunogenicity of antigens	-
Antimicrobial peptides#	X	?	●	●	○	○	●	Broad-spectrum, target MDR pathogens	Resistance, toxicity
Probiotics	●	●	○	●	●	○	?	Safe, multiple mechanisms of action	Standardization of dose, strain
Bacteriophages and lysins	X	○	○	X	○	○	●	High specificity, ease of administration	Integration in host genome, unstable
Phytochemicals	●	○	?	●	○	○	?	Immunomodulation	Poorly standardized products/toxicity
Antimicrobial enzymes	○	?	?	○	○	?	●	Specificity and strong catalytic activity	Denaturation, loss of activity
Heavy metals	○	○	?	●	○	○	●	Target multiple cellular processes	Metal toxicity, resistance
Nanomaterials	?	?	?	?	?	?	●	Improved pharmacokinetics	Toxicity
CRISPR/Cas	?	?	?	?	?	?	?	Re-sensitization of bacteria to antibiotics	Delivery option, target mutation
Predatory bacteria	?	?	?	?	?	?	?	Potential for resistance rare	Risk of gut microbiome alteration

*Efficacy varies with host species and reasons of use; ● Promising strategies (evidence of efficacy in systemic review, meta-analysis or review of authoritative organizations (WHO, FAO), commercially available; ○ Potential strategies (scientific evidence of usefulness available but not sufficient to justify large scale commercial use/market approvals); $Efficacy in direct health promotion not by prevention of infections; #Many AMPs such as colistin banned for animal use to save them for human use; ? Investigational strategies (approaches in pre-clinical/clinical research); X No efficacy. CRISPR/Cas, clustered regularly interspaced short palindromic repeats/CRISPR-associated; AMPs, associated molecular patterns

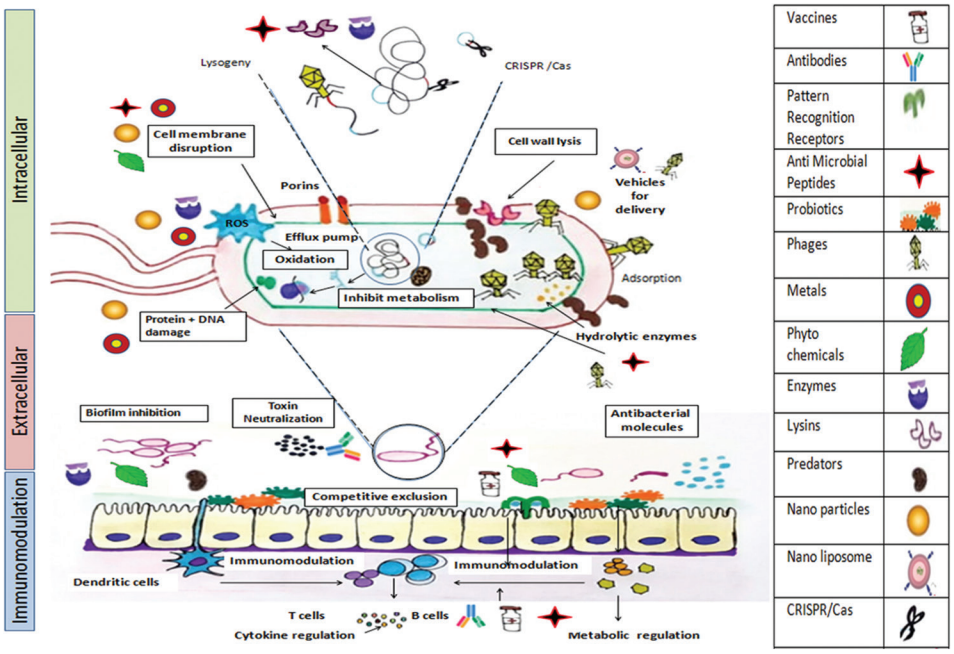

Figure. Major mechanisms of action of antibiotic alternatives wherein alternatives to antibiotics (shown as pictograms in the right panel) bring anti-microbial effect by intracellular action (bacterial cell damage), extracellular action (neutralization of microbe derived products/ toxins), production of antibacterial molecules, enhancing mucosal barrier, competitive exclusion by adherence to the mucosa/epithelium, biofilm inhibition and immunomodulation (metabolic regulation and inducing protective cytokines). The antipathogenic action as discussed in this review is shown by the combination of pictograms with the mechanism of action as shown in boxes. CRISPR /Cas: Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated.

most of these target viruses with a paucity of vaccines for bacterial pathogens[16,17]. There is urgency to develop vaccines for pathogens in which AMR is of critical concern such as *E. coli* resistant to third-generation cephalosporin and fluoroquinolone; *Klebsiella pneumonia*e resistant to carbapenem; methicillin-resistant *Staphylococcus aureus* (MRSA); penicillin-resistant *S. pneumoniae* and fluoroquinolones-resistant non-typhoidal *Salmonella* and *Shigella* and *Neisseria gonorrhoeae*, with reduced susceptibility to third-generation cephalosporins[18,19]. Development of vaccines against Gram-negative infections can make a difference for diseases, such as burns and severe injuries, which need prolonged use of antibiotics[16,17]. Large-scale coverage for vaccine-preventable diseases is also an issue depending on the type of vaccine used as there are safety concerns (reversion to virulence) and difficulty in cold chain maintenance with live vaccines, parenteral route of administration with killed vaccines, suboptimum immunogenicity with subunit vaccines and risk of integration in host genome with DNA vaccines[16,17].

Future prospects: It is an arduous task to develop vaccines against a myriad of pathogens infecting humans and animals due to limited understanding of host–pathogen interactions, suboptimal challenge models and long, complex research in developing vaccines along with regulatory hurdles in licensing[17] However, there is some hope as some vaccines against AMR pathogens such as *Clostridium difficile*, *S. aureus*, *M. tuberculosis*, carbapenem-resistant and extraintestinal *E. coli* have entered clinical trials[17].

Polyclonal/monoclonal antibodies: Antibodies are produced by the host immune system in response to any foreign antigen to rapidly eliminate it by multiple mechanisms, such as preventing adhesion, neutralization, complement fixation and opsonization by phagocytes and antibody-dependent cellular toxicity[20].

Polyclonal antibodies: Passive immunization by transfer of polyclonal antibodies is a time-tested tool for prophylaxis and treatment of several human and animal diseases. These antibodies are derived from pooled plasma/serum of convalescent patients, immune people or animals[20].

Applications: Antibodies are used for prophylaxis and treatment of acute viral infections [hepatitis, measles, varicella, vaccinia, cytomegalovirus (CMV), *etc*.] and bacterial infections (tetanus, anthrax, botulism,

diphtheria, bacteraemia due to *S. aureus*, *etc*.) in humans[20]. There are some licensed polyclonal antibodies for passive immunization in livestock for *Arcanobacterium pyogenes*, clostridioides infections, tetanus, West Nile virus, septicaemia, *Rhodococcus equi* infections, *etc*[21].

Challenges: Polyclonal antibodies have limitations with regard to standardization of quality and quantity of antibodies as these are pooled from multiple animals or convalescent patients and there is a risk of cross-reactions and non-specific interaction within the antibody pool[20].

Monoclonal antibodies (mAbs): mAbs are being investigated for their prophylactic or therapeutic potential against bacterial infections as stand alone therapy, along with antibiotics, or as adjuvant because of high specificity and low risk of development of resistance in acute infections[22]. These hold promise in emerging rare/fatal diseases and immunocompromised states where passive infusion might bring immediate protection by neutralizing the foreign antigens[22,23]. Many mAbs are approved for the treatment of cancers, autoimmune diseases and chronic diseases, but much success has not been achieved for infectious diseases[22].

Applications: mAbs showed reduced mortality in Ebola virus outbreak but did not get approval in view of safety and efficacy concerns[24]. Palivizumab was the first-approved mAb for respiratory syncytial virus infection in high-risk infants[22]. Subsequently, bezlotoxumab and obiltoxaximab were approved to reduce the recurrence of *C. difficile* infection (CDI) and prevention of inhalational anthrax, respectively[22]. Obiltoxaximab was approved on compassionate grounds only based on animal studies in view of high mortality due to anthrax[22]. Itolizumab was recently approved for emergency use for the treatment of moderate-to-severe complications of SARS-CoV-2[25]. mAbs are being extensively explored for prophylactic and therapeutic use for animals, but currently, there is no approved product for commercial use[26].

Challenges: Economic viability due to high production cost of mAb is a concern coupled with poor return on investment due to efficacy against single disease target/antigenic site. Moreover, gathering adequate data for rare, emerging/fatal diseases for conducting randomized clinical trials is a strenuous task because of unpredictability of outbreaks, unspecified epidemiology, difficulty in inclusion of patients and high fatality rates[22]. There is also risk of neutralization escape if mutation occurs in the targeted single antigenic site[22].

Future prospects: Research for finding effective human mAb has gained impetus for nosocomial bacterial pathogens; *S. aureus*, *Enterococcus faecium*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Acinetobacter baumannii,* and *C. difficile*[22,26]. There is a considerable possibility of mAbs cocktails, multivalent antibody and multiple variable region constructs of different specificities to reach market in the near future[22,26].

Targeting pattern recognition receptors (PRRs): PRRs such as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain proteins and nucleotide-binding domain leucine-rich repeat-containing receptors are important components of innate immune system[27]. These are expressed either on the cell surface or in the endosomal compartment of a wide variety of cells such as spleen, peripheral blood leucocytes, in lungs and gut epithelium[27]. Microbial cell surfaces and damaged cells have a defined molecular signature called pathogen-associated molecular patterns, which are recognized by PRRs[27]. After recognition, PRRs get activated and induce the expression of various pro-inflammatory, anti-infective molecules with activation of T and B lymphocytes, macrophages and dendritic cells to boost immune response and combat infections[27]. Thus, TLR agonists are promising candidates to boost the adaptive immune response to eliminate pathogens and attenuate pro-inflammatory immune response and as adjuvant to increase immunogenicity of vaccines[27].

Applications: Currently, only one TLR4 agonist (monophosphoryl lipid A) is licensed for use as an adjuvant in hepatitis B virus (HBV) and human papillomavirus vaccine[27].

Future prospects: Several TLR agonists with potential of limiting chronic inflammation and tissue damage caused by viral pathogens [HCV (ANA773, PF-487861), HIV (GS-620), influenza (VAX102), CMV (CBLB502) and melioidosis (CRX527)] are in preclinical and phase 1 clinical trial[27]. In addition, several TLR agonists alone or in combinations (CpG DNA, Pam3CSK4) are being evaluated as adjuvant in vaccines[27].

Host defence peptides (HDPs) [antimicrobial peptides (AMPs), defensins]: Host defence peptides (HDPs) are components of innate immune defence, which are produced constitutively or induced in response to any

foreign insult by almost all species from prokaryotes, insects, plants to higher animals[28]. Antimicrobial peptides (AMPs) are positively charged amphipathic small peptides (approximately 12-50 amino acids), which are produced in abundance at all sites exposed to pathogens such as skin and mucosal epithelium[28,29].

Mechanism of action (MOA): AMPs act by bactericidal and/or modulating host immune response against a broad range of pathogens (bacteria, fungus, virus and MDR pathogens) and have potential to inhibit/eradicate biofilms[14,29]. Bactericidal action is brought mainly by two mechanisms: (*i*) alteration of cell membrane permeability leading to spore formation with leakage of cell contents or blockage of membrane respiration and cell death, and (*ii*) by intracellular damage to mitochondria and other organelles, DNA fragmentation and inhibition of macromolecules synthesis[10,29]. Immunomodulation is brought by balancing of pro- and anti-inflammatory immune response, with stimulation of adaptive immunity[29,30]. These molecules bring local non-inflammatory resolution of infections by suppression of pro-inflammatory cellular response[29].

Applications: AMPs are selectively toxic to bacterial cells as these interact with negatively charged cell membranes sparingly uncharged or neutral eukaryotic cell membranes[10,14]. Many AMPs such as bacitracin, dalbavancin, daptomycin, enfuvirtide, oritavancin, teicoplanin, telaprevir, telavancin, vancomycin, polymyxin B and colistin are highly efficacious last resort anti-infectives for serious/life-threatening infections by MDR pathogens as topical, oral or systemic preparations[11,28,30,31]. Despite limited research on animal use, AMPs gained extensive popularity for growth promotion and therapeutic purposes in food animals, poultry and aqua-culture, thereby resulting in imposition of bans for their use (particularly, colistin and vancomycin) in animals in several countries, to preserve these antibiotics for human use[32]. AMPs are also used commercially in the food industry (spheniscin, protamine, magainins, nisin, bacteriocins, *etc*.) as preservatives to prevent the growth of food-spoilage organisms, to increase shelf life of dairy, meat, fish, *etc*[33].

Challenges: Development of resistance to AMPs is a major challenge as bacteria evade these molecules by increasing their net positive charge, thus not allowing AMP interaction with cell membranes, by producing proteases for peptide degradation or activating efflux pumps[34]. Besides, there are issues due to instability, degradation by endogenous proteases, short half-life, toxicity, high costs and unreliable pharmacokinetics[34].

Future prospects: Many synthetic long-lasting AMP analogues and short peptides are being evaluated to avoid peptide degradation and to reduce production costs[14,35]. Unique delivery mechanisms to deliver AMPs inside bacterial cells such as liposome encapsulation are being explored to enhance stability and reduce toxicity[35]. In addition, various inducers of natural AMPs (butyrate, histone deacetylase inhibitors, vitamin D3, *etc*.) are in different phases of clinical trials for local/systemic AMR pathogens and inflammatory disorders[35]. These inducers offer some distinct advantages such as lowered potential for emergence of AMR, requirement for fewer doses and thus reduced cost and toxicity to host cells[35].

Pro-, pre-, post- and syn-biotics: Gut microbiome consists of a variety of commensal organisms (>1000 types) which promote human and animal health by energy metabolism, digestion and immune functions and by regulating gut–brain axis[36,37]. Probiotics refer to live microorganisms when administered in adequate amounts bring health benefit to host[38]. These include organisms such as *Lactobacillus*, *Bacillus*, *Bifidobacterium*, *Saccharomyces boulardii*, non-pathogenic strains of *E. coli*, *Bacillus*, *Clostridioides, Veillonella*, *Peptostreptococcus* and certain *Enterococci*[36-38]. Probiotics have anti-pathogenic, anti-inflammatory, antidiabetic, anticancer, anti-allergic, anti-obesity and angiogenic properties along with modulation of gut–brain axis[8,37].

Prebiotics, probiotics and post biotics act by similar mechanisms. Prebiotics are naturally occurring non-digestible products such as fibres, natural sugars, vegetables and fruits, which act as food for commensal bacteria and stimulate their growth. Post biotics refer to metabolic by-products of probiotics such as bacteriocins, ethanol, organic acids, diacetyl, acetaldehydes and hydrogen peroxide that bring biologic activity in the host[37]. Synbiotics are a combination of prebiotics and probiotics in definite proportion[37].

MOA: These agents bring anti-pathogenic effect by several ways: (*i*) interfering with pathogen attachment and entry into gut mucosa by enhancing mucosal barrier, (*ii*) producing antibacterial substances such as bacteriocins and organic acids, (*iii*) destruction of toxins produced, (*iv*) restoration of gut dysbiosis,

and (*v*) bringing in immunomodulation by inducing protective cytokines (IL-10, TGF-beta) and suppressing pro-inflammatory cytokines (TNF)[8,36,37].

Applications: Probiotics meet health promotive, preventive and therapeutic uses. These improve intestinal health and enhance immune response and prevent superinfections by organisms such as *C. difficile* and AMR pathogens by maintaining microbiota composition in patients treated with antibiotics[8,36-39]. These are also being used for prevention of certain diseases (travellers' diarrhoea, antibiotic-associated diarrhoea, vaginitis, sepsis, atopic dermatitis); treatment of acute (CDIs, diarrhoea, constipation) and chronic diseases (irritable bowel disease/ syndrome, hepatic encephalopathy) and preventing side effects after chemotherapy or standard antibiotic therapies[40,41].

Faecal microbiota transplant (FMT), wherein stool containing commensal microorganism, is introduced in the gut, has shown promise as the second-line therapy for recurrent CDI not responsive to standard therapies and clears colonization by many MDR pathogens and to prevent neonatal sepsis[42]. FMT offers decreased risks to the host and increased ease of administration with reduced production costs[42].

Several probiotics, prebiotics (fructo-oligosaccharides, malto-oligosaccharides, short chain fructo-oligosaccharides) and synbiotics are being commercially used to improve productivity and health of food animals[43]. By increasing feed conversion efficiency, these bring growth promotion and reduction in mortality in farm animals[43].

Challenges: Choosing the correct probiotic(s) product is challenging as their efficacy is strain-dependent, disease-specific and dose-dependent[44]. Probiotics act by different MOAs on different pathogens, and same probiotic strain or mixture of strains may be effective for one disease and yet not effective for other disease subtypes, for example, *Lactobacillus rhamnosus* GG, is effective for preventing paediatric antibiotic-associated diarrhoea but is not effective for disease subtypes such as Crohn's disease, CDIs, nosocomial infections or traveller's diarrhoea[44]. Standardizing the dose required is highly intricate as attaining the optimum number of viable cells for effective gut colonization depends on manufacturing processes, quality control, interaction among different bacterial species administered together, acid and bile tolerance[44]. Moreover, like antibiotics, there is a risk of horizontal transfer of AMR-resistant genes from probiotic strains to other co-infected pathogens and vice versa[40].

Future prospects: Probiotics hold a promising future to reduce antibiotic use, but over-the-counter availability of a large number of probiotic combinations of doubtful efficacy along with irrational usage and undefined doses may be problematic[39,43].

Phage therapy: Bacteriophages are viruses that infect bacterial cells and can be genetically engineered for a range of antibacterial activities by several mechanism of action as mentioned below[36,45].

MOA: Lytic phages enter inside bacterial cell by binding to variety of receptors and multiply therein using host cell machinery and bring out cell lysis, while lysogenic phages integrate with bacterial genome and replicate passively without producing virions but can switch to lytic cycle, thereby killing host cell[45]. Lysogenic phages/phagemids (plasmids carrying bacteriophage genes) are genetically engineered to express certain proteins, enzymes, AMPs or toxins to disrupt the normal metabolic processes in bacteria and remove virulence or resistance genes. Bacteriophages encode a variety of peptidoglycan hydrolases such as endolysins and holins to digest bacterial cell wall for phage entry inside cell or release before cell lysis[45].

Applications: Bacteriophages are highly specific and replicate only in target bacteria without disrupting normal microbiome[46]. They offer favourable safety profiles, tolerability and ease of administration and are cheaper than antibiotics[47]. These have been extensively evaluated for safety and therapeutic efficacy in treatment of several bacterial infections in humans and animals, following topical or oral administration[48]. Bacteriophages have shown efficacy in burns, wound infection, diabetic foot ulcer and also for treatment of systemic infections by several pathogens[48]. Their potential for synergistic activity with antibiotics and for treatment of MRSA, MDR *P. aeruginosa*, *A. baumanii* and *K. pneumoniae* has met with considerable success[48]. These have also shown promising results for disease prevention and treatment in food animals[6].

Some phage cocktails were initially licensed in a few countries only for clinical, animal and environment use in view of equivocal efficacy but are slowly gaining recognition in other countries as well[45]. Some phage preparations are available for selected indications for topical use in chronically infected cutaneous wounds/diabetic ulcers refractory to systemic antibiotic treatment, for the treatment of purulent infections,

for oral use in diarrhoea and dysentery, *etc*[45]. Phage therapy is currently approved in the United States, only for emergency treatment under compassionate grounds ('off-license' approval) for terminally ill patients in the absence of any effective antibiotic or alternative treatment[49,50]. A recent compendium of case series for compassionate use has revealed phage therapy to be safe and clinically efficacious therapy, resulting in eradication of pathogens[50].

Bacteriophages have also been approved for prophylaxis and treatment of infections due to *Salmonella* (PLSV-1™) and *Clostridium perfringens* (INT-401™) in poultry in the USA[51]. Fixed phage mixtures are commercially available for biocontrol of food-borne pathogens such as *E. coli Salmonella* serotypes and *Listeria monocytogenes*, *Shigella* and for surface disinfection in various parts of the world including USA[51,52]. Lysins are most evaluated hydrolases used extensively in food industry for preventing food spoilage of cheese, meat and fresh cut fruit and inhibiting biofilm formation[36].

Challenges: High phage specificity, difficulty in characterization and standardization of phage dosage limit their therapeutic usefulness. Phage preparations are unstable, and resistance emerges easily by modifications of phage binding targets in bacterial cells, by integration of antibiotic resistance genes by lysogenization of phage DNA into bacterial genome (transduction)[47]. Tetz and Tetz[53] have highlighted a contradictory picture, in which phages themselves act as pathogens by killing the normal microbiome, by interacting with host cells, proteins and thus have proposed that their abundance may also be a target for therapeutic intervention This aspect needs to be explored further.

Future prospects: U.S. Food and Drug Administration (FDA) has accepted a new drug application for an intravenously administered bacteriophage-based therapy[54]. Phages are being researched to act as a vehicle to deliver bacterial biofilm/capsule-degrading enzymes, proteins to repress the DNA repair mechanism, antibiotics, photosensitizing agents, *etc*. directly inside bacterial cells, thereby avoiding binding to targets amenable to mediate resistance[45]. These can also act as vehicles for delivery of novel RNA-guided endonucleases to selectively cut, destroy the DNA sequences encoding for virulence and AMR in MDR Gram-negative pathogens [clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated (CAS) system discussed later][10,55]. Phage cocktails promise a propitious future to target mixed bacterial populations based on signals of safety and therapeutic efficacy[45].

Phytochemicals: Phytochemicals (phytobiotics, phytogenics, herbal medicines or functional foods) are natural bioactive compounds such as carotenoids, curcumin, organosulphur compounds, phytosterols and flavonoids that are derived from a variety of plants, fruits, vegetables, legumes, whole grains, *etc*. Besides antioxidant, anticancer, immune stimulant, anti-inflammatory, provitamin, enzymatic, anti-stress and hormone regulation properties, these are also being evaluated for antimicrobial (antibacterial, antifungal, antiviral, antiparasitic) activity[56,57].

MOA: Immunomodulation to boost host cell immunity is the most explored and validated mechanism of phytochemicals for health and growth promotion[57]. Different compounds exert antibacterial activity by different mechanisms, *i.e.* by damaging bacterial membranes, suppressing virulence factors, interacting with membrane proteins, leakage of ions, coagulation of the cell content, inhibiting enzymes/toxins or bacterial biofilm formation[57]. Some also modulate antibiotic resistance mechanisms in bacteria and can bring synergistic effect with antibiotics, thereby reducing the dose of antibiotics or making them more effective[58-62].

Applications: Phytochemicals such as *ashwagandha*, curcumin and lycopenes are extensively used as nutraceuticals to bring a variety of health benefits in humans and livestock[6,59]. A large number of herbal drugs [*Ocimun basilucum* (basil), *Caryophyllus aromaticus* (clove), *Achillea millefolium* (yarrow), *Rosmarinus officinalis* (rosemary), *Melissa offficinalis* (lemon-balm), *Punica granatum* (pomegranate)]; phytochemicals (carvacrol, benzoic acid, cinnamic acid, eugenol and farnesol, *etc*.) and essential oils (cinnamon, oregano, thyme, Lemon grass, Sage, Caraway, Nutmeg, *etc*.) have shown good antimicrobial activity (for Gram-positive, Gram-negative and MDR pathogens) *in vitro* and are being used for their anti-inflammatory, prophylactic and therapeutic potential in infections without definitive evidence of efficacy and safety[58,59,61-63]. Topical application of combination of *Aloe vera*, *C. longa* and calcium hydroxide is currently used in sub-clinical mastitis in cattle because of broad-spectrum antimicrobial, anti-inflammatory and immunomodulatory activities[64].

Herbal medicines have shown potential in food units to control food spoilage by a variety of Gram-positive and -negative bacteria[65].

Challenges: Although herbal medicines, phytochemicals are being used alone or with antibiotics for prophylactic and therapeutic purposes, there are no methodological clinical trials for their safety and efficacy. Like antibiotics, there are reports of insensitivity/tolerance/resistance to herbal medicines, phytochemicals as well[65-67]. Herbal medicines have been believed to be safe and non-toxic compared to allopathic medicines, but there is increasing number of reports of unacceptable health risks to consumers because of contamination with toxic levels of metals and carcinogens[68].

Future prospects: There is a need for creation of comprehensive databases for natural product-based drug discovery[69]. An Indian database Indian Medicinal Plants Phytochemicals and Therapeutic uses has a subset of 60 potential druggable phytochemicals, and most of these are different from existing FDA-approved drugs[69]. There is hope that phytochemicals may emerge as a possible source of effective, cheap and safe antimicrobial agents.

Antimicrobial enzymes (enzybiotics): A wide variety of proteolytic (subtilin, lysostaphin, bacteriophage lysins), polysaccharide-degrading (lysozyme, amylase, dispersin B, alginate lyase), oxidative (myeloperoxidase, cellobiose dehydrogenase, horseradish peroxidase, quorum-quenching enzymes (acyl homoserine lactonase, acylase, *etc*.) are being explored for their antimicrobial action[70]. Several enzyme-based products are used in healthcare specifically for their anti-inflammatory properties (hyaluronidase), in food and biomedical industries[71].

MOA: Enzymes bring bactericidal effect by degrading structural components of microorganisms; cell oxidation by inducing production of hydrogen peroxide, by catalytic reactions, by quenching quorum sensing, thereby preventing biofilm formation[70].

Applications: Exogenously administered enzymes bring catalytic breakdown of food into smaller substances for better digestibility and nourishment. Enzymes have selective bactericidal action, non-toxic to normal flora with rare potential for emergence of AMR[70,71]. These have demonstrated good antimicrobial activity against *Pseudomonas, Streptococcus, Bacillus* and MRSA *in vitro* but have not shown conclusive evidence of efficacy and safety in clinical studies[70]. However, a combination of xylanases and beta-glucanases is commercially used for growth promotion in food animals and for prophylactic purposes for some diseases in chickens as feed enzymes[71].

Challenges: Major disadvantage of enzymes is propensity to get denatured in extreme conditions during storage, transport, sterilization of enzyme-coated devices and cost-intensive purification processes.

Future prospects: Research to design different enzyme formulations to increase their stability, improve pharmacokinetics, administration by different routes, with decreased side effects and toxicity has gained impetus.

Heavy metals: Metals such as iron, cobalt, manganese, copper, molybdenum and zinc are useful for a variety of physiochemical and biological functions of the body in permissible amounts[72]. These are natural ingredients of many foods and used in a variety of formulations as micronutrients to bring health and growth promotion in humans and food animals[72]. Deficiency of these metals predisposes to infectious diseases by impairing immune defence mechanisms[72]. Metals such as copper and silver have been evaluated for a number of antimicrobial effects and have shown efficacy as monotherapy or with antibiotics (additive and/or synergistic effect) to inhibit a variety of Gram-positive and -negative bacteria[73].

MOA: Metals bring bactericidal effect at very low concentration. These act by targeting multiple cellular processes of bacterial cells, such as damage to cell membranes, oxidative damage by generating free radicals, inhibiting essential enzymatic activities and breakdown of nucleic acids[72,73]. Metals also lead to deactivation, precipitation of bacterial cellular proteins, thereby cell death[73].

Applications: Metals have been an integral component of a large number of Ayurvedic medicines (Indian Traditional Health System) for health-promoting, prophylactic and therapeutic uses but have recently gone in disrepute due to reports of metal toxicity[72,73]. Silver is used for health and growth promotion, for bactericidal activity in wound dressing and burns, as topical application[72]. Although metals are being evaluated for preventive and therapeutic antibacterial uses as topical and systemic preparations, there are not sufficient randomized controlled trials to assert efficacy and safety[74]. These are being explored as disinfectants on coatings of medical devices such as urinary catheters, intrauterine devices and hip

prosthesis and have shown inconsistent efficacy to warrant large-scale use[75]. Metals have gained prominence for use as surface coatings in hospitals, food industry and water disinfection systems to prevent growth of pathogenic microorganisms[75].

Challenges: Metals offer very low permissible limits and slight increase in exposure results in metal toxicity with interruptions of intracellular homeostasis due to free radical-induced damage to lipids, proteins, enzymes and DNA[76]. Studies have shown that some metals containing implantable medical devices predispose individuals to local or systemic inflammatory immune reactions[77]. Striking a fine balance between metal dose and toxicity is highly intricate. Furthermore, microbes develop resistance to metals by reducing uptake, activating efflux, extracellular/intracellular sequestration, metabolic bypass, repair of oxidized molecules, chemical modification, *etc*[78]. These also lead to cross-resistance to other metals (multimetal resistance) and antibiotics as the genes encoding for antibiotic resistance and heavy metals are genetically linked[78].

Future prospects: Metal complexed antibiotics and metallic nanoparticles are being evaluated to improve the biocompatibility, bioavailability, synergistic effect and circumventing drug-resistant mechanisms. In addition, microbes capable of producing metal nanomaterials may hold promise in mitigating metal pollutants with simultaneous antibacterial properties[79].

Nanomaterials: Nanostructured materials such as nanoparticles (NPs) and liposomal/polymer-based nano-drug carriers are attractive options to combat AMR as these can permeate cell membranes because of their ultra-small size[80]. NPs can be alloyed with metals such as silver, gold, aluminium and copper to bring bactericidal action and can also act as vehicles to deliver antibiotic/(s), antibiotics formulation with metals, immunomodulators/silencing agents safely inside host cells circumventing drug resistance mechanisms[80-83]. The slow release of antibiotics from encapsulated particles reduces the dose required and side effects along with favouring pharmacokinetics, therapeutic index and cost-effectiveness[81]. Silver NPs are being used as disinfectants in water filters, textiles, medical masks and food packaging industry with some success in animal husbandry and human use[84].

MOA: NPs bring antibacterial action by several mechanisms: (*i*) triggering generation of reactive oxygen species thereby leading to oxidative damage, (*ii*) disruption of cell wall, (*iii*) inhibiting enzymes mediating AMR, (*iv*) inhibiting biofilm formation, quorum sensing, (*v*) protein deactivation, (*vi*) DNA damage, (*vii*) damaging bacterial efflux pump, and (*viii*) plasmid curing[82,83].

Applications: Many metal-based NPs have been tested for efficacy and safety *in vitro*, in animals and clinical studies with mixed results[84]. However, silver NPs have been found safe and are commercially used as disinfectant coatings on wound dressings, polyurethane ventricular catheter, hand gels, intravenous catheters, cavity fillers, *etc*.[84]. In addition, these are used for surface disinfection in food industry[85].

Challenges: It is difficult to calibrate dose and identify appropriate routes of administration because of the highly efficient cellular uptake of NPs across cells, tissues and organs as it has potential to attain toxic levels quickly[84]. Several studies have shown accumulation of NPs in liver, heart, lungs and spleen following intravenous or inhalation administration[81].

Future prospects: Currently, many NP-based metal alloys, liposomal nanomer-carrying antibiotics and other novel compounds are being explored for effectiveness against MDR Gram-negative bacteria and inhibition of biofilm formation. NPs coating of implantable devices such as heart valves, dental implants and catheters have been studied to reduce seeding and growth of bacteria[82]. Some of these may prevent emergence of resistance in bacteria or revert antibiotic sensitivity.

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas)-mediated gene disruption: CRISPR/Cas is an important component of the bacterial adaptive immune system. This is basically an array of short repeated sequences separated by spacers. Short repeats encode proteins (*e.g.* Cas 3/5/9) to bring functional activity (*e.g.* endonuclease activity to cut and remove the undesired sequences). Spacers are unique DNA sequences which are acquired from bacteriophages, plasmids or transposons and serve to counter any future attack with similar nucleic acids sequences. CRISPR array is transcribed and processed into short CRISPR RNAs that guide Cas nucleases to destroy target nucleic acids in case of any subsequent attack[8,86].

MOA: CRISPR/Cas protein is employed for gene editing to remove AMR genes from bacteria, thereby reversing their sensitivity to antibiotics. A single

guide RNA consisting of a crRNA sequence (specific to the DNA target encoding virulence factors/ antibiotic resistance/foreign DNA) with a transfer crRNA sequence (that interacts with the Cas protein) is delivered by means of engineered bacteriophages/ plasmids. crRNA sequence binds to the target DNA and Cas protein with DNA endonuclease activity brings cleavage in double stranded DNA followed by error prone DNA repair resulting in disruption of gene functions[55,86,87].

Applications: The gene editing is being explored for precise removal of virulence and antimicrobial-resistant genes in *S. aureus*, *E. coli*, carbapenem-resistant *E. coli* and enterohemorrhagic *E. coli* with re-sensitization of bacteria to antibiotics[55,86,87].

Challenges: Choosing effective delivery options in highly complex environmental populations, to evolution of resistance as a result of mutations in target sequences, loss of Cas activity and uncertainty due to legislative and social issues are some of the challenges[87].

Future prospects: There is a ray of hope with the beginning of the first antibacterial clinical trial for treating urinary tract infections using phages to deliver CRISPR-Cas3[88].

Predatory bacteria: *Bdellovibrio* and like organisms, *Micavibrio* and others are bacteria found ubiquitously in soils and water and can eat up bacteria completely without spilling any of the bacterial contents in the extracellular milieu (unlike phages), thereby averting inflammatory immune response[89,90].

MOA: These bacteria bring bactericidal action by releasing hydrolytic enzymes to digest the bacterial cell content either by entering inside or attaching to the cell surface. These attack a range of Gram-negative bacteria including *Salmonella*, *E. coli* and some AMR pathogens and may also break biofilms, thereby allowing antibiotics to act[89,91].

Applications: These have demonstrated efficacy as bactericidal agents in experimental and *ex vivo* studies from periodontal, sub-gingival, ocular and respiratory samples[91,92]. Moreover, predators can restore gut dysbiosis and bring biological control by preying on MDR pathogens[91]. Emergence of resistance is unlikely because killing does not involve binding to any specific receptor or protein[89].

Future prospects: These are in early stage of research and are associated with inherent risk of altering gut microbiota besides manufacturing and regulatory challenges[89].

Conclusion

Vaccines appear to be the most promising alternatives to antibiotics for several viral and bacterial diseases and can substantially reduce pressure on antibiotics. While there is a need for developing new vaccines, the focus should be more on utilization and expanded coverage of existing vaccines. Probiotics offer an optimistic choice for the treatment of a variety of infective gastrointestinal disorders, particularly CDIs, and acute diarrhoeal diseases but need stringent quality control to standardize the dose and species used. Although AMPs are highly efficacious anti-infectives for treating serious, life-threatening infections mainly by MDR pathogens but have a high potential for developing resistance, hence should be reserved for only human use. Bacteriophages are also getting acceptance for the treatment of many bacterial diseases including MDR pathogens and life-threatening illnesses. Herbal medicines and phytochemicals have been used since antiquity for health promotion, prophylactic and therapeutic uses but need methodologically rigorous clinical evaluation for safety and efficacy to warrant large-scale prophylactic and therapeutic use. Others like PRR agonists, metals and NPs, CRISPR/Cas and predatory bacteria are in early stages of development and require intensive research before being used as antibiotic alternatives. Like with antibiotics, enforcement of regulations and monitoring systems would be critical to prevent abuse of these alternatives leading to safety concerns and risk of emergence of resistance to some of these strategies.

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