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Antibiotics, Antibacterial Resistance and Alternatives To Control Infections.

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Introduction

The introduction of antibiotics to clinical therapy is a great advance in human medicine, since Fleming's great discovery. However, the successful use of antibiotics is gradually compromised by the development of antibiotic resistance. Currently, the problem of antibiotic resistance is receiving unprecedented attention all over the world. The global collection of resistance genes in clinical and environmental samples is the antibiotic "resistome" and is subject to the selective pressure of human activity. The origin of many modern resistance genes in pathogens is likely environmental bacteria, including antibiotic producing organisms that have existed for millennia. Most antibiotics in medical or agricultural use are derived from or produced by a group of soil-dwelling bacteria called the Actinomycetes (the most notable genus for antibiotic production being *Streptomyces*). These organisms are prolific producers of specialized metabolites (so-called "natural products"), including the antibiotics streptomycin, tetracycline, chloramphenicol, erythromycin, and vancomycin. The antibiotic resistance has a long history that is comparable to the discovery of antibiotics, and the resistance even appeared prior to the clinical use of the drugs (Yongfel et. al. 2017). Antibiotic resistance is a One Health problem, i. e., one that intricately links humans, animals, and the environment (Robinson et. al., 2017). The investigation of novel approaches for tackling the antimicrobial resistance crisis must be part of any global response to this problem. One such promising avenue of research involves so-called antibiotic resistance breakers (ARBs), capable of re-sensitising resistant bacteria to antibiotics. (Law et al. 2019). As more antibiotic are rendered ineffective by drug resistance bacteria, focus must be shifted toward alternative therapies for treating infection. Although several alternative

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already exist in nature, the challenge is to implement them in clinical use. Antibiotic resistance is a One Health problem, i. e., one that intricately links humans, animals, and the environment (Robinson et. al., 2017). The recognition that the environment is a nearly boundless reservoir of antibiotic resistance has resulted in several studies that seek to estimate the risk of gene transfer to pathogen (Manaia CM. 2016). The evidence is now clear that the environment is the single largest source and reservoir of resistance. Soil, aquatic, atmospheric, animal-associated, and built ecosystems are home to microbes that harbor antibiotic resistance elements and the means to mobilize them. (Surette *et. al.* 2017). The investigation of novel approaches for tackling the antimicrobial resistance crisis must be part of any global response to this problem. One such promising avenue of research involves so-called antibiotic resistance breakers (ARBs), capable of re-sensitizing resistant bacteria to antibiotics. (Law *et al.* 2019). As more antibiotic are becoming ineffective by drug resistance bacteria, focus must be shifted toward alternative therapies for treating infection. Although several alternative already exist, the challenge is to implement them in clinical use.

Mechanism of antibiotic resistance in producer organism

Antibiotic resistance poses a tremendous threat to human and animal health. To overcome this problem, it is essential to know the mechanism of antibiotic resistance in antibiotic-producing and pathogenic bacteria.

1. Antibiotic modification or degradation
2. Antibiotic Efflux
3. Antibiotic Sequestration
4. Target Modification/Bypass/Protection Mechanism

In case of aminoglycosides, chloramphenicol, and beta lactam antibiotic modification is commonly used strategy to make its ineffective. The aminoglycoside antibiotics are biosynthesized by Actinobacteria, so these prokaryoates must protect themselves against attacks by their own biosynthetic products. A large number of aminoglycoside modification enzymes (AMEs) including N-acetyl tranferases (AAC), O-phosphotransferase (APH), and O- Aden transferase (ANT) that acetylate, Phosphorylate or adenylate the aminoglycoside antibiotic respectively in producer

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organism. There is structural and sequence similarities between AMEs of producer and Cellular metabolic enzymes Modification enzymes may be co-opted from housekeeping enzyme. In contrast to modification beta lactam antibiotic is normally degraded by β-lactamase hydrolysing enzyme. This enzyme widespread among streptomyces and similar enzyme are found in pathogenic and non pathogenic bacteria, they all constitute the 'β-lactamase superfamily'.

Efflux of antibiotic is another commonly used mechanism for self resistance, although it usually occurs in conjunction with other mechanism'. The best studied example of antibiotic efflux among producer is Streptomyces peucetius which produced two closely related anticancer antibiotic, daunorubicin (Dnr) and doxorubicin (Dox). . These two antibiotics joined with DNA preventing further round of replication. Efflux of these antibiotics occur by ABC family transporter (ATP binding cassate). Another example is OtrC found in oxytetracycline producer Streptomyces rimosus. It exhibit multidrug specificity. Self resistance in S. rimosus is conferred by two efflux protein: OtrB located in the biosynthesis cluster, and OtrC located outside the cluster. OtrB belong to major facilator superfamily (MFS) of transport family.

Sequestration involves the function of drug-binding proteins, which prevent the antibiotic from reaching its target. In producers of the bleomycin family of antibiotics, the primary mechanism of resistance involves sequestration of the metal-bound or the metal-free antibiotic by binding proteins TlmA, BlmA, and ZbmA in *S. hindustanus*, *S. verticillus*, and *Streptomyces flavoviridis*, respectively (Rudolf *et al.,* 2015). Each bleomycin-family producer member has one or more genes related to ABC transporters in their biosynthesis clusters which may be used to remove the antibiotics bound to binding proteins. (Pozzi *et. al*. 2016). The three self-resistance-related genes, *blmA*, *blmB* and *orf7* encoding the bleomycin-binding protein, the bleomycin acetyltransferase, and ABC transporter respectively, are present at the end of the cluster. *orf29*, which is located at another end of the cluster, may also be involved in the self-resistance by transporting the drug. (Sanchez *et. al* 2000)

Target modification acts as a self-resistance mechanism against several classes of antibiotics, including β-lactams, glycopeptides, macrolides, lincosamides, and

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streptogramins (MLS), and aminoglycosides. The β-lactam antibiotic has a similar structure to PBP substrates (peptidoglycan precursors), thus allowing the antibiotic to associate and cause acylation of the active site serine resulting in its inhibition. The producer *Streptomyces* species, despite being Gram-positive, are highly resistant to penicillins, which is due to either overproduction of PBPs or synthesis of low-affinity PBPs (Ogawara. 2015). Analysis of the biosynthesis cluster of ß-lactam producing bacteria showed that they contained gene for PBPs suggesting their role in self resistance

Origin of Antibiotic Resistance in Clinical Isolates

It was based on the observation that the aminoglycoside-modifying enzymes found in actinomycetes exhibit biochemical activities similar to the enzymes found in pathogenic strains. Another striking example of a strong connection between antibiotic resistance genes in clinical isolates and those found in antibiotic producing bacteria is provided by the *van*HAX genes, which show considerable protein sequence similarity as well as a conserved arrangement and organization of genes within the cluster. Despite strong indications that transfer from producer organisms to the pathogenic strains might occur a direct link between producers and pathogens has, however, been hard to establish. This is primarily due to the fact that resistance genes in producers show high sequence divergence and a very different G+C content as compared to determinants in pathogens even when they use similar mechanisms, however, now seem to suggest that resistance genes found in non-producer environmental bacteria may have played a more important role in shaping the evolution of antibiotic resistance in pathogens. Overall, it is safe to conclude that both producer and non-producing environmental organisms represent rich pools of resistance genes which could potentially be mobilized to the clinically relevant strains, A few reports of direct genetic exchange from producer to non-producer organisms and from environmental organisms to clinical pathogens are indeed available. In one report, otrA and otrB gene sequences, found in the oxytetracycline biosynthesis cluster in Streptomyces, were identified in mycobacteria variants. Interestingly, the same study also provided

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evidence for the presence of S. aureus tetracycline resistance genes Tet(K) and Tet(L) in Streptomyces and mycobacteria variants

Overall, these examples suggest that both producer and non-producer environmental bacteria play a role in dissemination of resistance genes although recent direct transfers to clinical strains seem to have mainly occurred from non-producer environmental bacteria.

Enrichment of Antibiotic Resistance Genes

It is well-recognized that the environment itself plays an important role in the acquisition of antibiotic resistance by pathogenic organisms. This process go through four stages:

1. Emergence of novel resistance genes
2. Mobilization
3. Transfer to pathogens
4. Dissemination

While emergence and mobilization events likely occur all the time, environmental factors, such as selective pressure, fitness cost, and dispersal, determine whether these events actually result in establishing novel genes in populations. Of these, selection is perhaps the single most important factor which plays a critical role in maintenance of resistance genes. The most important source of selective pressure, however, is the widespread and indiscriminate usage of antibiotics by humans, which results in dominance of resistant and multiply resistant strains of bacteria not only among human pathogens but also in environments where human activities (such as antibiotic manufacturing facilities) result in pollution with antibiotics **(Larsson, 2014**). Other settings, considered to be hot-spots "Role of HGT in Transfer of Antibiotic Resistance Genes" Where human-associated and environmental bacteria co-exist, also provide significant opportunities for exchange of resistance genes as well as selection for resistance. Other settings, considered to be hot-spots "Role of HGT in Transfer of Antibiotic Resistance Genes" Where human-associated and environmental bacteria co-exist, also provide significant opportunities for exchange of resistance genes as well as

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selection for resistance. Soil bacteria contain antibiotic resistance genes responsible for different mechanisms that permit them to overcome the natural antibiotics present in the environment. This gene pool has been named the 'resistome', and its components can be mobilized into the microbial community affecting animal and humans because of the participation of genetic platforms that efficiently facilitate the mobilization and maintenance of these resistance genes. Resistance to antibiotics can occur either by mutations or by acquisition of resistance genes via horizontal gene transfer (HGT), of which the HGT is considered to be the most important factor in the current pandemic of AMR.

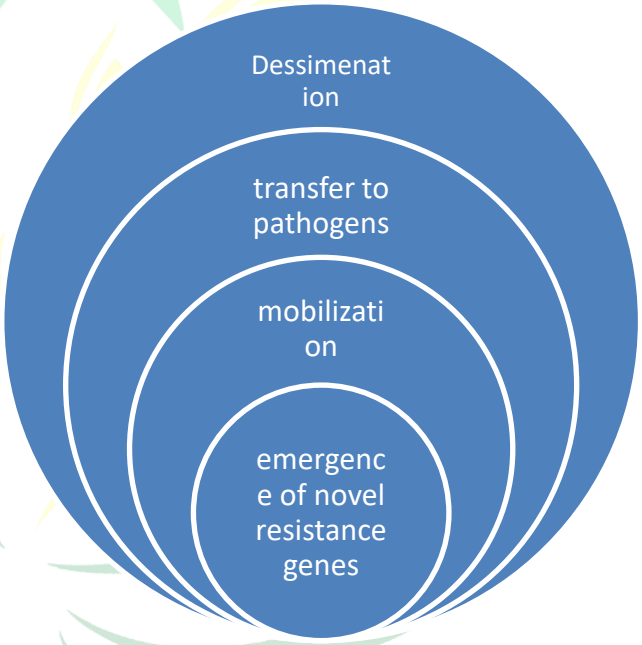

Fig 1. Emergence of new antibiotic resistome

Reservoirs of Antibiotics Resistance

In order to understand the mobilisation and dissemination of antibiotic resistance, it is necessary to map the resistome of various environments. In recent years there has been increasing interest in this matter, as many studies have used various techniques to sample the resistome of environments such as, but not limited to, soil, wastewater, and human and animal gut microbiota (Pehrsson et al., 2013; Penders et al., 2013; Rizzo et

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al., 2013; von Wintersdorff *et al.*, 2014). It has since become clear that ARGs, including clinically relevant ones, are widespread in such environments (Wright, 2010). It is generally accepted that the ARGs are as old as the natural-product antibiotics in bacteria, for the simple reason that the antibiotic producers should equip themselves with resistance genes to protect themselves (Davies 2010). As the antibiotic biosynthetic pathways emerged several hundred million years ago, the ARGs probably had circulated for a long time in bacterial communities before their "flourishing" in recent decades. The natural environments, therefore, is reasonably regarded as the first reservoir of ARGs (Martinez *et al.*, 2008; Allen *et al.*, 2010). Another study further demonstrated that antibiotics can serve as a sole carbon source to support the growth of soil bacteria, and many of the phylogenetically diverse bacteria are found closely related to human pathogens and resistant to multiple antibiotics (Dantas *et al.*, 2008). Along with terrestrial environment, aquatic environment is another reservior for ARGs. Unlike soil, the water environments that are easily accessible to human and animal, may be more affected by anthropogenic activities. The containing of animal and human pathogens and industrial pollutions like antibiotics and disinfectants, sewage wastewater released constantly in human activities contributes greatly to the spread and accumulation of ARGs in water environment (Baquero *et al.*, 2008) In addition, the heavy use of prophylactic antibiotics in aquaculture, results in the frequent occurrence of antibioticresistant bacteria and a rapid dissemination of the antibiotic resistance determinants in water environment (Cabello *et al.*, 2006) Along with natural environment, host-associated environment that is gut microbiota is a more complex antibiotic resistome reservoir due to more frequent exposure to antibiotics of the gut bacteria. One of the important host-associated environment is the gut bacteria of farm animals. It is estimated that, in the United States, nearly 80% of antibiotics were used in animals for growth promotion, disease prophylaxis and treatment purpose. (Yap. 2013). It is therefore, not unexpected, that the animals and their related environments constitute a huge reservoir of ARGs. The effect of antibiotics on the alteration of the human gut bacteria as well as the resulting antibiotic resistance has long been recognized (Anderson, 1968. ; Weinstein, 1954) The ARGs are silently circulated among natural environments, animal, and human beings, i. e., in the one health settings,

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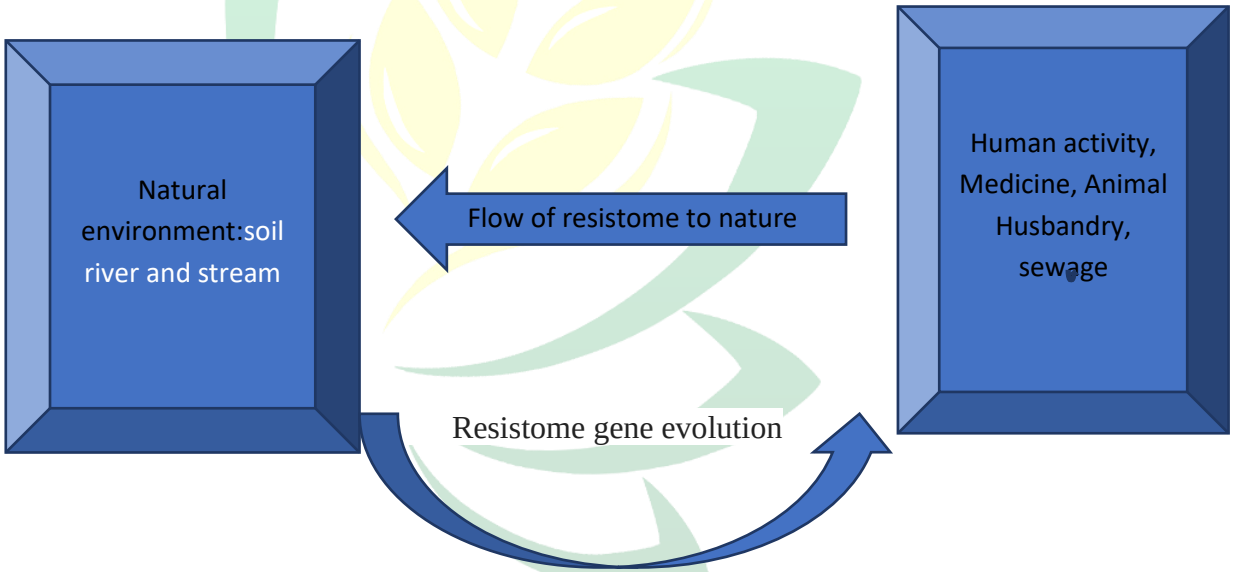
and increasingly accumulated. The first report of antibiotic resistance transfer was published in 1969, demonstrating that the R factors from animal or human E. coli can be transferred to the resident E. coli in the alimentary tract of a human being (Smith., 1969) Another important reservoir in the spread of antibiotic resistome among different environments is wild animal. The wildlife in proximity to the contaminated ecosystems is get exposed to resistant bacteria and ARGs. Once the wild animals acquired ARGs, there is a potential for them, especially the highly mobile species, birds migratory birds to spread the ARGs around the world. Very recently, Vittecoq et al. reviewed more than 210 studies concerning antibiotic resistance in wildlife (Vittecoq et al., 2016). They concluded that a strong link exists between human activities and the carriage of antibiotic resistant bacteria in wildlife; omnivorous, and carnivorous species are at high risk of acquiring and disseminating resistance bacteria; aquatic environments are container for resistance bacteria exchange and are potential site for the spread of antibiotic resistance.



- Fig 2-The antibiotic resistome: gene flow in environments, animals and human beings

Alternative to antibiotic

The antibiotic resistance problem is caused by the evolution and transfer of genes that confer resistance to medically and veterinary important antibiotics. The acquisition of such resistance genes by pathogens complicates disease treatment, increases health care costs, and

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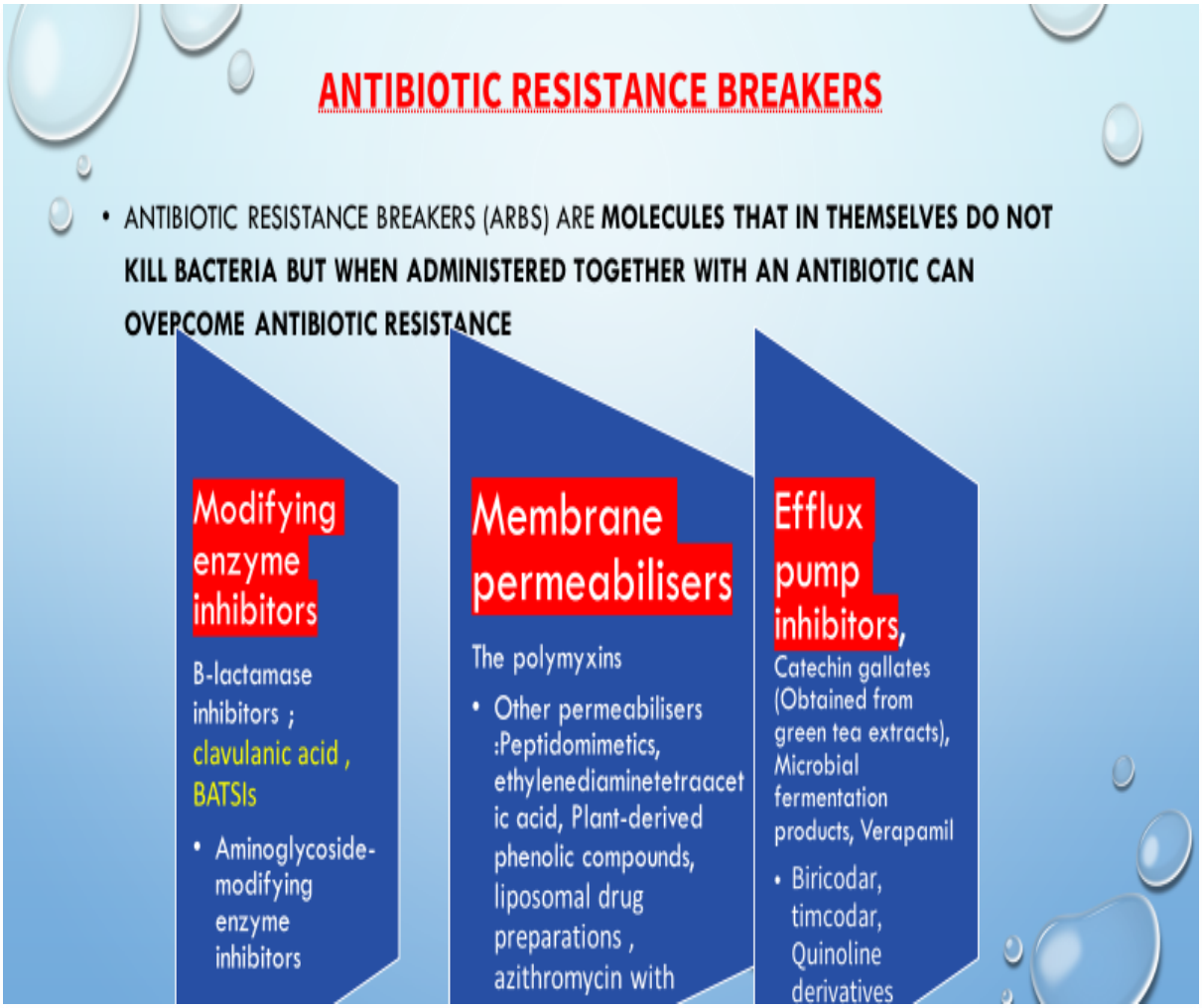
increases morbidity and mortality in humans and animals. Reducing or preventing the dissemination of antibiotic resistance genes into clinical pathogens is currently of high international importance. Novel therapeutic alternative to antibiotic are three type:



Naturally occurring alternative

1. **Phage Therapy**: Bacteriophages (phages), or viruses that 'eat' bacteria, were used to treat infected livestock before conventional antibiotics were used. Phage are known to select between mixed population of bacteria Thus exploitation of lytic cycle it can be develop a selective approach to target pathogenic bacteria. Advantages over conventional antibiotics; Self-replicating pharmaceuticals, Selective towards specific strains of bacteria, Amenable to genetic engineering. Possible disadvantages: Immunogenicity Pharmacokinetics Release of bacterial

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endotoxins Inadequate preparations – failure to remove endotoxins and pyrogenic substances Resistance development.

2. **Antimicrobial Peptides:** As the first line of defence, antimicrobial peptides (AMPs) and host-defence peptides (HDPs) are produced by many multicellular organisms against invading pathogens [9–11]. They have diverse activities ranging from antibacterial, antifungal, antiviral, anticancer, antiplasmodial, antiprotistal, insecticidal, and spermicidal to immunomodulation. Advantages : Not prone to resistance development Broad-spectrum activity is an advantage, depending upon application. Disadvantages: Expensive large-scale production Susceptible to proteolysis toxicity

3. **Bacteriocins**: In order to prevent competition and enhance survival, several bacteria produce small AMPs that act against other bacteria within the population. These ribosomally synthesized peptides, called bacteriocins, are often active against drug-resistant pathogens of clinical importance. Broadly classified into two groups, bacteriocins that undergo rigorous post-translational modification belong to class I, and the unmodified belong to class II. The mechanism of action of bacteriocins is similar to that of AMPs in that they target the cell membrane. Advantage: Specificity towards pathogenic strains of bacteria Resistance to heat and UV. Disadvantages: Expensive large-scale production Susceptible to proteolysis. Bacteriocins suffer from the same problems as AMPs, but their use in certain cases can offer more advantages. Conventional antibiotics and AMPs rarely select between commensal and pathogenic bacteria. However, like bacteriophages, bacteriocins can also have selectivity against particular bacterial strains.

4. **Alteration of the Gut Microbiota:**

   a. ***Probiotics***: The mammalian gut microbiota comprises over 1000 microbial species, including bacteria and yeast. Bifidobacteria, lactobacilli, streptococci, and nonpathogenic strains of E. coli, Bacteroides sp., Clostridiodes sp., Fusobacterium sp., Eubacterium sp., Peptococcus sp., Peptostreptococcus sp., are some of the predominant bacterial genera found in the intestines Although bacteria belonging to the genera Lactobacillus and Bifidobacterium have been

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used for the treatment of various gastrointestinal infections. Treatment with probiotics and prebiotics is advantageous over antibiotics as these agents are safe for long-term consumption and do not cause side effects or allergies.

b. ***Fecal Transplant Therapy****:* Fecal transplant treatment (FTT) is an alternative strategy involving the introduction of the microbiome from a healthy donor into the diseased gut. It is used to treat bacterial infections or other cases involving gastrointestinal dysbiosis. . However, the investigation of the efficacy of FTT against these pathogens is limited

5. **Predatory Bacteria**: The use of predatory bacteria such as Bdellovibrio and like organisms **(BALOs)** has been considered a promising alternative to antibiotics. These organisms are d-proteobacteria that multiply only upon entering other Gram-negative pathogens such as E. coli, Salmonella, Legionella, Pseudomonas. BALOs degrade prey cells by lysing them with various hydrolytic enzymes (such as DNAses and proteases). Such enzymes can also penetrate into bacterial biofilms, giving them an advantage over conventional antibiotic. The lipopolysaccharide (LPS) of bdellovibrio lacks the negatively charged phosphate group, resulting in reduced binding affinity to the LPS receptors in human immune cells. Thus, they exhibit minimal inflammatory response (TNF-a and IL-6) [43]. The failure of these bacteria to multiply within mammalian cells indicates their potential in the treatment of bacterial infections. Upon oral administration of Bdellovibrio to chicks infected with a gut-colonizing Salmonella enterica serovar Enteritidis phage type 4, Atterbury et al. found a significant reduction in the bacterial load in bird gut cecal contents and reduced cecal inflammation.

6. **Antibodies** : To fight the invasion of pathogens, the immune system produces antibodies – proteins that recognize specific components of the pathogen and neutralize them. Antibodies are thus useful alternatives for the treatment of intractable bacterial infections. They could be used to treat bacterial infections either by directly targeting the bacterial surface or indirectly by neutralizing the bacterial toxins and the virulence factors that are responsible for infection. A major drawback of using antibodies for antibacterial therapy is the cost of the production and poor shelf life.

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Synthetically Designed Strategies

1. Synthetic Mimics of Antimicrobial Peptides (SMAMPs); Scientists have also designed molecules in an attempt to mimic the properties of antimicrobial peptides. It yield excellent results with some able to re-sensitize drug-resistant bacteria to conventional antibiotics.

2. Innate Defence Regulatory Peptides; It is peptide with no antibacterial activity but with antiendotoxin and immunomodulatory activities. IDR peptide are promising alternative to conventional antibiotic and one of them has completed phase one clinical trial. (SGX942).

3. Antibacterial Oligonucleotides;It is based on gene silencing therapy. Silencing of essential and resistance causing genes by use of antisense oligonucleotides with sequences complementary to the target mRNA is an alternative strategy for tackling multidrug resistance bacteria.

4. Inhibitors of Bacterial Virulence. : The inhibition of expression of virulence factors, which interfere with the interaction between the bacterium and its host, is another strategy for tackling infection. Although it was not antibacterial itself, it exhibited synergism with antibiotic.

Biotechnology-Based Approaches

1. **Genetically Modified Bacteriophages:** Recently upon treatment with these engineered phage both biofilm and the bacteria that are embedded within the biofilm were lysed.

2. **Lysins (Endolysins, Exolysins, and Autolysins)**: These are attractive candidate for alternative therapy because of their direct antibacterial activity. Since these enzyme are genetically encoded, they are amenable to production using genetic engineering.

3. **CRISPR-Cas 9:** The CRISPR(clustered, regularly interspaced short palindromic repeats)-Cas9(CRISP- associated protein 9). These are key components of bacterial immune system wherein 20nt small RNA acts as guide for cas9 to cleave foreign genetic elements such as those present in plasmid and phage at specific sites,

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4. **Antibiotic Inactivators:** The use of antibiotic disrupts the gut microbiome, thereby providing an opportunity for the growth of pathogenic strain. In order to reduce this possibility, antibiotic in activator could be used, e. g. Ribaxamase

Antibiotic Resistance Breakers

- To tackle the increasing emergence of AMR, alternative treatment strategies have been designed with the collective aim of reducing the number of antibiotics used:
- **Modifying enzyme inhibitors** Modifying enzyme inhibitors are used to disrupt bacterial detoxification enzymes, increasing the effectiveness of a co-administered antibiotic. Two major classes are the BLIs and aminoglycoside-modifying enzymes.

  1. B-lactamase inhibitors:example is a) clavulanic acid, commonly sold as the combination products co-amoxiclav b) co-ticarclav (combined with ticarcillin)
  2. Aminoglycoside-modifying enzyme inhibitors:several antimicrobial peptides against the aminoglycoside-modifying enzymes(APH(3')-IIIa (EC 2. 7. 1. 95), AAC(6')-Ii and AAC(6')-APH(2″) (EC 2. 3. 1. 81)), The bovine peptide indolicidin.

- **Membrane permeabilizers** : It iscompounds that make the Gram-negative OM more permeable to facilitate increased antibiotic influx. Membrane permeabilisers can function by chelating and removing divalent cations from the OM and/or (in the case of permeabilisers with a net cationic charge) associating with the negatively charged OM to disrupt it, causing a breakdown of OM structure (Zabawa et al. 2016). use of liposomal drug preparations (Torres et al. 2012). Polymyxins including polymyxin B and polymyxin E (colistin), are antibiotics that function through disruption of the Gram-negative OM. Lin et al. found that azithromycin, ineffective against Gram-negative rods, showed synergy with colistin; the combination was effective against MDR-isolates of P. aeruginosa, K. pneumoniae and A. baumannii (Lin et al. 2015). Peptidomimetics are synthetic compounds that mimic the membrane permeabilization mechanism of action of antimicrobial peptides, but are stable to enzymatic degradation. Another example of a permeabiliser is ethylenediaminetetraacetic acid, a chaotropic agent that has been shown to release a large proportion of LPS from the

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OM. Plant-derived phenolic compounds, a group of secondary metabolites abundant in fruit, vegetables and berries, have been shown to possess membrane permeabilising activity. Li and co-workers have demonstrated that cholic acid derivatives are suitable alternatives to polymyxins as ARBs.

- **Efflux pump inhibitors :** A diverse array of EPI compounds have been reported to date. Catechin gallates : Obtained from green tea extracts, catechin gallates have been shown to reverse β-lactam resistance in MRSA. Abietane diterpines : Isolated from the herb *Rosmarinus officinalis*, carnosic acid and carnosol act as potentiators of erythromycin and tetracycline against *S. aureus* strains containing Msr(A) and Tet(k) pumps. Methoxylated flavones and isoflavones: Baicalein, isolated from the leaves of *Thymus vulgaris.* flavones have shown activity against Gram-positive bacteria, but few reports have been made on their interaction with Gram-negative bacteria (Mahmood *et al*. 2016). Microbial fermentation products :Compounds EA-371α and EA-371δ were originally isolated from *Streptomyces* fermentation extracts. At 0. 625 µg mL^{-1}, both compounds caused a four-fold decrease in the MIC of levofloxacin against a strainof *P. aeruginosa.* Homoisoflavonoids :Bonducellin, a homoisoflavonoid purified from the roots of *Caesalpinia digyna*, is another compound that has shown potential for use as an EPI.
- Alkaloids :reserpine, an indole plant alkaloid extracted from the roots of *Rauvolfia serpentina* and *Rauvolfia vomitoria*, has been shown to be effective in inhibiting
- Biricodar, timcodar: rBiricodar (formerly VX 710) and timcodar (formerly VX 853) were originally developed by Vertex Pharmaceuticals as anticancer agents, but have more recently found applications in prokaryotic efflux inhibition.
- **Calcium channel blockers** :Verapamil, a drug used to treat cardiac disorders through inhibiting mammalian efflux transporters such as P-glycoprotein, has also been shown to inhibit the ATP-dependent ABC-type prokaryotic efflux systems. It is capable of potentiating a number of antibiotics (including rifampicin, fluoroquinolones and macrolides) against strains of M. tuberculosis (Pule et al. 2016, Chien, Yu and Hsueh 2017).

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- **Quinoline derivatives** :Quinoline compounds and their derivatives have been shown able to inhibit efflux of various antibiotics in MDR isolates of Klebsiella aerogenes.

Conclusions

- The antibiotic resistome in natural environments, animal and human microbiomes is more complex than we expected. It is known that the ARGs are from natural environments, however, the human activities using antibiotics lead to a significant amplification of the original ARGs in human clinical settings. Then, antibiotics as well as the ARGs, which are now considered to be new pollutants, are released to the environments by various physical or biological forces during anthropogenic activities.

  Therefore, from an ecological point of view, the scenario for the resistance gene flow is that the ARGs are "from the natural environments" and "to the natural environments" Human and animals, as intermediate recipients and disseminators, contribute greatly in such a circulation from the beginning to the end. Alternative approaches have been developed to combat antibiotic resistance and treat bacterial infection.

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