A STUDY ON MECHANISM OF MULTIDRUG RESISTANCE IN BACTERIAL PATHOGENS

Dissertation submitted in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

By

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UNDER THE GUIDANCE OF

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MAY-2021

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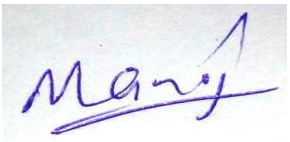
DECLARATION BY THE SCHOLAR

I hereby declare that the review of literature reported in the M.Sc thesis entitled **"A Study on Mechanism of Multidrug Resistance in Bacterial Pathogens"** submitted at **Jaypee University of Information Technology, Waknaghat, India**, is an authentic record of work done by me (Manoj Kumar-197802) carried out under the supervision of **Dr. Rahul Shrivastava** (Associate Professor) Department of Biotechnology and Bioinformatics. I have not submitted this work elsewhere for any other degree or diploma.



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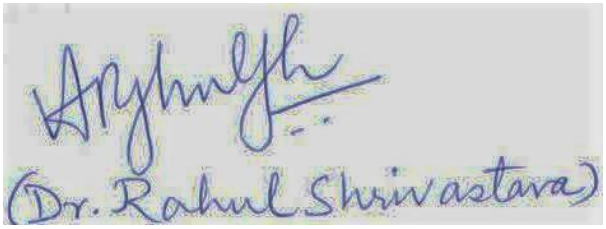
SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the M.Sc. thesis entitled **"A Study on Mechanism of Multidrug Resistance in Bacterial Pathogens",** submitted by **Manoj Kumar (197802) at Jaypee University of Information Technology, Waknaghat, India,** is a bonafide record of his original work carried out under my supervision. The duration of the project was from January 2021 to May 2021. This work has not been submitted elsewhere for any other degree or diploma.



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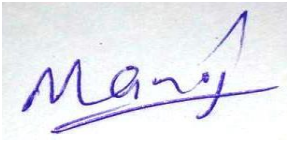
Last but not the least, I would like to thank all individuals whose names could not be mentioned who were generous enough to help me throughout the Duration of the project.

I bow my head before the **Almighty God** whose blessing gave me the strength to make this successful venture and I dedicate my work and achievement in his lotus feet.



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LIST OF ABBREVIATIONS

MDR	Multi drug resistance
WHO	World health organisation
MRSA	Methicillin-resistant Staphylococcus aureus
AMR	Anti-microbial resistance
VRE	Vancomycin resistant enterococci
CDC	Centers for disease control and prevention
ICMR	Indian council of medical research
AMRSN	Antimicrobial Resistance Surveillance & Research Network
FDA	Food and drug administration
AMP	Anti-microbial peptides

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ABSTRACT

Discovery of antibiotics revolutionized the field of medicine but the generation of resistance among bacteria averts the use of these wonder drugs. Since 1940s, resistance has been widely recognised among bacterial pathogens. Since then, researchers have analysed the nature and method of origin of resistance in various bacterial species. Study on several strains of bacteria showed that the origin of resistance can be innate or adaptive and resistance generation is a tactic response to the abundance of antibiotic in bacterial surroundings. Bacterial resistance originates on physiochemical basis or on the genetic level. Divers classes of bacteria share resistance mechanisms and exhibit the resistance against one or more than one type of antibiotic. A well-known cause of resistance generation is the overuse and misuse of antibiotics by human population. Our environment contains a high concentration of antibiotics and pollutants, which assists in selection of resistance bacteria over sensitive one. A considerable complication with resistance is the spread between different species and classes of bacteria. Proliferation of resistance among bacteria is causing escalation of the frequency of resistance pathogens in environment. Some known strains, as VRE and MRSA, that are predominant pathogens to cause hospital-acquired infections, are identify to be resistant against all commonly available antibiotics. This study hence reviews the various causes, which aids in generation of resistance and mechanisms utilised by bacteria to eliminate the effectiveness of antibiotics. In addition, the study discusses the escalation of resistance pathogen frequency with increased antibiotic use and several unconventional technologies, which can act as antibiotic alternatives to combat resistant pathogens.

Keywords: Antibiotics, Anti-microbial resistance (AMR), Multi-drug resistance (MDR), Drug discovery, Antibiotic alternati

1. Introduction to MDR

In late 19th century, infectious agents were discovered which started the search for preventive measures against these agents. After half a century later, antibiotics were discovered which gives successful treatment against various infectious agents. Discovery of antibiotics was a revolution in field of disease and medicine.

In academic research and in pharmaceutical, discovery of antibiotics, their action mechanism and resistance mechanism originated in bacteria against antibiotics is a productive research topic [Fig1] [1]. Antibiotics are natural products and it has been intellectually challenging to understand chemical nature of antibiotics, their pathways of biosynthesis and their mode of action against bacteria. Since the discovery of first antibiotic, scientists with understanding of their nature and working mechanisms have discovered many novel therapeutics.

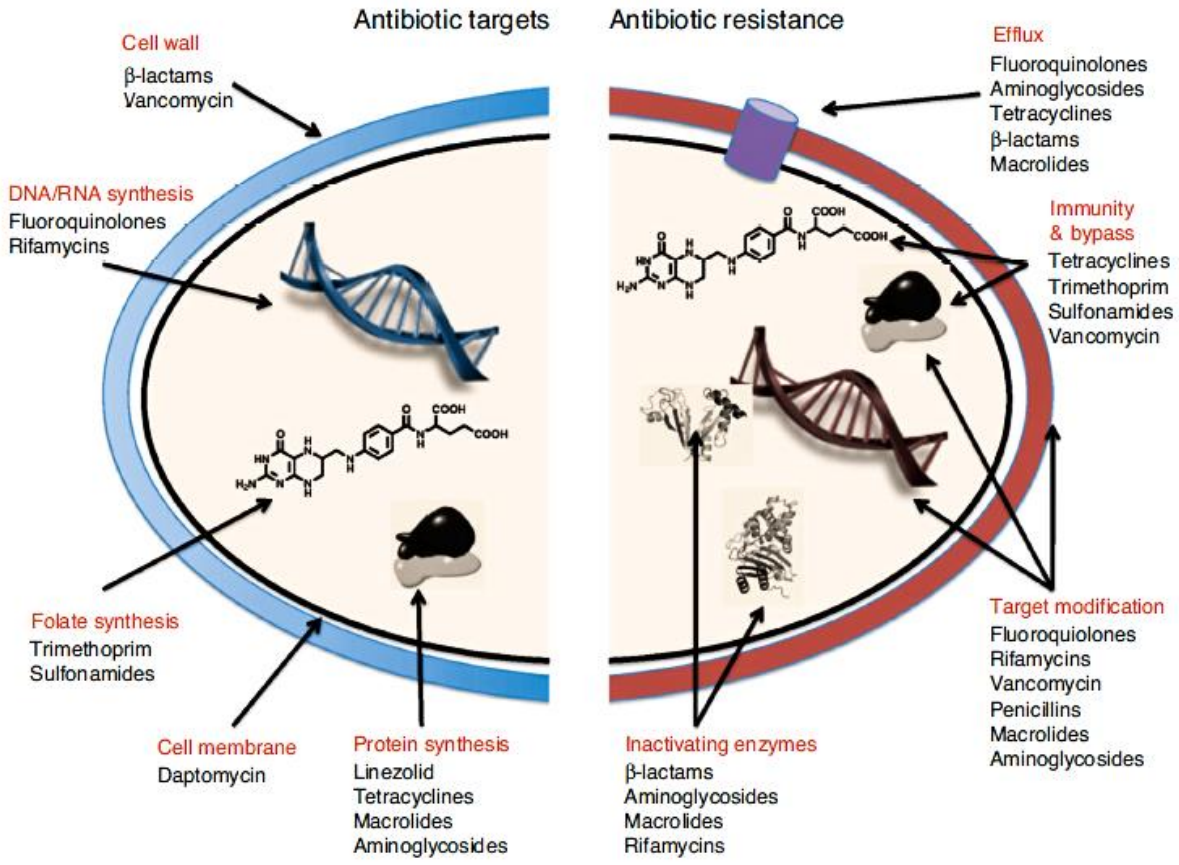


Figure 1: Target of antibiotics and resistance mechanism adapted by bacteria. *[Reference: D. Sheard, N. O'Brien-Simpson, J. Wade and F. Separovic, "Combating bacterial resistance by combination of antibiotics with antimicrobial peptides", Pure and Applied Chemistry, vol. 91, no. 2, pp. 199-209, 2019. Available: 10.1515/pac-2018-0707.]*

Although discovery of antibiotics revolutionized the field of medicine, the appearance of resistant against these wonder drugs has been seen in different bacterial strains. A major cause of resistance generation is the widespread use of antibiotics in hospitals and in research facilities, which in turn select MDR bacteria over sensitive strain [2]. The microbes, which are in constant contact with antibiotics, tend to originate a resistant mechanism and the selection pressure due to extensive use of antibiotics causes distribution of these resistant mechanism and resistant strains [Fig2]. Generation and proliferation of resistant is not a natural process but a situation created by men via underuse, misuse and overuse of antibiotics. This is a classic example of Darwin's natural selection law.

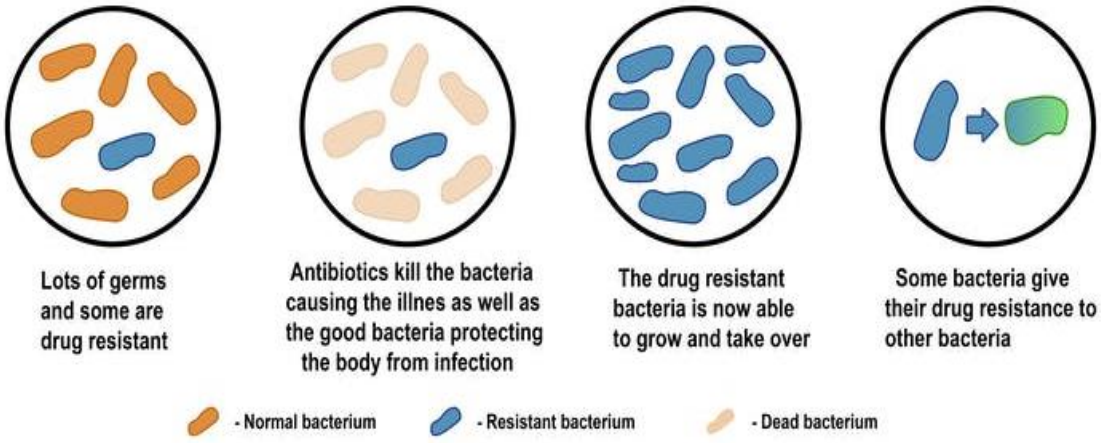


Figure 2: Overuse of antibiotic can help in natural selection of resistant bacteria over sensitive one. *[Referece: "How to train the body's own cells to combat antibiotic resistance", The Conversation, 2021. [Online]. Available: https://theconversation.com/how-to-train-the-bodys-own-cells-to-combat-antibiotic-resistance-106052. [Accessed: 17- May- 2021].*

There are many mechanism; physical or biochemical, are responsible for the resistance against antibiotics in bacteria. The resistance process can be simple or it can be complex [Table1] and we lack some basic information about resistant generation and proliferation. That is the reason why we have been not achieving any significant results while preventing generation and proliferation of the resistant among bacterial strains [3].

Resistant against antibiotics among bacterial strains is not a new topic but the emergence of resistant against effective drugs in the strain, which are already resistant against various other

type of antibiotic, is problematic for us. There are many disease agents, which we once can treat by antibiotics, are coming with resistant against antibiotics [4].

Table 1: commonly used antibiotics, their biochemical target, examples, commonly target bacteria and resistant mechanism generated against these antibiotics in bacterial strains.

Antibiotic class and biochemical target	***Example***	***Common target bacteria***	***Mechanism of resistance***
Aminoglycosides (Translation)	Gentamicin, Streptomycin, Neomycin, Amikacin, Tobramycin	Gram negative bacteria	Phosphorylation, acetylation, nucleotidylation, efflux, altered target
β-Lactams (Peptidoglycan biosynthesis)	Penicillins, Cephalosporins, Carbenicillin, Nafcillin, Ampicillin, Oxacillin	Gram positive and gram negative bacteria	Hydrolysis, efflux, altered target
Glycopeptides (Peptidoglycan biosynthesis)	Vancomycin, Decaplanin, Teicoplanin, Bleomycin	Gram positive bacteria	Reprogramming peptidoglycan biosynthesis
Tetracyclines (Translation)	Minocycline, Doxycyclins, Tigecycline, Oxytetracyclins	Gram positive and gram negative bacteria	Monooxygenation, efflux, altered target
Quinolones (DNA replication)	Ciprofloxacin, Levofloxacin, Moxifloxacin	Gram positive and gram negative bacteria	Acetylation, efflux, altered target
Sulfonamides (Folate synthesis)	Sulfamethoxazole, Sulfadiazine, Sulfasalazine	-	Efflux, altered target
Lipopeptides (Cell membrane)	Daptomycin, Bacillomycin, Mycosubtilins	Mostly Gram positive Bacteria	Altered target
Macrolides (Translation)	Erythromycin, clarithromycin, Azithromicin, telithromycin	Mostly Gram positive bacteria	Hydrolysis, glycosylation, phosphorylation, efflux, altered target

There are various approaches has been researched to combat the problem of antibiotic resistant in disease causing microbes. One method is combination therapy. We can either combine two antibiotics in which one antibiotic will be effective against resistant mechanism for the first antibiotic or we can add an adjuvant with antibiotic that can alter its biochemical mechanism. Some other mechanism like phage therapy, use of CRISPR cas 9 technology, use of bacterial toxins etc. can also be used to combat this problem.

2. A brief history

Drug resistance is first reported in military hospitals in 1930s where most of the antibiotics were being used. First antibiotic resistant microbe reported was *Streptoccoccus pyogenes, which* was resistant to sulfonamides. In 1940s *Staphylococcus aureus* resistant to penicillin were reported in London civilian hospitals. Streptomycin resistant *Mycobacterium tuberculosis* was reported shortly after the discovery of this antibiotic [Fig3].

Multiple drug resistant in bacteria was first reported in late 1950s to early 1960s in enteric bacteria like *Shigella, Salmonella* and *Escherichia coli* [5]. These strains were very harmful for human health and can pose risk to human lives. Their impact was more in developing countries then in developed countries. In 1970s, ampicillin resistant microbes like *Haemophilus influenza* were reported which cuses respiratory diseases. At the same time, *Neisseria gonorrhoeae*, which causes gastrointestinal diseases, also reported to have resistant against ampicillin [6]. *Haemophilus* was also reported to have resistance against chloramphenicol and tetracycline [7]. In developing countries, the spread of antibiotic resistance was more because antibiotics were available in stores without prescription. In addition, due to lack of money these countries are not able to buy latest more expensive antibiotics which are more effective against various pathogens. A new tuberculosis strain was reported in 1980s, which are resistant to many drugs and to treat the disease caused by this strain, sometime six to seven different antibiotic is being used [8].

There are some strains, which are resistant against almost all the antibiotics that are available commonly. An example is methicillin resistant *Staphylococcus aureus* (MRSA). This strain is resistant against Methicillin, aminoglycosides, macrolides, tetracycline, chloramphenicol and lincosamides. This strain is also resistant from disinfectants. This strain is a common factor of hospital-acquired infection nowadays [9].

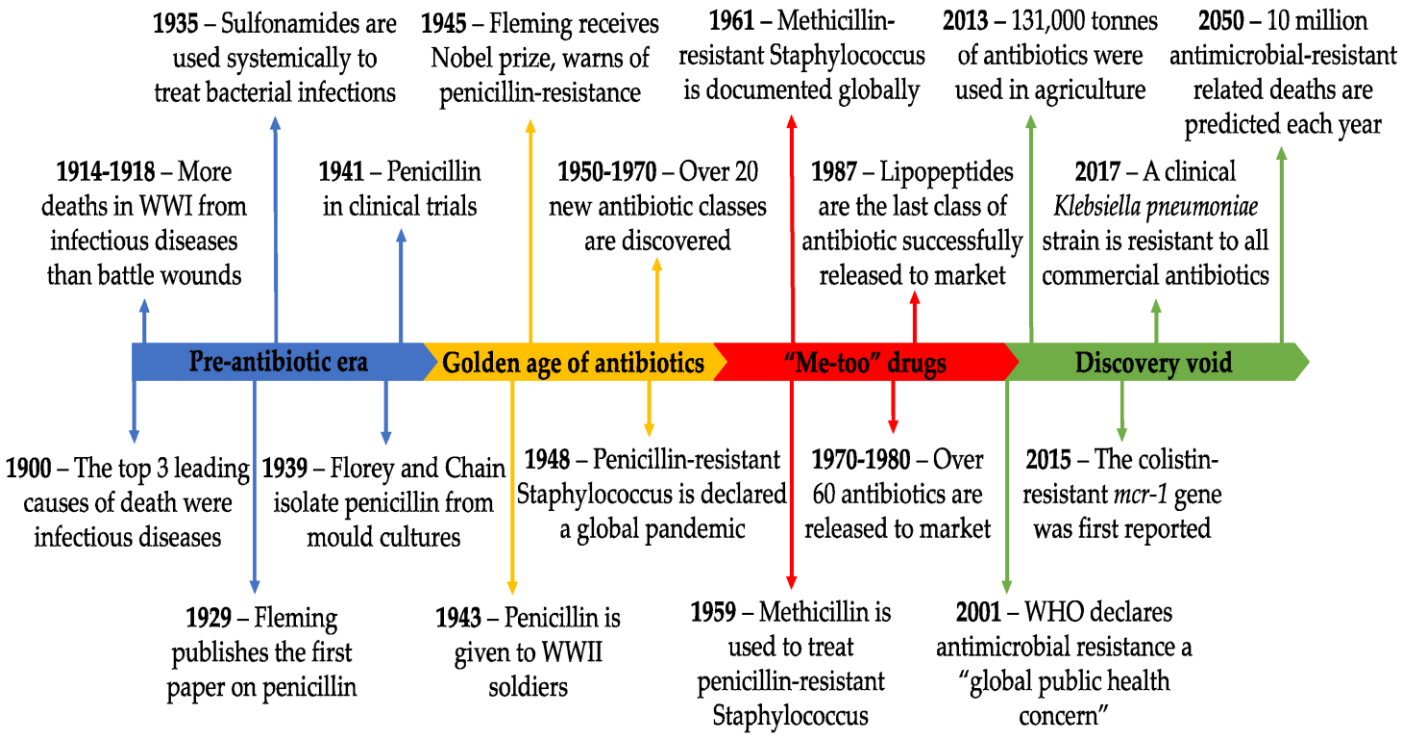


Figure 3: A brief history showing various era of discovery and development of antibiotics with the generation of resistance in bacterial strains. *[Reference: K. Browne et al., "A New Era of Antibiotics: The Clinical Potential of Antimicrobial Peptides", International Journal of Molecular Sciences, vol. 21, no. 19, p. 7047, 2020. Available: 10.3390/ijms21197047.]*

It has been estimated that about 100,000 tons of antibiotics are being manufactured annually in the world and how we are using these drugs are affecting bacterial life. Since their discovery, a very large amount of antibiotic has been produced for variety of purposes other than medicinal use. Production of antibiotics has been modified since time to make it less costly to make it more reachable for the common public, which in turn enhances overuse and misuse. The saturation of the environment with these toxins is selecting resistant strain of bacteria over susceptible one. This selection in turn causing a threat to healthcare system, global economy and food security [10]. In United States over 23,000 deaths are caused by antimicrobial resistance illness. It has been reported that resistance microbes in United States cause over 2 million illness annually and this problem cost around 20 billion dollars annually [11]. It has been estimated that by 2050, antimicrobial resistance will cost global economy around $100 trillion. In addition, by 2050, the AMR problem will kill more people than diseases like cancer and diabetes [Fig4].

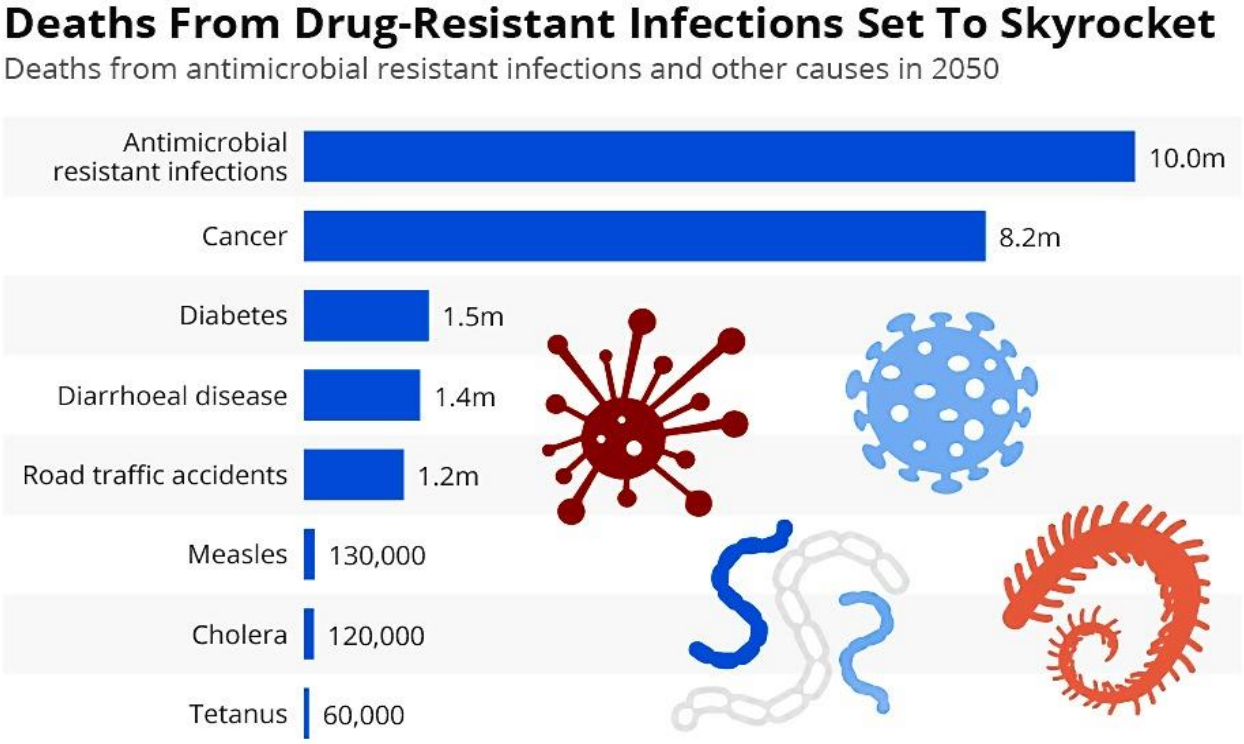


Figure 4: The statistical prediction reveals that AMR will cause most deaths by 2050. *[Referece: "Infographic: Deaths From Drug-Resistant Infections Set To Skyrocket", Statista Infographics, 2021. [Online]. Available: https://www.statista.com/chart/3095/drug-resistant-infections/. [Accessed: 17- May- 2021].*

3. Epidemiology of MDR

Treatment of resistant diseases can be costly and sometimes the treatment can be unsuccessful. In the developing world, many antibiotics are failing in treating some pathogens [12]. According to a report by WHO, as we develop new antibiotics there are emergence of new mechanisms of resistant among bacteria and with widespread use of antibiotic the new mechanisms are spreading globally. The spreading of resistance is leading to prolonged illness and death due to common infectious diseases. Those patients, which are infected with resistant strains, are under risk of severe illness or death and they need more resources in healthcare facilities then non-resistant strain of same disease [13]. In 2017, WHO released a list of bacteria highlighting with high priority pathogens that are resistant to multiple antibiotics and urgently need new therapeutic approach [Table2].

Table 2: Priority list of pathogens by WHO. *[Reference: D. Bloom and D. Cadarette, "Infectious Disease Threats in the Twenty-First Century: Strengthening the Global Response", Frontiers in Immunology, vol. 10, 2019. Available: 10.3389/fimmu.2019.00549.]*

Pathogen	***Resistance***
PRIORITY 1: CRITICAL	
Acinetobacter baumannii	Carbapenem-resistant
Enterobacteriaceae	Carbapenem-resistant, 3rd generation cephalosporin-resistant
Pseudomonas aeruginosa	Carbapenem-resistant
PRIORITY 2: HIGH	
Staphylococcus aureus	Methicillin-resistant, vancomycin intermediate and resistant
Campylobacter	Fluoroquinolone-resistant
Enterococcus faecium	Vancomycin-resistant
Neisseria gonorrhoeae	3rd generation cephalosporin-resistant, fluoroquinolone-resistant
Helicobacter pylori	Clarithromycin-resistant
Salmonella *species*	Fluoroquinolone-resistant
PRIORITY 3: MEDIUM	
Haemophilus influenza	Ampicillin-resistant
Streptococcus pneumoniae	Penicillin-non-susceptible
Shigella *species*	Fluoroquinolone-resistant

Klebsiella pneumoniae is a bacterium, which causes pneumonia, infection in new-borns and blood stream infections in intensive care patients in hospitals. It is a major cause of hospital-acquired infection. Common drug to treat the infections caused by this bacterium is carbapenem and resistance against this antibiotic is found widespread. Resistance against the drug increased from 13% in 2008 to 56% in 2017. For polymixins, resistance increased from 2% in 2012 to 17% in 2017.

Fluoroquinolones resistance *E. coli* is very widespread that causes Urinary tract infection. In many countries around world, fluoroquinolones is effective in only 50% of the patients infected with this strain of *E. coli*. In 2008, it was found that resistance against fluoroquinolones is 83% in 2008 in India, which increased to 86% in 2017 [Fig5].

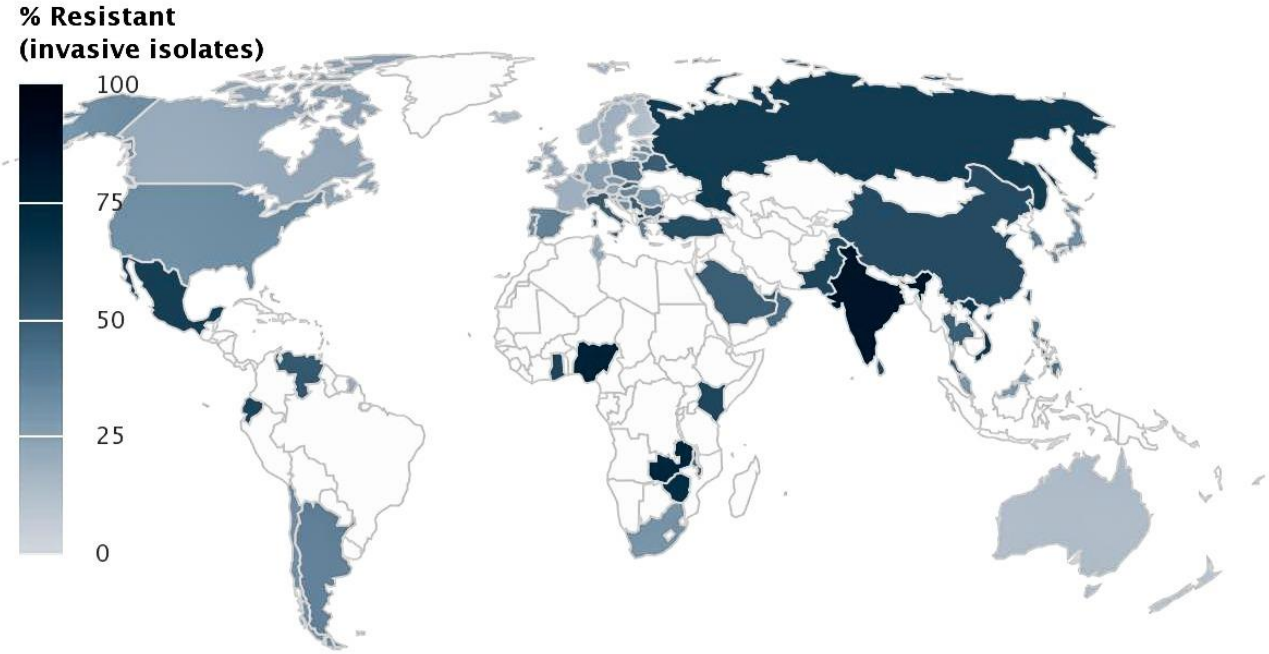


Figure 5: E. coli resistance distribution against Fluoroquinolones drugs worldwide. [Reference: *The Center for Disease Dynamics, Economics & Policy. ResistanceMap: https://resistancemap.cddep.org/AntibioticResistance.php. Date accessed: May 6, 2021].*

For gonorrhoea disease, third generation cephalosporin is used as treatment but resistance generation against this antibiotic has been confirmed in at least 10 countries (Australia, Austria, Canada, France, Japan, Norway, Slovenia, South Africa, Sweden and the United Kingdom of Great Britain and Northern Ireland). Resistance against drugs like tetracyclin, penicillin, macrolides, fluoroquinilones and early generation cephalosporins are emerging in *N. gonorrhoeae.* The only remaining therapy for the disease in many countries is injectable extended-spectrum cephalosporin (ESC) ceftriaxone. A study from Delhi hospitals showed that *Neisseria gonorrhoeae* resistant to different drugs increased to 83.3% from 2002 to 2008.

WHO estimates that, in 2014, Multidrug resistant tuberculosis (MDR-TB) has around 480,000 new cases worldwide. This strain of tuberculosis is resistant to two of the most powerful TB drugs used against the bacterium. Only about a quarter of these (123 000 cases) were detected

and reported. Treatment of MDR-TB takes longer time and effectiveness of treatment is less then susceptible TB. In 2014, patients that are completely traded from MDR-TB is half of the people actually got sick with the disease. People who are treated with non-resistant TB are getting new TB disease with resistant strain. Extensively drug-resistant tuberculosis (XRD-TB), which are resistant to at least 4 drugs is also prone in the people which are diagnosed with MDR-TB. In 2017, around 10 million new cases were reported with MDR-TB from which 1.6 million people died [Fig6].

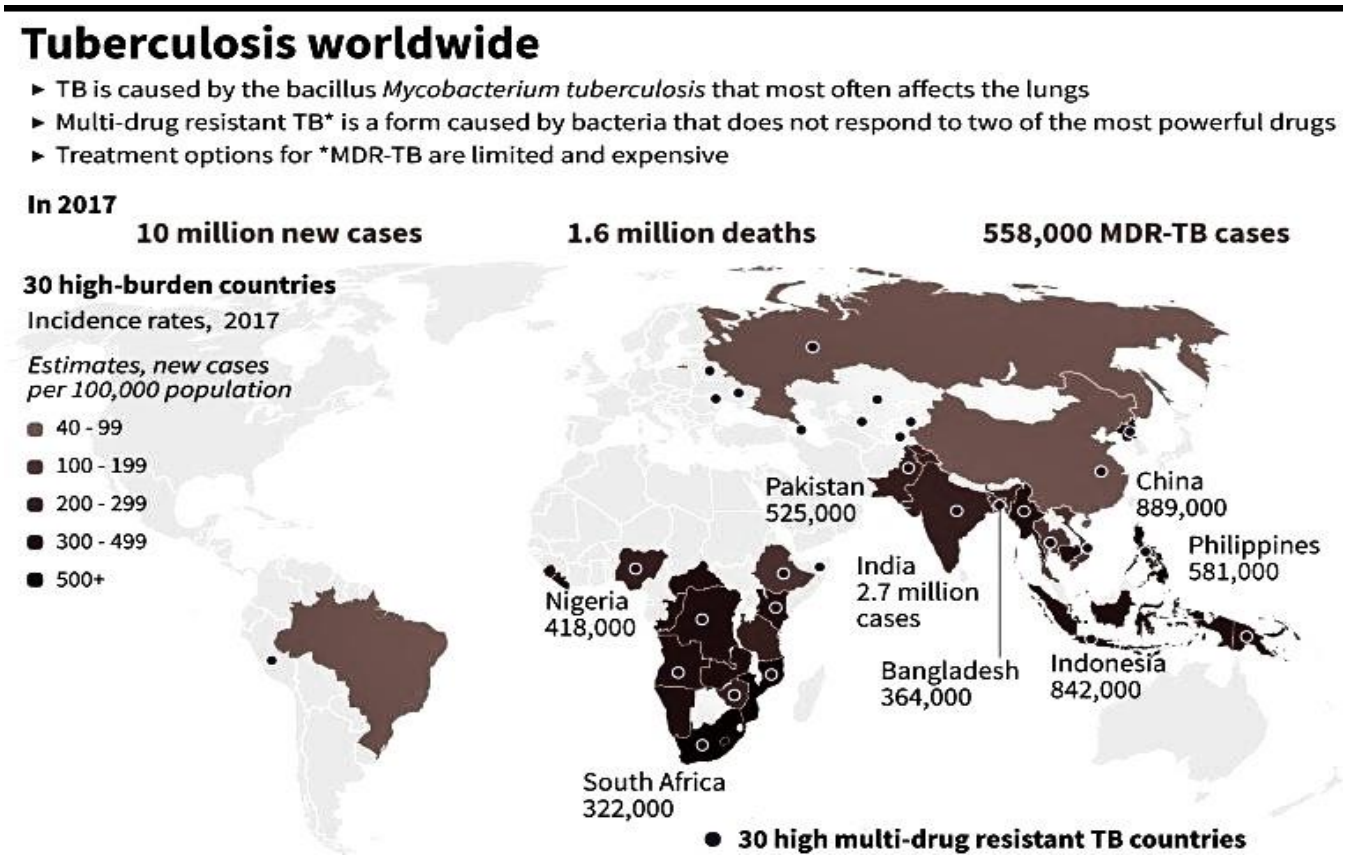


Figure 6: The figure shows the 2017 MDR-TB data obtained from WHO website. Also, 30 countries, which show high MDR-TB cases. India is one of the country where MDR-TB is Prominent. *[Reference: 'Short' drug-resistant TB regimen could cut treatment time by more than half", Medicalxpress.com, 2021. [Online]. Available: https://medicalxpress.com/news/2019-03-short-drug-resistant-tb-regimen-treatment.html. [Accessed: 17- May- 2021].*

MRSA strain is also widespread and causes hospital acquired infections. People with MRSA are estimated to be 64% more likely to die than people with a non-resistant form of the infection. Resistance against fluoroquinolones increased from 39% in 2008 to 85% in 2014 in India. Against Aminopenicillin, resistance increases from 78% in 2008 to 94% in 2014.

Artemisinin (Also called ACTs) which is a combination therapy is used to treat malaria caused by *P. falciparum*. In July 2016, resistance against these drugs in protozoan has been found in 5 countries the Greater Mekong subregion (Cambodia, the Lao People's Democratic Republic, Myanmar, Thailand and Viet Nam) [14].

Vancomycin resistant enterococci (VRE) which are harmless when present in gut of humans are usually harmless but can cause diseases like UTIs and blood infections. These are resistant to many antibiotics. Dalfopristine is the antibiotic, which was active against these bacteria, but resistance against this antibiotic has been found in VRE strain.

4. Origin and mechanism of Resistance

With increase in the use of antibiotics, the resistance among bacterial strains is increasing. The frequency of resistance in bacterial population is escalating specially in developing countries. The reason of this increase is the easy availability of antibiotics without prescription. In addition, the sanitation conditions in developing countries is poor which is helping spread of resistance. There is also a problem of budget for healthcare in developing countries. Due to this, these countries cannot afford to buy new effective drugs and forced to use old drugs against which resistance is already been developed in bacteria.

Generally, we can group the resistance mechanism into three groups, innate resistance, acquired resistance and adaptive resistance [Fig7]. In innate resistance, the genes responsible for resistance are present in the bacteria that produces antibiotic as secondary metabolite. In case of acquired resistance, the bacteria obtains the resistance genes from other bacteria and needs a certain concentration of antibiotic to sustain the resistance mechanism genetically. However, the adaptive resistance arises due to environment of bacteria, which causes changes in bacteria genetics to provide bacteria resistance mechanism like efflux pumps, target enzyme modifications etc.

The problem of resistance in bacteria can be depicted by two simple components; one component is the antibiotic itself, which is helping in selection of resistance microbe over susceptible one. Another is the resistance genes present in bacteria [15]. When these two components come together, they give rise to resistance problem in clinical situations. There can be several methods of origin of Multi drug resistance in a bacterium, which can be biochemical, genetic or anthropogenic.

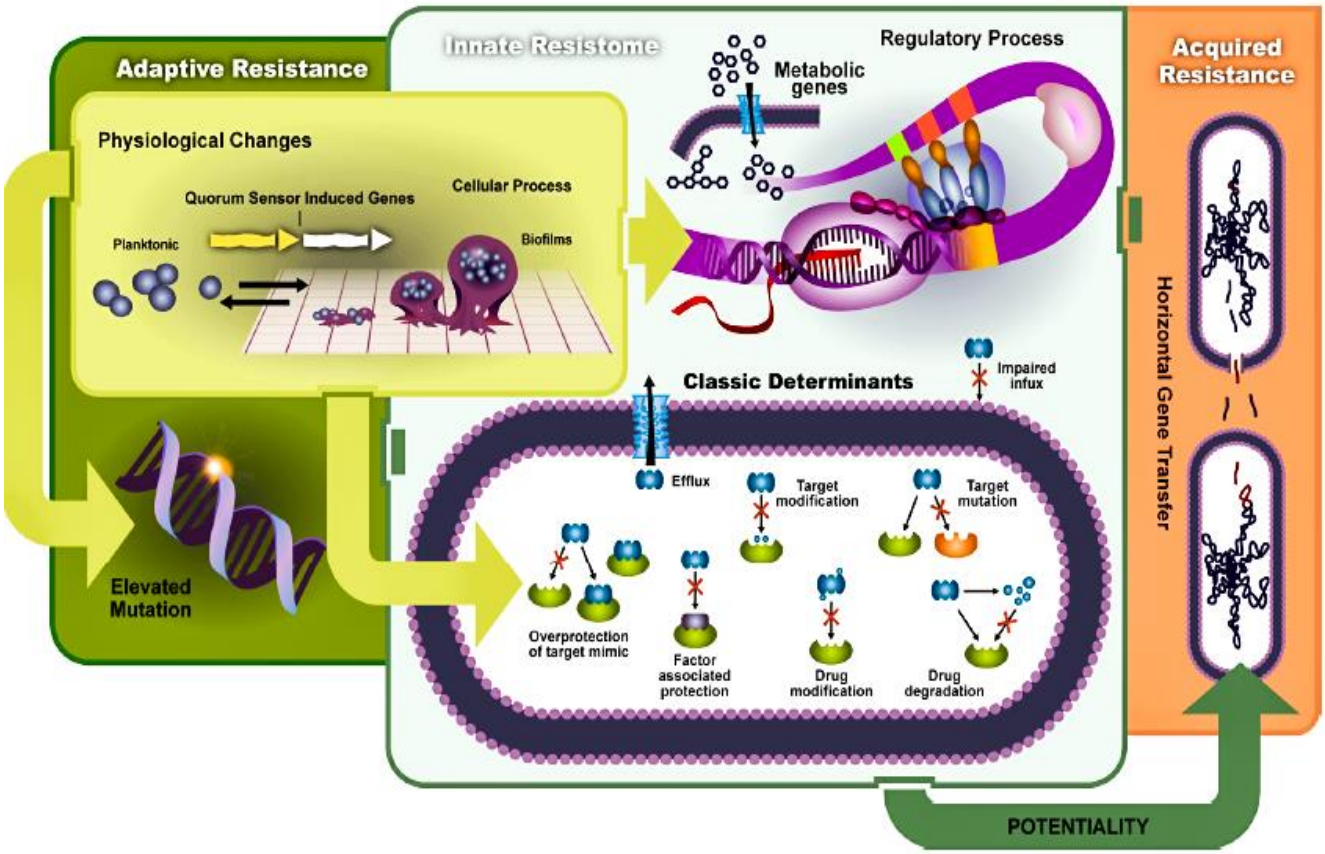


Figure 7: Adaptive, Innate and aquired type resistance in bacteria. The effect of environment factors can lead to mutation in gene, changes in metabollic processes and origin of resistance mechanism. *[Reference: M. Schroeder, B. Brooks and A. Brooks, "The Complex Relationship between Virulence and Antibiotic Resistance", Genes, vol. 8, no. 1, p. 39, 2017. Available: 10.3390/genes8010039.]*

4.1 BIOCHEMICAL MECHANISM OF RESISTANCE

Resistance is originated in bacteria depends on its action mechanism inside the bacterial cell. There are several mechanism of resistance developed by some bacteria which was originally emerges against one particular drug but can also prevent action of other drugs which in turn leads to MDR condition. One of the example of this kind of mechanism is efflux pumps [16]. This system removes the antibiotic from inside the cell and in turn reduce intracellular concentration of antibiotic [Fig8]. This kind of mechanism can act against more than one type of drug.

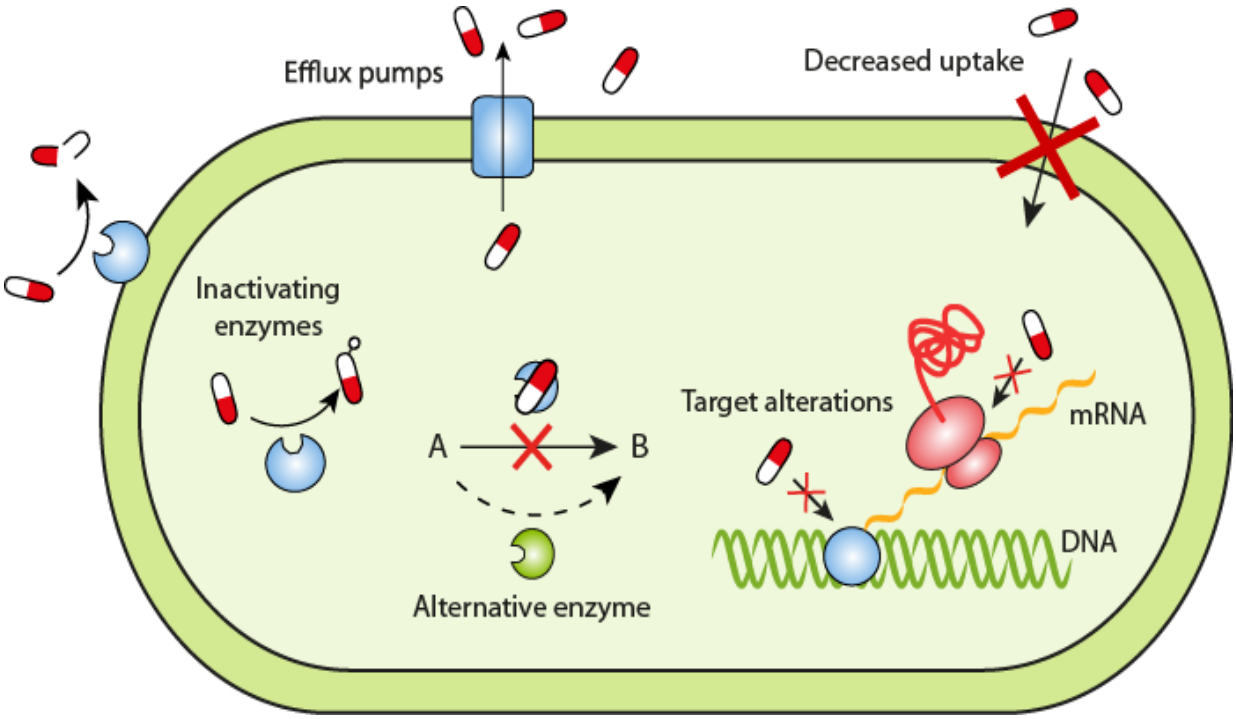


Figure 8: Various biochemical mechanisms of antibiotic resistance in bacteria. *[Reference: C. Pal, "Effects of biocides and metals on antibiotic resistance: a genomic and metagenomic perspective", Hdl.handle.net, 2021. [Online]. Available: http://hdl.handle.net/2077/48671. [Accessed: 17- May- 2021].*

4.1.1 Alter target protein by mutation

There are some drugs that are made chemically and are difficult to digest by enzymes but bacteria can mutate the target protein of such drugs and hence prevent binding of the drug with target protein. An example is fluoroquinolones drug, which act on topoisomerase enzyme that is essential for DNA replication. To generate resistance against this drug, Bacteria mutated its topoisomerase enzyme hence preventing drug action. This resistant mechanism is not prone to transfer from one strain to another because the gene-encoding enzyme is present in genomic DNA rather than Plasmid DNA hence preventing transfer via plasmid. However, selection pressure can select resistant bacteria over susceptible one.

4.1.2 Inactivate drug by using enzyme

Antibiotics, which are obtained from the nature, are most likely to be digested by enzymes. Some example of naturally derived antibiotics are aminoglycosides (tobramycin, amikacin and kanamycin). This antibiotic can be inactivated by mainly three methods: the phosphorylation by the enzyme aminoglycoside phosphoryl-transferases (APH), acetylation of drug by

aminoglycoside acyltransferases (AAC), or adenylation of drug by nucleotidyl transferases or adenyltransferases. There are many enzymes, which are found in bacteria that modifies aminoglycosides antibiotic. The modification in the drug via enzyme reduces net positive charge of polycationic antibiotic [18] [19]. In 1973, it was said that the source of the gene which encodes for enzymes which inactivate antibiotics are the organism which is used to produce antibiotics as these enzymes are already present in those organism as defence against antibiotics.

Another class of naturally derived drug is β-lactams, which includes penicillin, carbapenems and cephalosporins, and these drugs can be inactivated by the enzyme β-lactamases that hydrolyses the enzyme in periplasm. Genes, which encodes for these enzymes are present in plasmid so, the transfer of this resistant mechanism found is bacterial strains. *S. aureus* was the first organism that showed resistance against these antibiotics. The spreading of this resistance mechanism is widespread and this makes many antibiotics like ampicillin and methicillin useless against gram-negative bacteria [20].

4.1.3 Taking genes which codes for less susceptible protein from other species

There are proteins present in the bacteria known as penicillin binding protein (PBP) or DD-trans peptidase that are the target for penicillin antibiotic. *Streptococcus pneumonia* is an organism, which is resistant against penicillin as a mosaic PBP is present inside the bacterium in which penicillin is unable to bind. When sequencing of this protein is done, it was found that parts of this mosaic protein comes from other species of bacteria [21]. The pint to note here is that *S. pneumonia* have the ability to transform naturally. Another organism, which also naturally competent for transformation, is *Neisseria meningitides, which* is also resistant to penicillin by using same mechanism.

MRSA strain is also an example in this case. It contains a new PBT called PBP-2A, which provide MRSA resistance against methicillin antibiotic. Gene that encodes for this PBP is present in a large segment of DNA (30-60 kb) and this segment is not native to *S. aureus* species and came from other bacterial species [22].

4.1.4 Bypass the Target

To study this resistance mechanism, we can take example of the resistance mechanism in enterococci against vancomycin antibiotic. Vancomycin is obtained from *Streptomycetes* by fermentation and the action mechanism of this antibiotic is different from other drugs. This antibiotic binds with substrate lipid-linked disaccharide penta-peptide, which acts as precursor

for peptidoglycan present in cell wall. I susceptible bacteria, the end part of penta-peptide contains d-Ala-d-Ala, which acts as binding site for vancomycin. In resistant enterococcus, this structure is replaced by an ester structure d-Ala-d lactic acid, where vancomycin is unable to bind [23]. Enterococci contains resistance against β-lactams, aminoglycosides, macrolides, and tetracycline naturally and those strains, which are also resistant to vancomycin, are prevalent in hospital-acquired infections and are difficult to treat.

4.1.5 Preventing Drug to reach to Targets

In this method of resistance, some bacteria have developed various mechanism to prevent drug to reach to its target protein or enzyme. It can be done locally or by active efflux pumps. It can also be done by reducing influx of drug inside bacteria cell as used by gram-negative bacteria.

(i) Inhibition of drug access locally. Tetracycline binds with ribosome and prevent protein synthesis. Gram-positive bacteria produces protein like Tet (M) or Tet (S) which have high binding affinity with ribosomes and change the confirmation of the ribosome hence prevention binding of the drug. Qnr protein, which are encoded by plasmid, binds with topoisomerase enzyme and prevent binding of fluoroquinolones with the enzyme [24].

(ii) Efflux pumps specific to drug. Gram-negative bacteria contains a protein called Tet A which is responsible for the resistance against tetracycline antibiotic. This protein activates outward pumping of Tetracycline-Mg complex, which is dependent on proton-motive-force [25].

(iii) Inhibition of drug access (Non-specific). If we grow bacteria in laboratory condition with nutrient rich medium containing β-lactams, the colonies selected are mutants which contains less porins than normal [26]**.** Although due to less porins, these mutants also gets less nutrients because influx is less. These conditions are not commonly present in clinical samples. In some species of Enterobacteriacae like *Enterobacter aerogenes, Klebsiella pneumonia* this mechanism is present and acts in presence of those β-lactams antibiotics, which are not digestible by enzyme. It is also reported that the genes that encodes for porins have mutations, which encodes for different types of porins. These porins only allow influx of smaller molecules and prevent entry of heavy antibiotic molecules [27].

4.2 GENETIC METHOD OF RESISTANCE

Genetic method of resistance is the baseline of the MDR in bacterium. It includes several characteristics of bacterial genome like Mutation, recombination, genetic selection etc.

4.2.1 r (resistance) Gene

Bacteria contains r (resistance) gene, which is responsible for bacterial resistance towards antibiotics and transmission of resistance between Bacterium. The gene is present on an R-plasmid, which can contain more than one r-gene and act against various antibiotics and environment contaminants [Fig9]. Source of the r gene in the bacterium can be antibiotic producing organism or microorganisms present in environment like soil.

(i) Organisms which produce antibiotic

We obtain antibiotics from some bacteria species that produce these chemicals as secondary metabolite. For example, aminoglycosides are obtained from *Streptomycetes.* These kinds of organism contain several genes that encodes for proteins, which prevents the producing organisms from antibiotic effect. Similarly, β-lactams and vancomycin resistance is originated from the same way. Vancomycin resistant genes encodes for several enzymes, which participate in resistance mechanism. When vancomycin resistant enterococci were studied for the resistant genes, it was found that these genes were similar with the resistant genes present in *Streptomycetes* [28]. This shows that the resistance gene can be originate from antibiotic producing organisms.

(ii) Microorganisms present in the Environment, like Soil

Bacteria present in environment especially in soil can contain some genes, which takes part in resistance mechanism. An example is ampC gene that is present in genera *Enterobacteriaceae*, which contains *Enterobacter*, *Serratia*, and *Proteus.* A soil microorganism *Psudomonas aeruginosa* also contains this gene. *E. coli* also contains this gene but it lacks the mechanism of induction of this gene. *Salmonella* spp. Which are a pathogenic strain do not have this gene. It has been seen that microorganism which have abundant of antibiotic in their environment have resistant gene against one or several antibiotics [33]. There are several species of soil bacteria, which adapted in utilizing antibiotics as nutrient source and have enzymatic mechanism to degrade these drugs [29].

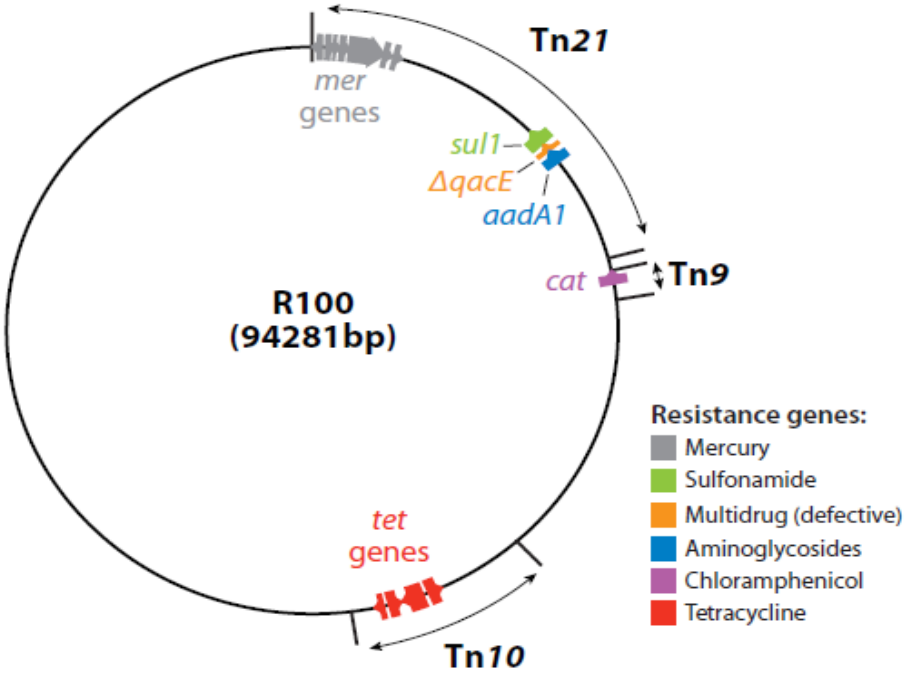


Figure 9: An early R plasmid (R100) map. It contains tertracyclin resistance and sulphonamide resistance gene. Tn represents a transposon. Cat is the chloramphenicol acyltransferase gene (cat). *[Reference: H. Nikaido, "Multidrug Resistance in Bacteria", Annual Review of Biochemistry, vol. 78, no. 1, pp. 119-146, 2009. Available: 10.1146/annurev.biochem.78.082907.145923.]*

4.2.2 Integrons

There are several gene acquisition elements called integrons. There was an association between these elements and r gene that are obtained from *Shigella* isolates. These isolates were characterized as transferable plasmid-mediated resistance containing in Japan in 1950s [30]. Integrons are not mobile by themselves but can take part in insertion and transfer functions. These elements act during pickup of r gene from environment and during their expression. They were found to be source of transferable r genes in γ *Proteobacteria* [31].

With the help of bioinformatics, we have been successful in identifying 3D structure of these elements and we now properly understood the mechanism of gene cassette acquisition [Fig10]. Many cassettes have been identified which are covering almost all the classes of antibiotics [32]. It has been thought that these elements are only present in gram-negative bacteria but these were also found in some gram-positive bacteria as well [33]. It has also been discovered

that there are many cassettes present in bacteria present in natural environment that do not code for any antibiotic.

4.2.3 Genetic linkage.

It has been seen that genes which are responsible for resistance against various antibiotics are ten to locate nearby in bacterial chromosome i.e. they are genetically linked. Because of this linkage during horizontal or vertical gene transfer, their sharing is happening together. In addition, they can be present in plasmids or as conjugated transposons, which makes it easier to share. In these cases, when any strain gains one resistance gene, it gets others as well.

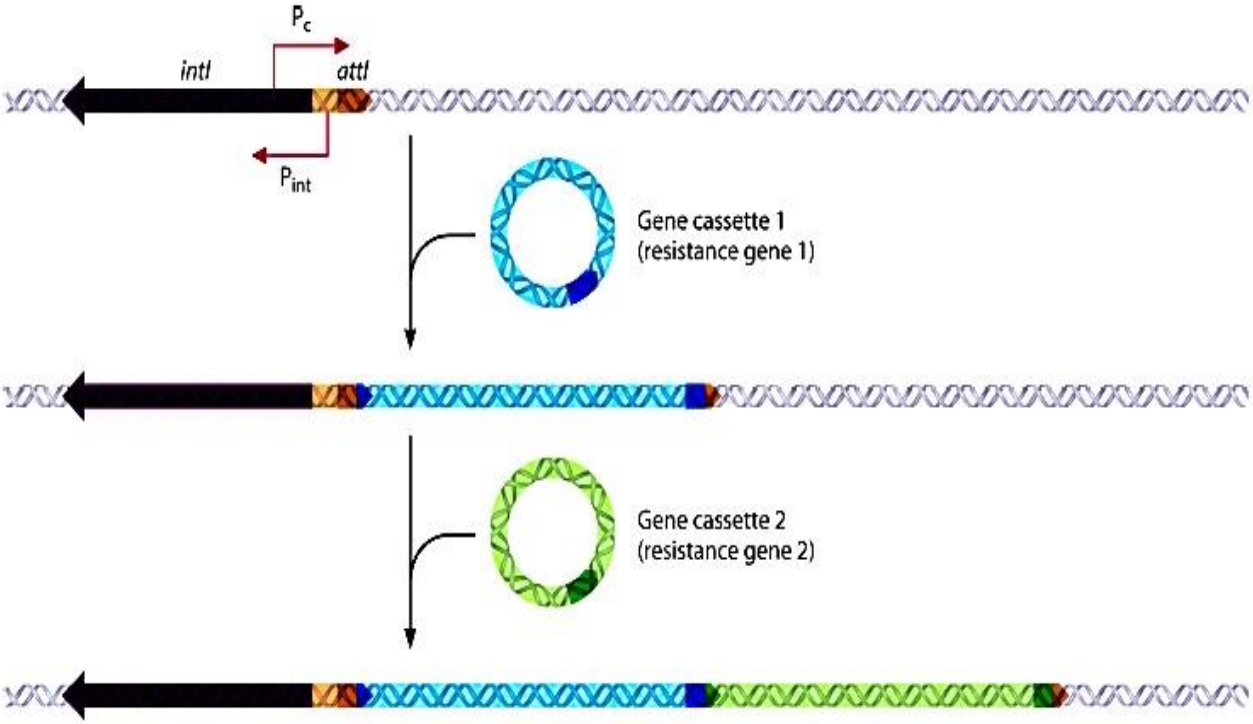


Figure 10: Structure of an integrin and how its mechanism of capturing a gene. Its structure contains promotors (Pint and Pc) at 3' end of gene along with an integrase (int) with insertion site (attI). Gene cassette attaches and integrated in insertion site to make an operon-like arrangement with Pc as strong promoter. *[Reference: J. Davies and D. Davies, "Origins and Evolution of Antibiotic Resistance", Microbiology and Molecular Biology Reviews, vol. 74, no. 3, pp. 417-433, 2010. Available: 10.1128/mmbr.00016-10.]*

Integrons, transposons and plasmids (Horizontally transmitted elements) can also incorporate new resistance element over time [34]. Transposons have gene encoding transposes that helps in incorporation of new element into genomic region. Through site-specific recombination

which is done by integrons themselves, they tend to gather various gene cassette with specific recombination site. Transposons as well as integrons might be present on plasmid or in bacterial chromosome. There are three primary method of sharing these elements within or between different species namely transformation, transduction and conjugation [18].

4.2.4 Bacterial lineages, which is highly mutable.

Different bacterial lineage can have different mutation rate. This is due to variation in genes, which are involved in proof reading mechanism of DNA replication. An example is mismatch pair system [35]. In addition, different bacterial lineage has difference in their ability to integrate and accept transforming DNA. Since, drug resistance genes are originating due to mutation and recombination, those bacterial lineages, which are more prone to mutation and recombination will have higher frequency of MDR determinant.

When vancomycin resistant *S. aureus* were first studied, the mutation which are present in chromosome only provide it intermediate resistance but after some time, the new strain was highly resistance and they have transposons which are present in *Enterococci* [36].

4.2.5 Genetic Jugglery

Most distributed resistance gene in world is gene, those codes for β-lactamase enzyme. Because of random mutation in gene, many modified enzymes have been found which provide extended spectra of resistance [37].

There are several antibiotics which acts on different sites on 50s ribosome subunit specifically in peptide exit tunnel. These antibiotics are macrolides and related drugs. Due to mutation in gene, modified rRNA is produced which causes resistance against all the antibiotics which have similar action mechanism [38].

In 1987, a new highly potent fluoroquinolones were introduced which acts on gyrase enzyme and act as its inhibitor. Experts predicted that it is highly unlikely to generate resistance against this antibiotic. However, it has been seen that resistance against this drug is originated by mutation in gene, that codes for gyrase enzyme. In addition, efflux pumps are also used against this drug by bacteria [39]. An enzyme aminoglycoside N-acetyltransferases modifies a secondary amine of this drug. This causes reduction in activity of drug.

4.3 ANTHROPOGENIC ACTIVITIES AIDING IN RESISTANCE EMERGENCE

Human plays predominant role in origin or resistance against various antibiotics in bacteria. Since discovery of antibiotics, their use is increased and because of their availability to public without prescription and their overuse, concentration of these drugs in environment is increasing. We do not have proper means to remove these drugs from environment. Their contact with microbes present in various environment is increasing which ultimately leading to generation of resistance against drugs in bacteria. Since large concentration of antibiotics helping these bacteria in selection resistant colonies, it is helping in proliferation of resistance. Use of antibiotics by humans is not only limited to medical purposes but these drugs are being used for various purposes like;

(i) Use as growth promoter in animal feed,

(ii) Use as therapeutics for humans, in aquaculture, household pets,

(iii) Use in agriculture for pest control,

(iv) Use in households cleaning products and toiletries as biocide,

(v) Use in research for cloning and selection.

If we see uses of antibiotics by human, less than half of the commercially produced antibiotics is used as therapeutic drugs for humans; rest of the antibiotics is being used for other operations. If we combine all the uses of antibiotics whether it is in agriculture, therapeutic or cleaning products, there are a large amount of antibiotic, which is being discarded in environment without any treatment. Many pharmaceuticals are dumping excess drugs into rivers for example a company in Hyderabad releases around 50Kg a day of ciprofloxacin into river [40]. These kinds of pollution in environment might be done in various parts of world, which are not reported. This kind of pollution not only helping in selection of resistance bacteria, but it also effects the native insects, animal, birds and human population [Fig11] [41].

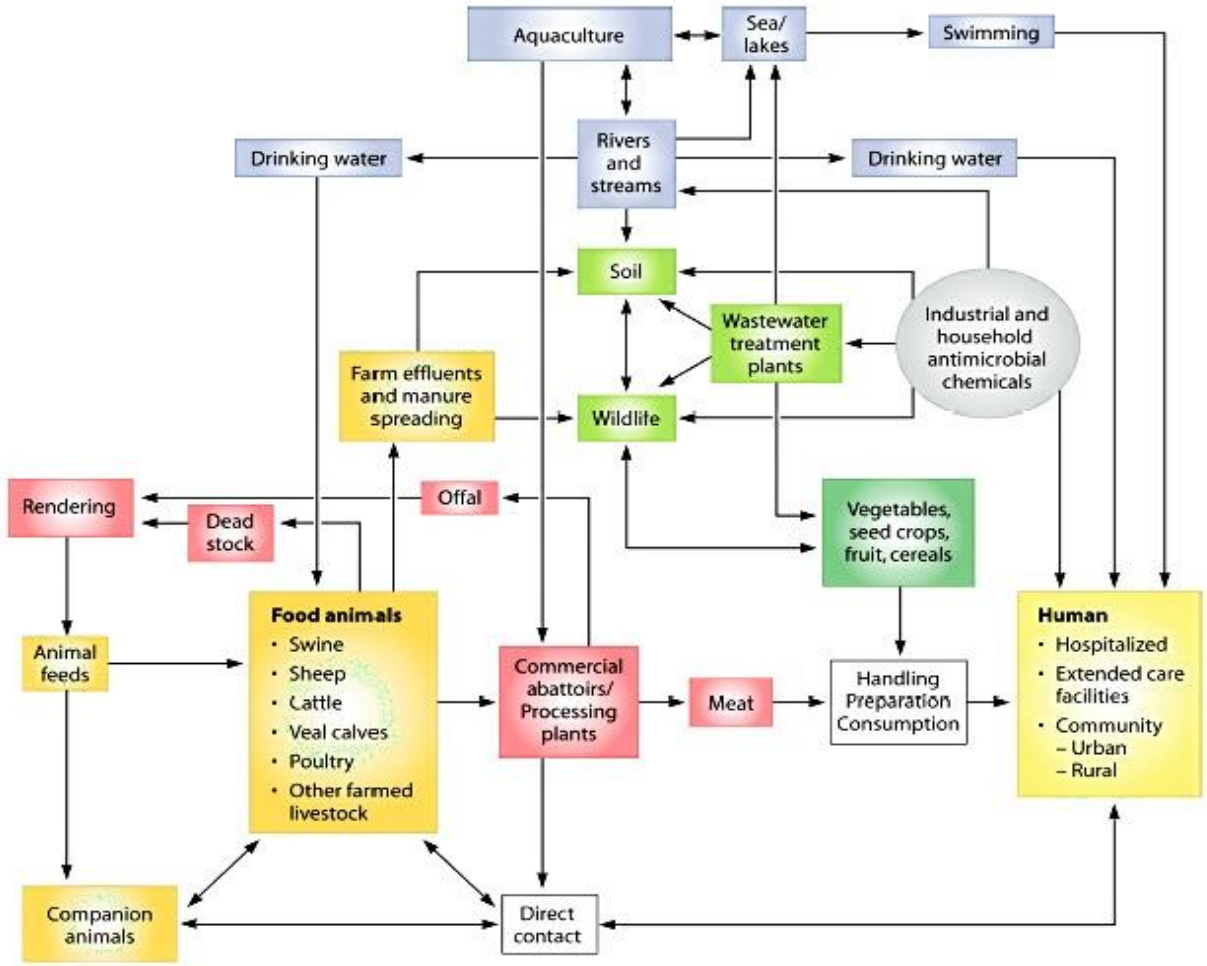


Figure 11: Dispersion of antibiotic resistance and antibiotics within community, agriculture, hospitals, wastewater treatment plants and other environments. *[Reference: J. Davies and D. Davies, "Origins and Evolution of Antibiotic Resistance", Microbiology and Molecular Biology Reviews, vol. 74, no. 3, pp. 417-433, 2010. Available: 10.1128/mmbr.00016-10.]*

When a genetic and genomic study is done to identify r genes in wastewater treatment plant, it has been found that these kinds of environment are rich in r genes [42]. These genes are present in plasmid that can be easily transfer from one bacteria to another.

5. Proliferation of MDR

Origin of antimicrobial resistance in a bacterium is a major problem in disease treatment but transfer of resistance from one bacterium to another is also a considerable issue. Transfer of resistance between bacteria happens on genetic level and mainly two components of bacterial genome takes part in the process; plasmid and transposons. Bacteria can uptake the resistance element directly from other bacteria (conjugation), uptake from environment (transformation)

or from bacteriophage (transduction) [Fig12]. There are various reasons for bacteria to uptake and retain resistance genes.

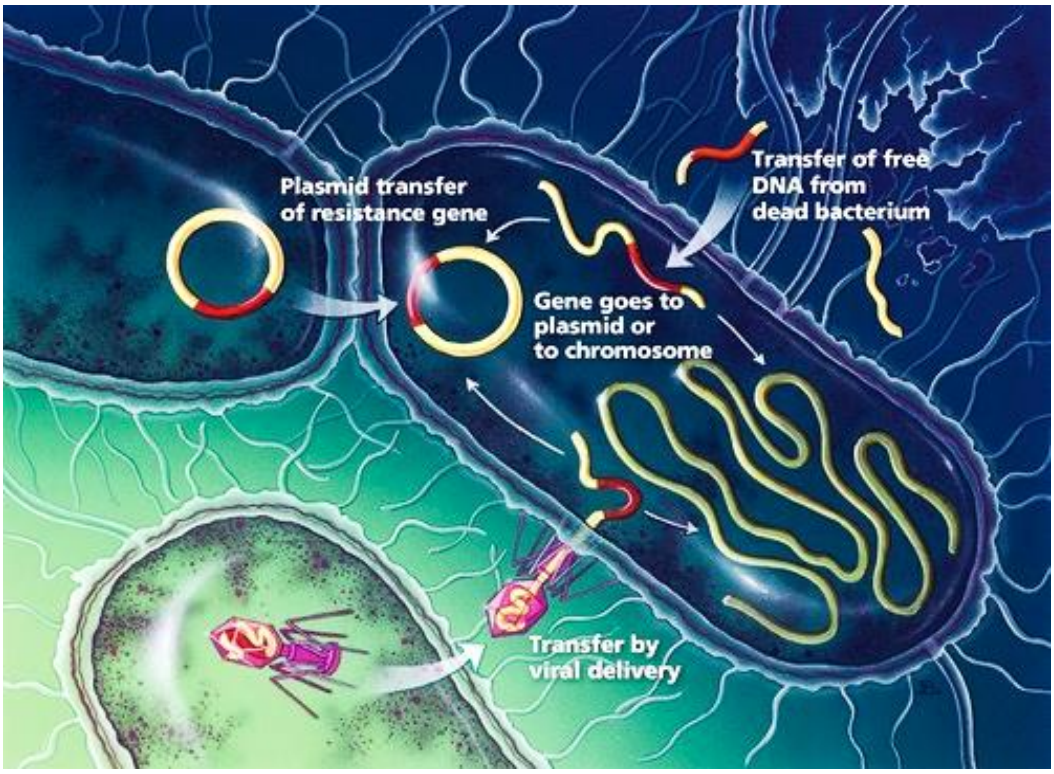


Figure 12: Uptake of resistance genes by bacteria directly from other bacteria (conjugation), bacteriophage as resistance gene carrier (transduction) and direct uptake of resistance gene from environment (transformation). *[Reference: C. Potera, "Germ Warfare? Strategies for Reducing the Spread of Antibiotic Resistance", Environmental Health Perspectives, vol. 121, no. 8, 2013. Available: 10.1289/ehp.121-a255.]*

5.1 Linkage of resistance genes.

Sometimes, resistance is proliferated not only for the gene, which is functional for resistance against a drug but for other genes that are responsible for resistance against a drug, which is not present in environment as well. This happens because the genes responsible for resistance are generally genetically linked i.e. are present close in bacterial genome. So, if resistance against a new drug originates in close proximity with resistance gene for older drug, in case of using new drug the resistance against older drug also proliferate with new gene. In addition, even if only one kind of drug is present in environment it will select those organisms, which are not only resistant with that particular drug but also resistant to other drug. It is highly likely that resistance gene against new drug will originate near older gene because the selection pressure due to old drug will help in continue spreading of resistance gene till we use new drug

[70]. An example is sulphonamide resistance in *E. coli* in United Kingdom. Even though sulphonamide prescription decreased in the country, the resistant *E. coli* number did not reduce [43]. One possible explanation for this is that suIII gene which is responsible for sulphonamide resistance is present in a plasmid that contains resistant genes for several other antibiotics [43]. Therefore, even if we use other antibiotic, the resistance against sulphonamide also proliferate with other resistance genes.

5.2 Selection of Bystander.

Bystander is an individual, which is present in the situation but do not take any part in it. Whenever we treat a disease, we have a selected target bacterium in mind but there are other microbes also present in the same environment, which might be affected by the drug. These bystander species of bacteria might develop multidrug resistance because of continues interaction with different kinds of drugs overtime. For example, microbes present in digestive tracts and upper respiratory tracts that comes in contact with antibiotic even though they are not the target species [44]. This condition is especially applicable during use of broad-spectrum antibiotic.

5.3 Interaction between determinants for drug resistant

Fitness is a criterion, which defines the ability to survive and produce offspring in a population. Sometimes, in absence of antibiotic, the resistance organism face difficulties to grow and survive [45]. Therefore, for a bacterium to maintain a resistance gene or multiple resistant genes, it is important to understand how these genes will interact with genetic background of the organism. If in case of MDR genes, the combined effect will give benefits to the organism, the strain that contains these genes will be selected over the strain, which only contain one resistant gene [46]. This is called epistasis where multiple alleles combine and give a fitness status to the organism, which is different from individual allele effect. If a drug resistance against drug A provide better fitness to strain than drug resistance against drug B, even if only drug B is present in the environment, the strain which have both drug A resistance and drug B resistance will be selected over the strain which have resistance only against drug B. An example for this condition is *P. aeruginosa* growing in streptomycin environment. It has been seen that cost of acquiring streptomycin resistance is more than acquiring rifampin resistance so even if only streptomycin is present in media, the strain which have both streptomycin and rifampin resistance is favoured over the strain which contain only streptomycin resistance.

5.4 Aggregation of resistance in a specific population

There are many drug classes, which are used against various microbes. These drug classes are used more for certain groups of population like for children, older people of for sexually active person where sexually transmitted infections are prominent [47]. In addition, certain environment has more use for these drugs like in hospitals, research labs. The abundance of MDR is more prominent in the places where the drug is mostly used. When we take combined population of a strain in various environment containing different concentration of drugs and resistant genes, the combine frequency of MDR containing organism becomes high. This is called Wahlund effect when we associate allele frequency of two fully or partially distinct population [48]. An example for this condition is high use of many antibiotic classes in hospital environment where these drugs are used mostly in intensive care unit but when we calculate frequency of resistance in hospitals, we associate whole population [49].

5.5 Accumulation of resistant determinant in bacteria

When a strain of bacteria comes in contact with a particular antibiotic for a very long time (more than 10 days), it has been found that the strain not only generate resistant to that a particular antibiotic but can accumulate resistance against other antibiotics as well. It has been seen for the intestinal and skin flora of individual in which tetracycline were being used for treatment of urinary tract infections [50]. The colonies of intestinal microflora after long term exposure to tetracycline were not only resistant to tetracycline but also for other antibiotics as well which were structurally not related to tetracycline. This condition is also being found in chickens where tetracycline was being used in feed. E. coli present in gut of chicken generate resistance against tetracycline in few days and by two weeks; they were resistant to many antibiotics [51].

5.6 Slow loss of resistant gene

Appearance of antibiotic resistant is fast in bacteria but their loss is slow even if antibiotic is not present in environment of growth. One of the reason for this is discussed above as the resistant genes can be linked with each other so in presence of some other antibiotic, the genes for other antibiotic resistance can get transferred [52]. Bacteria will attain the plasmid containing multiple resistant gene as long as it is providing survival advantage to bacteria. In addition, some resistant mechanism like efflux pumps provide some survival advantage during presence of compounds like ammonia. Therefore, the bacteria continue to use that gene for survival. This mechanism has been found in *S. aureus* [53].

6. Ecology of MDR

If only one person is taking antibiotic, for the duration of treatment, the drug will favour growth of resistant colony but after finishing treatment colonies, which are sensitive to, drug will once again compete with resistant colonies for survival. This was the resistant colony population will be dilute out. However, if a large population will use same antibiotic, drug susceptible colonies will get no opportunity to grow and resistant colonies will get an advantage for growth. This imbalance of growth will produce a resistant gene pool in environment [Fig13] [54].

The selection density of antibiotic favours the selection of resistance. Selection density of an antibiotic is the total amount of antibiotic, which is being applied for a defined number of people in a geographical area like a home, hospital or farm. In this scenario, each individual of this population acts as a factory for resistant bacteria and produces these bacteria into environment. The difference between different geographical areas provide information about the effect of antibiotic use in different conditions. This selective pressure will ultimately provide information about how many individuals are contributing in selection of resistant and how any sensitive colonies are surviving [5].

There are some societal drugs where, the individual using the drug will also affect other individuals, which are present in same environment [55]. For example, if one individual of a household is using antibiotic for acne treatment, the resistant colonies were found in that particular individual as well as other individuals of the household. Similarly, a study in Boston area shows that many individuals have resistant intestinal microflora [56].

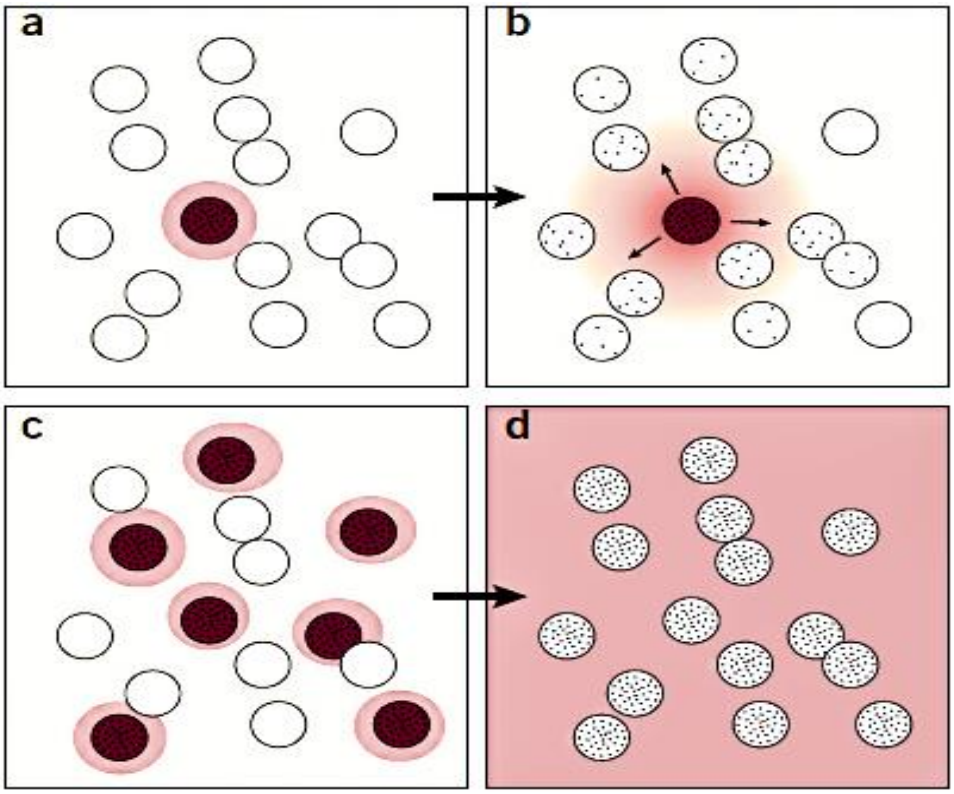


Figure 13: Dispersion of antibiotic after therapeutic use. (**a)**. while an individual is taking antibiotic, he/she will be the focal point of antibiotic effect and resistance bacteria, which is originated with use of antibiotic. (**b)**. the resistant bacteria spreads the resistance while he is in contact with low dose of antibiotic, which enters in environment through human waste or hospital disposal etc. (**c).** now the environment contains high concentration of resistant bacteria. (**d)**. selective pressure is continuing the resistant selection. *[Reference: S. Levy and B. Marshall, "Antibacterial resistance worldwide: causes, challenges and responses", Nature Medicine, vol. 10, no. 12, pp. S122-S129, 2004. Available: 10.1038/nm1145.]*

Also, environment have many contaminants which are either man made or produced by human activities for example, solvents, petroleum, industrial waste, garbage etc. Since industrialization, many kinds of organic waste are dumped in rivers, landfills, ocean and air. Heavy metals are also present in high amount I environment. Many compounds like arsenic, iodine, mercury that were used as medicines are also being added in environment. Many bacteria have a multivalent pumping system, which is like efflux pumps. These mechanisms sometimes helps bacteria in the environment-containing antibiotic as well. The systems helps bacteria survive in the area containing these pollutants [57]. Bacteria, which produce

antibiotics, also contains efflux systems to remove antibiotic from the cell [58]. There is a coexistence between production and antibiotic and resistant mechanism in environment.

It has been studied that antibiotics persist in environment mostly in intact form. In a study of wastewater, it has been found that it contains large frequency of products, which contribute in selection of resistant [59]. This gives an approach to prevent antibiotic resistant is to develop drugs which digest in environment easily hence preventing resistant selection.

Resistant mechanism can be pleotropic in nature i.e. it can affect more than one phenotypic character of the microbial community. Sometimes this property leads to better survival opportunities like formation of biofilm or maintaining efflux or influx pumps [60]. A phenotype which is responsible for resistance might not be originally develop in response to antibiotic for example a mutation in ribosome can also be done for temperature sensitivity, phage propagation etc. but this mutation can give resistant to bacteria.

6.1 Use of antibiotics in food animals and agriculture

In many countries like European Union, use of antibiotics as animal feed is ban but in countries like United State, this practice is still being used. Even if low level of antibiotic use is allowing for animal feed, its effect in generation of resistant is still prominent [45]. There are many enteric organisms like *E. coli*, *Salmonella*, *Campylobacter*, *Listeria* and *Enterococci*, which are present in animal gut and in other environment as well. These microbes can transfer from means like food and animal waste [61]. Overall, animal can contribute in the production of resistant strain even if it is not a significant contribution; it still effects the overall MDR production [62].

7. Strategies for the prevention of MDR

Many knowledge experts and international groups like WHO and CDC have been proposing different solutions for the problem of MDR. There are proposals for controlling use of antibiotics, prevent use of antibiotics without prescription, controlled and effective use during treatment, animal feed and agriculture. These proposals are given to governments and to public to prevent misuse of antibiotics and prevent spread of resistance among those bacteria that are still sensitive to drugs.

7.1 Tracking resistance frequency.

A surveillance system is proposed which monitor the resistance frequency and drug susceptibility in local, national and global level. The surveillance will help in making decisions

about which drug to be used for which region. It also helps in alerting officials about origin of new resistant pathogen and its possible treatment options. An Antibiotic Resistance Data project is formed which takes data from global surveillance systems and provide help to allied countries [5].

In India, Indian council of medical research (ICMR), New Delhi created the Antimicrobial Resistance Surveillance & Research Network (AMRSN) in 2013 [Fig14]. The main objective of this network is to collect national wide data related with resistant infections. To address the problem of Resistance, it is vital to understand the molecular mechanism of resistance, how bacteria is able to evolve the mechanism and transmit the resistance [63]. The main objective of ICMR-AMRSN is;

(i) to monitor clinically important microbes and their trends in antimicrobial susceptibility by creating a network of hospitals;

(ii) To understand mechanisms of resistance and identifying causable agents by using molecular techniques;

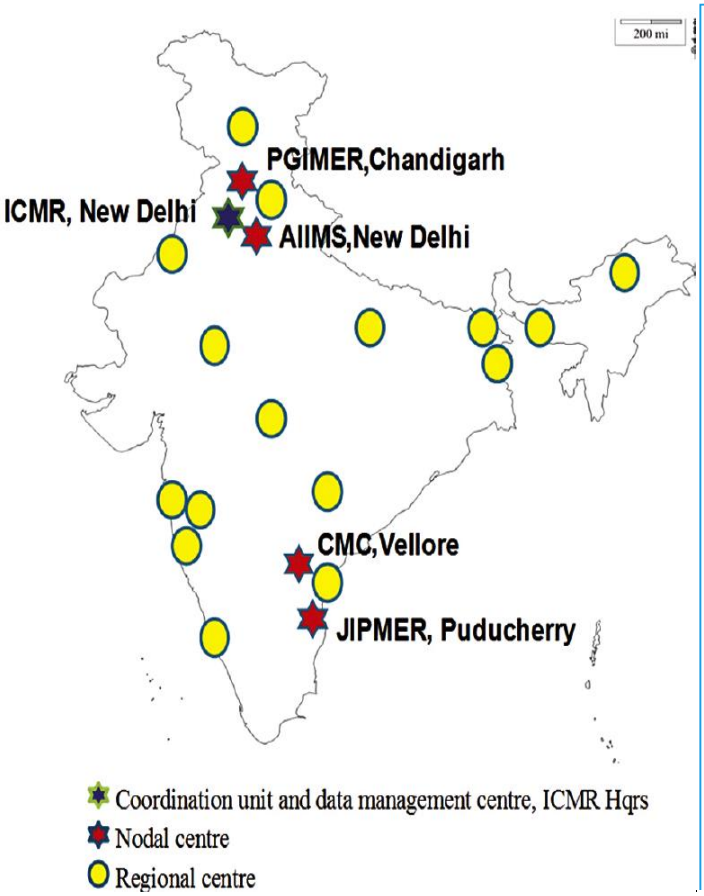
(iii) Circulate information about AMR pathogens to help in reduction of AMR; and

(iv) *To* create a system to manage, collect and analyse data.

The structure of AMRSN contains three levels; *(i)* The administrator, which is ICMR, New Delhi; (ii) The nodal centres and (iii) The regional centres. Each centre have its roles and responsibilities, the data is collected, and Principle office (ICMR) analyse the data and issues guidelines.

7.2 Identify and isolate patients with resistance pathogen

In Australia hospitals MRSA levels are lowest because they opted a process in which they identify and isolate patients with MRSA infection [64]. These patients are treated separately and the waste produced from these wards are treated to eliminate MRSA strain. In USA, patients infected with MRSA and Vancomycin Resistant *Enterococci* are isolated in separate microbiologically isolated rooms. Although this method does not prevent spread of resistant strain permanently, it has some positive outcome in preventing the MDR pathogen spread [65].



Box 1. Time line of expansion of AMRSN network
Phase 1: Establishment of six Nodal Centres in 2013-2014
AIIMS, New Delhi: Enteric pathogens
CMC, Vellore: GNNF, Diarrhoeal pathogens
JIPMER, Puducherry: Gram-positives
PGIMER, Chandigarh: *Enterobacteriaceae*, Fungal pathogens
Phase 2: Inclusion of six Regional Centres in 2017
AFMC, Pune
Apollo, Chennai
Hinduja Hospital, Mumbai
MGIMS, Sevagaram
Sir Ganga Ram Hospital, New Delhi
Tata Medical Center, Kolkata
Phase 3: Inclusion of 10 Regional Centres in 2018
AIIMS, Bhopal
AIIMS, Jodhpur
Assam Medical College, Dibrugarh
IPGMER, Kolkata
KGMU, Lucknow
Kasturba Medical College, Manipal
Nizam Hospital, Hyderabad
Regional Institute of Medical Sciences, Imphal
LTMGH, Sion, Mumbai
SKIMS, Srinagar

Figure 14: Nodal and regional centres for ICMR-AMRSN, India. *[Reference: K. Walia et al., "Establishing antimicrobial resistance surveillance & Research network in India: Journey so far", Indian Journal of Medical Research, vol. 149, no. 2, p. 164, 2019. Available: 10.4103/ijmr.ijmr_226_18.]*

7.3 Introduction of new therapeutic approaches.

There is no significant achievement in producing new drugs to treat MDR infections so; the only option remaining is to use the already existing drug more prudently. This practice can reduce resistance and can make drug effective eventually [66]. Appropriate utilisation of drug can help in treating already resistant pathogen and it will prevent origin of new resistant pathogens. Decreasing the use of drugs in places like hospitals is effective for repopulating sensitive drugs in these environments. This process is slow and sensitive colonies have to compete with MDR strain because in MDR colonies are resistant against more than one antibiotic and the genes can also provide several other kind of survival advantages to resistant strain [56]. 'High dose for shorter time' strategies should be used to reduce selection pressure. New antibiotics development is essential which either block the resistance mechanism or directly attack target pathogen. New drugs can also be produced against the genes that are

responsible for infection. This kind of inhibition will not reduce growth and hence sensitive colonies can grow in the presence of drug.

7.4 Use of combination therapy

Most used method of resistance among environmental microbe and their pathogen relatives is their ability to pump out antibiotics i.e. efflux pumps. We can use some compounds with antibiotic, which block efflux pumps and hence prevent excretion of antibiotic [67]. Combination of drugs is also being used to treat resistant pathogens in which each drug has different action mechanism [Fig15]. For example, fluoroquinolones and macrolide in which first drug inhibit DNA synthesis while the later one inhibits protein synthesis. Another example is combination of β-lactam with tetracycline in which first, one inhibit cell wall synthesis and the later one inhibits translation. Combination therapy is also used to treat diseases like cancer and HIV infections. However, it is important to study pharmacodynamics of drugs to make proper standardised combination [Table3].

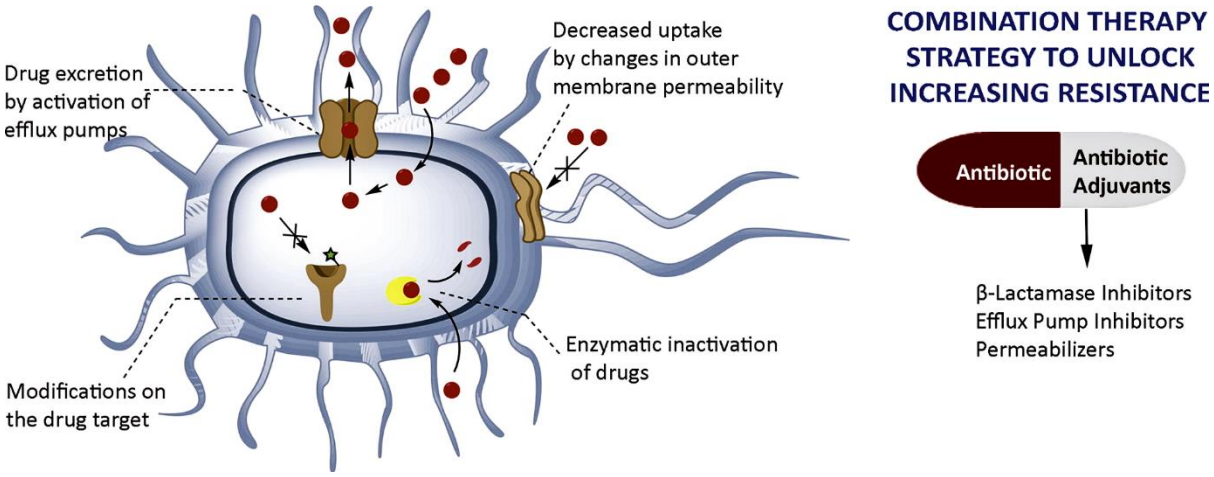


Figure 15: Mechanism of resistance used by bacteria and combination of drug with drug or drug with adjuvant to kill resistant bacteria. *[Reference: C. González-Bello, "Antibiotic adjuvants – A strategy to unlock bacterial resistance to antibiotics", Bioorganic & Medicinal Chemistry Letters, vol. 27, no. 18, pp. 4221-4228, 2017. Available: 10.1016/j.bmcl.2017.08.027.]*

Table 3: Combination of drugs approved by FDA to treat bacterial and malarial infections. *[Reference: Malik, M. A., Wani, M. Y., & Hashmi, A. A., "Combination therapy: Current status and future perspectives. In Combination Therapy Against Multidrug Resistance", 2020.]*

Trade name	***Combination***	***Treatment***
For bacterial Infection		
Ceftobiprole	Zevtera, Mabelio	For the treatment of communityacquired pneumonia and hospitalacquired pneumonia (HAP) in adults
Zerbaxa	Ceftolozane-tazobactam	For the treatment of complicated intraabdominal and urinary tract infections and bacterial pneumonia
Recarbrio	Imipenem, cilastatin, and relebactam	Treating adults with complicated urinary tract infections and complicated intraabdominal infections
Avycaz	Ceftazidime-avibactam	For the treatment of complicated intraabdominal infections in combination with metronidazole and urinary tract infections
Dalbavancin	Dalvance, Xydalba	For treatment options for methicillinresistant *S. aureus* (MRSA) infections
For malarial infection		
Fansidar	Sulfadoxine and Pyrimethamine	For the treatment of acute, uncomplicated *P. falciparum* malaria
Coartem	Artemether and Lumefantrine	For treating nonsevere malaria
Coarsucam or ASAQ	Artesunate/amodiaquine	Recommended by the WHO for

		uncomplicated falciparum malaria
Ariplus or Amalar plus	Artesunate and sulfadoxine/ pyrimethamine	Recommended by the WHO for uncomplicated falciparum malaria
Malarone	Atovaquone and Proguanil	Prevent malaria by interfering with the growth of parasites in the red blood cells of the human body
Artequin or ASMQ	Artesunate and Mefloquine	Recommended by the WHO for uncomplicated falciparum malaria
Pyramax	Pyronaridine and Artesunate	For the treatment of *P. falciparum* and *P. vivax*

Different strategies can be utilise to avoid, inhibit or bypass the resistance mechanisms used by pathogens. One of the successful example is combination of clavulanic acid with β-lactam antibiotic. Clavulanic acid inhibits β-lactamase enzyme and in turn inhibits the resistance mechanism against β-lactam drugs [85]. However, some bacteria have developed β-lactamases, which have different structure hence prevent attachment of clavulanic acid with enzyme [68]. Vaccine is also an effective alternative to antibiotics. Encapsulated *H. influenzae* type b and *Pneumococcus* can be used to kill bacteria and hence prevent use of antibiotics. The only problem with this system is that vaccine development and its delivery is quite difficult. In addition, in immunocompromised individual, microflora can act as pathogen and activity of vaccine against this microflora can destroy natural defence mechanism against recognised pathogens.

7.5 Drug Cycling

It has been discussed that drugs needs to be cycled to try to decrease selection pressure. The drugs, which are being utilised to treat a disease, need to replace with other drug after some time [69]. This method is not hundred percent effective and it does not remove resistance but it can help sensitive colonies against the drug to grow over time and the frequency of resistant strain will reduce.

7.6 Use of Non-drug therapy

There were propositions to use compounds that inhibit bacterial virulence and these compounds can prevent disease. This way there is no requirement for the use of antibiotics. This method has a significant advantage over antibiotics because we do not need to kill microbes, the survival of microbe will not be halted and resistance will not generate. There were attempts to use this kind of method and it has been successful in animal models [70]. Other approaches involve the use of innate immunity of host by stimulating it to remove disease-causing pathogen. Some studies show that gut microflora can also help in innate immunity in humans and scientists are studying for how we can utilise it to treat diseases.

7.7 Alternatives to conventional drugs.

As we have discussed, many pathogenic bacteria have developed resistance mechanisms against different kinds of antibiotics. Many alternatives have been proposed by experts to treat the disease, which are caused by MDR pathogens. Some of the alternatives are listed in this study, which can be act as possible alternatives for conventional drugs [Table4].

Table 4: Some strategies, which can be used as alternatives to conventional antibiotics, their advantages and disadvantages.

Strategy	***Advantages***	***Disadvantages***
Phage therapy	• Can replicate by itself • Selective treatment as phage can infect specific kind of bacteria • Genetic engineering can be utilised to modify phage particles.	• Can act as immunogenic • Bacterial endotoxins can be releases during lysis • Can produce some endotoxins and pyrogenic substances • Some bacteria have resistant mechanism against Phage.
Lysins	• Genetic engineering can be utilised to modify • Selective treatment for a specific kind of bacteria • Difficult to develop resistance against lysins	• Difficult to produce • There is lack to knowledge that can hinder during utilisation

CRISPR/Cas9	• Can be utilised for variety of applications • No need to use antibiotics • Can be design to act against specific bacteria	• Large scale production is expensive • Can be toxic to humans
Antimicrobial peptides	• Difficult to develop resistant • Can show broad spectrum activity	• Large scale production is expensive • Peptide can be digested by enzymes and structure can be disrupted by various reasons • Can be toxic to humans
Bacteriocins	• Selective treatment for a specific kind of bacteria • Show resistance towards heat and UV	• Large scale production is expensive • Peptide can be digested by enzymes and structure can be disrupted by various reasons
SMAMPs (Synthetic mimics of antimicrobial peptides)	• Synthesis is easy • Difficult to develop resistant • Can show broad spectrum activity	• Can be toxic to humans • Difficult to administer inside bacterial cell
IDR peptides (innate defence regulatory peptides)	• Utilise host immune system • Since it does not have antimicrobial property, there is no chance of resistance development	• Large scale production is expensive • Peptide can be digested by enzymes and structure can be disrupted by various reasons
Probiotics	• Easily available	• Mostly applicable in intestinal infection only
Antibodies	• Selective treatment for a specific kind of bacteria • No action against indigenous microflora	• Cost of production is high and takes long time. • Very low shelf life of antibodies

7.7.1 Naturally Occurring Alternatives

(i) Phage Therapy

This method uses bacteriophage, a virus that infects and kills bacteria. This method was used before the introduction of antibiotics to treat several diseases. Life cycle of bacteriophage includes three primary steps; first is their attachment with bacterial cell, and then they inject their genomic material inside the bacterial cell. Phage, then takes over bacterial machinery and starts replicating its genomic material and translate its protein part. New phage viruses released from infected cell that will spread in environment and infect other bacteria [Fig 16].

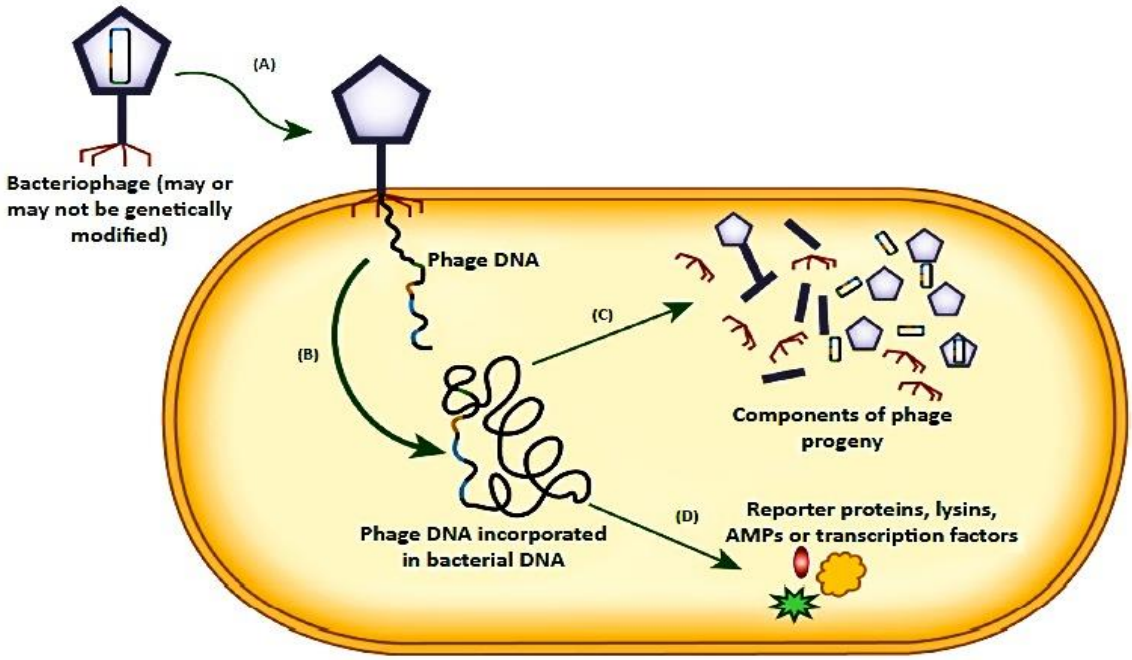


Figure 16: working of Bacteriophage therapy. (A) Selective bacteria targated by GM bacteria. (B)Integration of bacteriophage DNA with host genome and replication of phage DNA using host macinary. (C) Assembly of phage particles in host cell. (D) Lysins, antimicrobial peptides and toxins will damage host cell and release of phage particles. *[Reference: C. Ghosh, P. Sarkar, R. Issa, and J. Haldar, "Alternatives to conventional antibiotics in the era of antimicrobial resistance," Trends Microbiol., vol. 27, no. 4, pp. 323–338, 2019]*

During this infection cycle, lysis of bacterial cell takes place during release of phage progenies. There is an advantage of using phage is that they can select their host in a mixed colony of bacteria. Therefore, there can be effective use for this selective property of bacteriophage to kill specific kinds of pathogen inside body [71]. Many bacterial pathogens like *Shigella, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella* infection can be treated by

using bacteriophages [Table5]. This treatment is also studied for the resistant cystic fibrosis infections [72].

Table 5: Bacteriophages having approval or are undergoing clinical trials for human use. *[Reference: C. Ghosh, P. Sarkar, R. Issa, and J. Haldar, "Alternatives to conventional antibiotics in the era of antimicrobial resistance," Trends Microbiol., vol. 27, no. 4, pp. 323–338, 2019]*

Product	***Company***	***Condition***	***Phase (status)***
EcoShield	Intralytix	Food industry (*Salmonella enterica*)	Approved
ABPA01	AmpliPhi	Chronic rhinosinusitis and cystic fibrosis (*P. aeruginosa*)	Preclinical
Phagesti	Biochimpharm	Treatment and prophylaxis of bacterial purulent–inflammatory infections (multiple microorganisms)	Approved
ListShield	Intralytix	Food industry (Listeria monocytogenes) Food industry (*Escherichia coli*)	Approved
PHOSA	Multiple centres	Treatment and prophylaxis of gastrointestinal infections (multiple organisms)	Approved
Phagyo	Biochimpharm	Treatment and prophylaxis of bacterial purulent–inflammatory infections (multiple microorganism)	Approved
Phagepy	Biochimpharm	Treatment and prophylaxis of bacterial purulent–inflammatory infections (*P. aeruginosa*)	Approved
Intesti-Phage	Micro-gen	Bacterial dysentery; salmonellosis; dyspepsia; dysbacteriosis; enterocolitis, colitis	Approved

SalmoShield	Intralytix	Burn infections (*Pseudomonas aeruginosa* and *E. coli*)	Approved
Pyo-Phage	Eliava Institute	Urogenital infections; pyo-inflammatory gynecologic diseases; enteric infections; dysbacteriosis, surgical infections	Approved
ENKO-Phage	Eliava	Pyo-inflammatory and enteric infections caused by *Shigella*, *Salmonella*, different types of *E. coli*, species of pathogenic *Staphylococci*	Approved
Phagetyph and Phagesal	Biochimpharm	Treatment and prophylaxis of enteric fever and salmonellosis (*Salmonella*)	Approved
Fersisi-Phage	Eliava	Pyo-inflammatory and enteric infections caused by *Staphylococci* and *Streptococci*	Approved
Phagedys	Biochimpharm	Treatment and prophylaxis of dysentery (*Shigella*)	Approved
ABSA01	AmpliPhi	Chronic rhinosinusitis, bacteremia, endocarditis, prosthetic joint infections, osteomyelitis, diabetic foot-ulcers (*S. aureus*)	Phase I
SES-Phage	Eliava	Pyo-inflammatory and enteric infections caused by *Staphylococci*, *Streptococci*, and entero-pathogenic *E. coli*	Approved
PhagoBurn	Multiple centres	*E. coli* or *P. aeruginosa* burn wound infections	Phase I/II

Although, phage therapy has several advantages it also has some limitations as antimicrobials. There have been report about resistance in bacteria against phage. So, it is crucial to study if bacteria are susceptible to phage or not. Also, bacteriophage can be immunogenic inside humans. Our body will recognise it as immunogenic component and it will remove out from

the body. This problem can be resolved by using advance genetic engineering methods [73]. Phage can have endotoxins and substances that can act as toxins in body and these needs to be removed before using phage as effective alternative to antibiotics.

(ii) Antimicrobial Peptides or host-defence peptides

Antimicrobial peptides (AMPs) also called host-defence peptides (HDPs) are the proteins produced by the host body against pathogens. These peptides can act as antifungal, antimicrobial, antiviral, antiprotozoal, anticancer etc. [74]. Most of these peptides have positive charge but some negative charge peptides have also been found. These peptides are amphiphilic in nature and their cationic part involve with negative charge present in bacterial cell surface. Hydrophobic part of the peptide attach with lipids present in bacterial cell membrane. By these interactions, bacterial membrane disintegrates hence killing bacteria [Fig17] [75]. Other proteins present in mammals are zwitterionic in nature hence they do not interact with AMPs. This makes AMPs selectively toxic against bacteria.

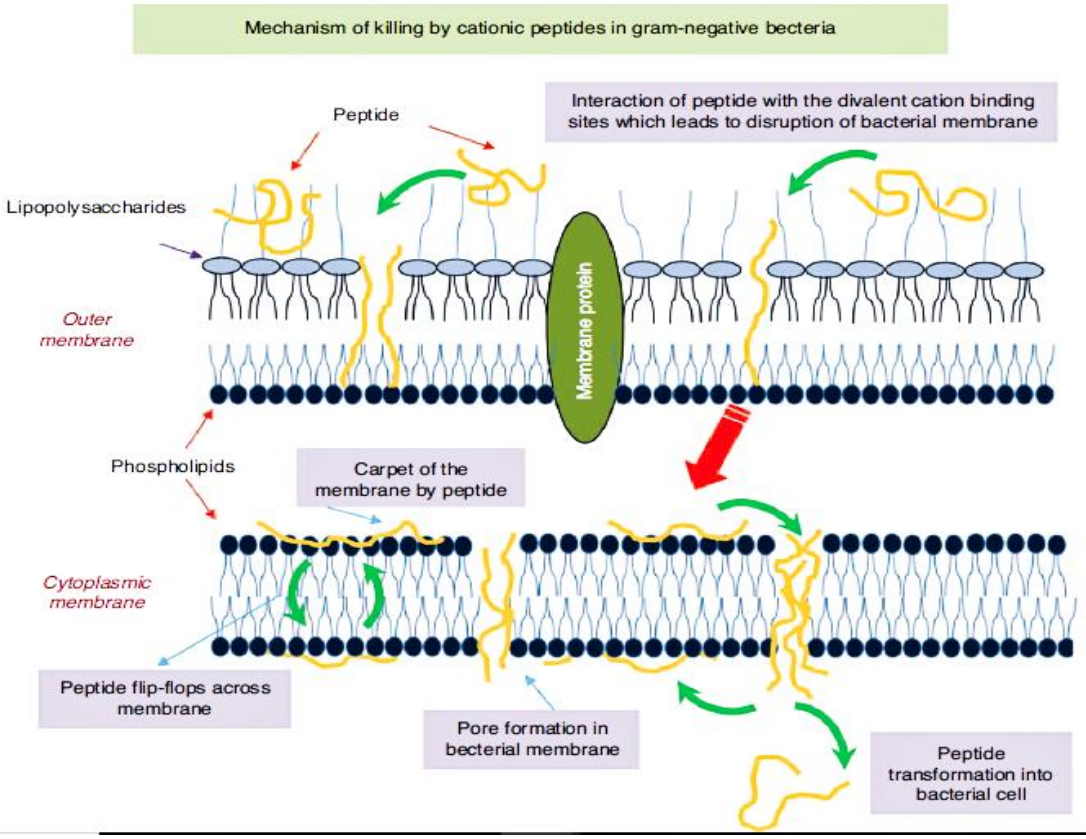


Figure 17: Working mechanism of antimicrobial peptide while interacting with gram-negative bacteria. For gram-positive bacteria, interaction of antimicrobial peptide with membrane occurs through techoic acid. *[Reference: M. M. Moghaddam, H. Aghamollaei, H. Kooshki, K. A. Barjini, R. Mirnejad, and A. Choopani, "The development of antimicrobial peptides as an*

approach to prevention of antibiotic resistance," Rev. Med. Microbiol., vol. 26, no. 3, pp. 98–110, 2015.]

Several antimicrobial peptides like Polymyxin B and colistine (Polymyxin E) a lipopeptide isolated from *Bacillus polymyxa,* gramicidin, a linear polypeptide obtained from Bacillus bravis, are available for clinical use as antibiotics. However, AMPs are not completely successful during clinical use. Enfuvirtide, is a biomimetic peptide which is approved by FDA to use as a combination therapy (Fuzeon) to treat HIV infections. Clinical trials are going on for several antimicrobial peptides to overcome challenges that hinder the efficient use of AMPs [Table6].

Table 6: Antimicrobial peptides (AMPs), Synthetic mimics of antimicrobial peptides (SMAMPs) and Lysins of AMPs in clinical trials. *[Reference: C. Ghosh, P. Sarkar, R. Issa, and J. Haldar, "Alternatives to conventional antibiotics in the era of antimicrobial resistance," Trends Microbiol., vol. 27, no. 4, pp. 323–338, 2019]*

Product	***Company***	***Indication***	***Status***
AB103	Atox Bio	Necrotizing soft-tissue infections	Phase III
Locilex (Pexiganan)	Dipexium Pharma	Diabetic foot ulcers	Failed Phase III
LL-37	M. D. Anderson Cancer Centre	Melanoma	Phase II
CLS001 (Omiganan)	Cutanea Life Sciences	Rosacea, acne, atopic dermatitis, and genital human papillomavirus	Phase III–Phase II
LL-37	Promore Pharma	Chronic leg ulcers	Phase II
SGX 942	Soligenix	Oral Mucositis	Phase II (Fast track)
Avidocin and purocin	Pylum Biosciences	Narrow-spectrum antibiotic	Preclinical
NP213	Novabiotics	Onychomycosis	Phase II
AP 138	Adenium Biotech	MRSA implant infections	Phase I

P-113	Pacgen Lifesciences	Oral candidiasis	Phase II
AMC-109	Amicoat AS	Impetigo Nasal decolonization	Phase II
Plectasin	Adenium Biotech	Gram-positive infections	Preclinical
HB 1345	Helix Biomedix	Acne	Preclinical
NP432	Novabiotics	Multibacterial infections	Preclincial
HB 1275	Helix Biomedix	Trichophyton infections	Preclinical
OG-716	Oragenics	Clostridium difficile infections	Preclinical
CSA-13	N8 Medical	Bacterial infections/fracture	Preclinical

(iii) Bacteriocins

Bacteriocins are the toxins (small peptides) produced by one bacteria against others in the population to enhance its survival. These kind of peptides generally produced by drug sensitive bacteria against resistant bacteria to enhance the survival chances of sensitive bacteria [76]. Bacteriocins can be broadly classified into two classes; Class I, which undergoes a post-translational modification and Class II, which do not undergo with post-translational modification [77]. Bacteriocins have same mechanism of action as mammalian AMPs i.e. they attach with cell membrane, disintegrate it hence killing bacteria. Apart from this, they can have several other action mechanisms like inhibiting synthesis of peptidoglycan [Fig18]. Nisin is a bacteriocins which is commercially used in food industry to inhibit peptidoglycan biosynthesis in bacteria.

Bacteriocins have several advantages such as; they can selectively target a bacterial species like bacteriophage. For example, thuricin is a bacteriocin, which target *Clostridiodes difficile* (*Clostridium difficile*) and do not act on commensal bacteria. In addition, like AMPs, resistance generation against Bacteriocins is difficult. The biggest advantage in using bacteriocins is their resistance to harsh conditions like heat and UV.

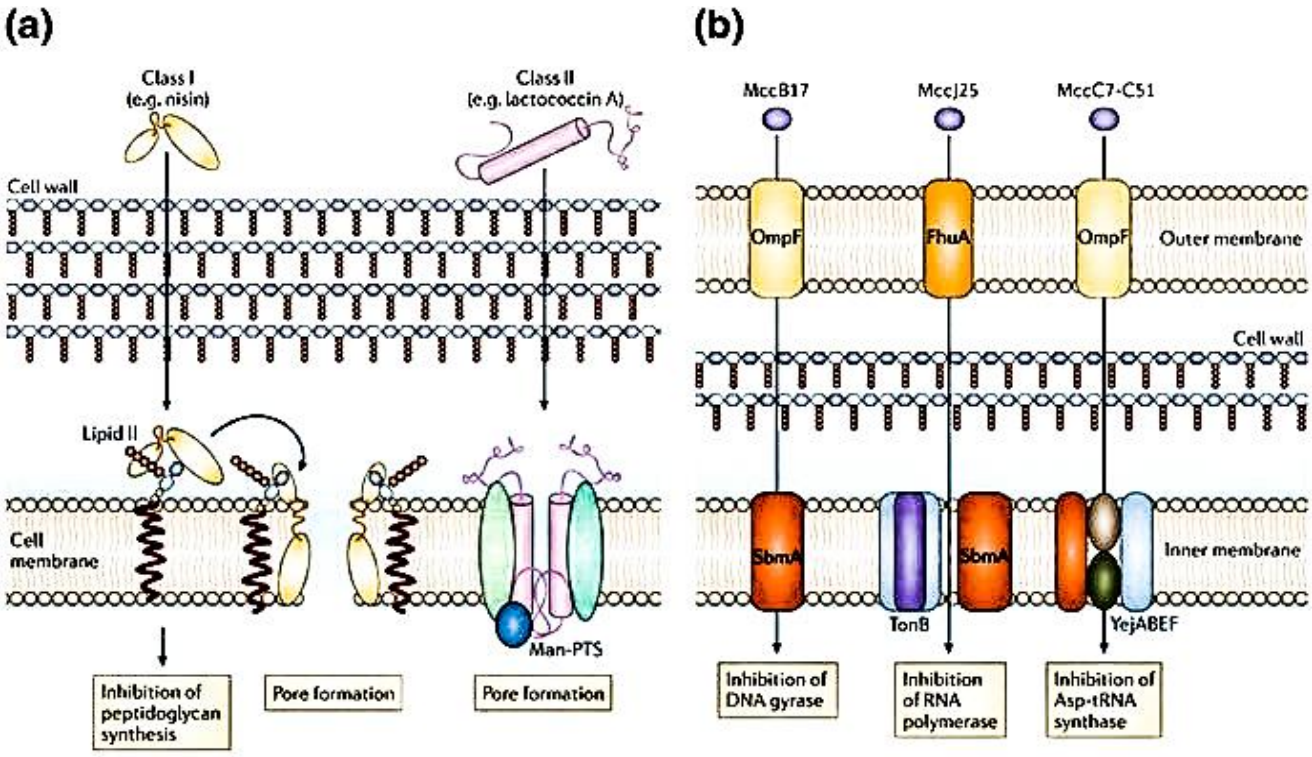


Figure 18: Action mechanism of Bacteriocins (a) on gram-positive bacteria, and (b) on gram-negative bacteria. *[Reference: S. W. Zuhainis, A. A. Hassan, M. Singh, and R. Mohamad, "Microbial surfactant for preservation of natural rubber latex," in Beneficial Microorganisms in Agriculture, Aquaculture and Other Areas, Cham: Springer International Publishing, 2015, pp. 101–128.]*

(iv) Probiotics

Human gut contains thousands of microbial species, which includes bacteria, yeast, viruses etc. Lactobacilli, Bifidobactera, Streptococci and non-pathogenic strains of Clostridum sp., Fusobacterium sp., Eubacterium sp., E. coli, Veillonella sp., are some of the most dominant bacteria found in the gut [78]. Recent laboratory studies have found that the gut microbiota plays an important role in metabolism and immune function. Use of antibiotic disturbs the population density of microbiota residing in gut, which can lead to growth of drug resistant colonies. In addition, reduced number of gut microbiota can increase the susceptibility of gut for pathogenic bacteria.

So, prebiotics and probiotics are being promoted to use in the gastrointestinal infections to reduce antimicrobial colonies accumulation in gut. Probiotics are also being used to prevent infections in gut and to help maintain healthy concentration of beneficial microbes throughout

the gut. Bacteria like *Saccharomyces boulardii* and non-pathogenic strains of *E. coli* has been studied to prevent *C. difficile* infections in-vivo [79].

The principle behind the use of probiotics is that, by using probiotics, we can balance the growth of gut microbiota and the growth of commensal bacteria can outgrow the pathogenic bacteria [80]. The commensal bacteria will Provide resistance to the humans by two methods; either by directly interacting with pathogenic bacteria or by helping immune system to eliminate pathogenic bacteria [Fig19].

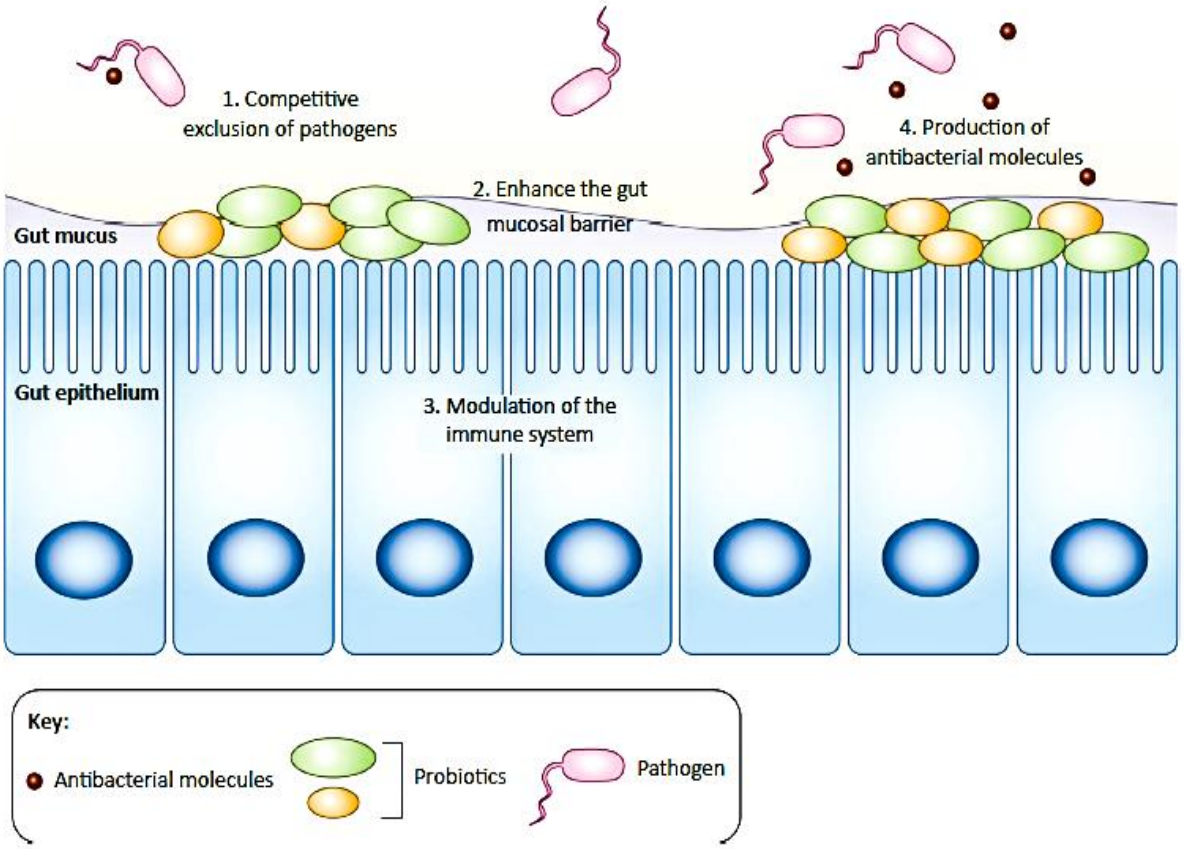


Figure 19: Mechanism of protection by probiotics against bacterial infections. *[Reference: C. Ghosh, P. Sarkar, R. Issa, and J. Haldar, "Alternatives to conventional antibiotics in the era of antimicrobial resistance," Trends Microbiol., vol. 27, no. 4, pp. 323–338, 2019.]*

The advantages of using probiotics and prebiotics is that they can be utilised for long term and they have no side effects. Many probiotics are approved for clinical trials for various infections [Table7].

Table 7: Probiotics that are approved and are going clinical trials for human use. *[Reference: C. Ghosh, P. Sarkar, R. Issa, and J. Haldar, "Alternatives to conventional antibiotics in the era of antimicrobial resistance," Trends Microbiol., vol. 27, no. 4, pp. 323–338, 2019.]*

Product	***Company***	***Condition***	***Phase (status)***
RBX 7455	Rebiotex	Recurrent C. difficile infection	Phase I
RBX2660	Rebiotix	Recurrent C. difficile infection	Phase III
SER-109	Seres	Recurrent Clostridium difficile infection	Phase III
SER 262	Seres	Recurrent C. difficile infection	Phase Ib
FIN 403	Finch therapeutics	Recurrent C. difficile infection	Phase II
VP20621	Shire (Viropharma)	C. difficile	Phase II

(v) Predatory bacteria

A promising alternative to conventional antibiotics is to use Bdellovibrio and like organisms (BALOs) also known as predatory bacteria. These organisms can multiply only when they enter in other gram-negative bacteria (d-proteobacteria) like *E. coli*, *Legionella*, *Pseudomonas*, *Salmonella* etc [Fig20] [81]. They use hydrolytic enzymes like DNase and protease to degrade host bacteria. These hydrolytic enzymes can enter in biofilms where conventional antibiotic cannot hence giving it a significant advantage. They form a localized pore in periplasm of prey bacteria to enter and make a hybrid called a bdelloplast. Bdellovibrio's lipopolysaccharide (LPS) lacks the negatively charged phosphate group hence it cannot bind to the LPS receptors present in human immune cells. This causes minimal inflammatory response while present in human body [82]. In addition, these bacteria cannot multiply in mammalian cells hence they cannot infect humans.

In addition, they can act against wide range of gram-negative bacteria and have low immunogenicity and low toxicity, which makes them a potential candidate as alternative to conventional antibiotics.

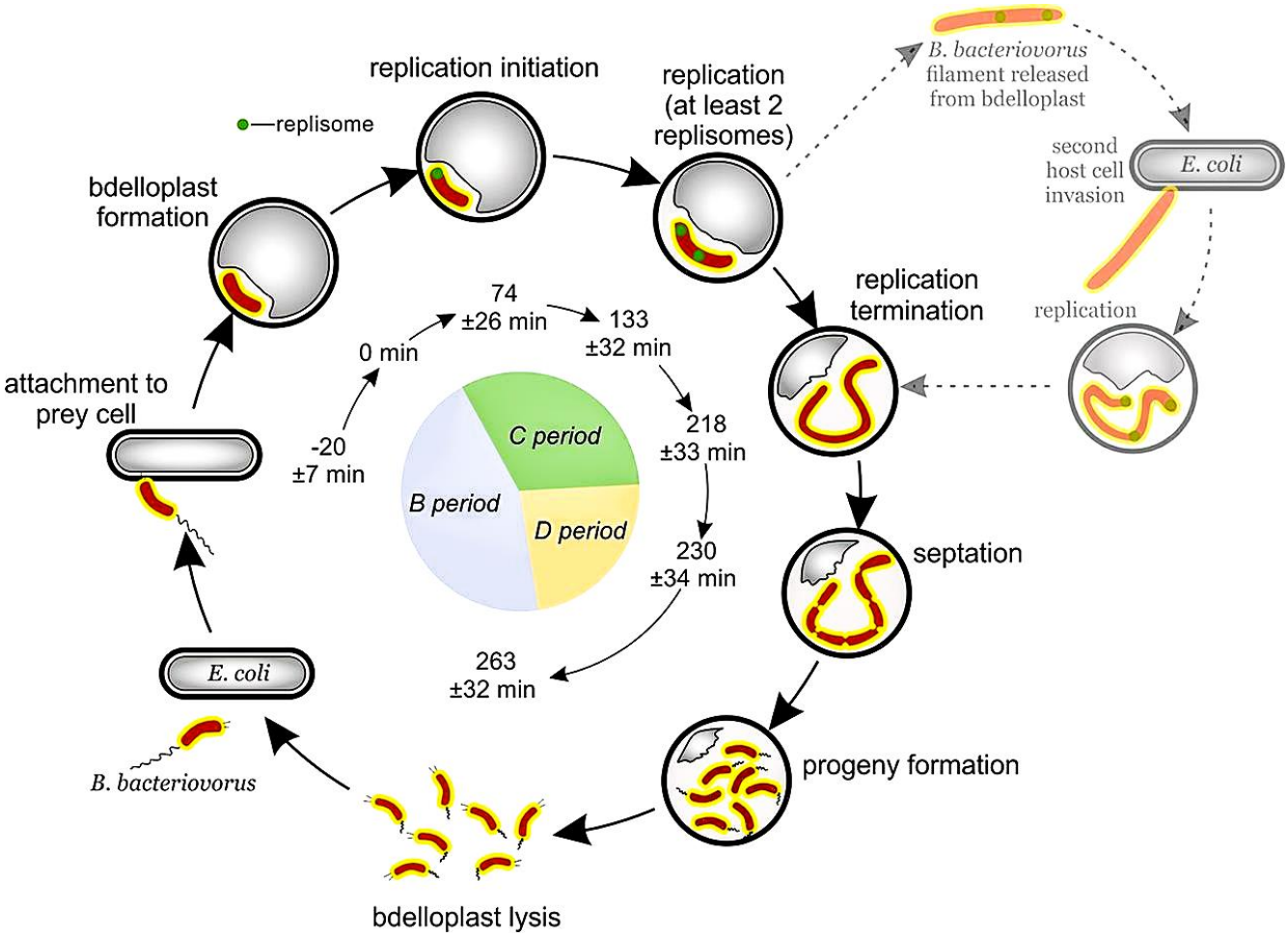


Figure 20: Life cycle of Bdellovibrio (orange) inside Prey gram –negative bacteria *E. coli* (Gray). T=0 time represents the formation of bdelloplast. Inner circle represents the bacterial life cycle period; B- time between progeny formation and chromosome replication, C- replication of chromosome and D- time between replication termination and filament septation. *[Reference: Ł. Makowski et al., "Dynamics of chromosome replication and its relationship to predatory attack lifestyles in Bdellovibrio bacteriovorus," Appl. Environ. Microbiol., vol. 85, no. 14, 2019.]*

(vi) Antibodies

Antibodies are produced by immune cells against pathogens like bacteria, fungi, viruses etc. Antibodies are proteins, which helps immune system by either neutralizing pathogen or by neutralizing the toxins produced by pathogen. Advantage of using antibodies is they specificity as they are highly specific and selective against a particular type of pathogen or toxin. The major drawbacks in using antibodies is their poor shelf life and high cost of production. In addition, the process of production is time consuming. Various antibodies are undergoing

clinical trials and are approved by FDA to use against various pathogens like *P. aeruginosa, Bacillus anthracis,* and *C. difficile* [Table8].

Table 8: Antibodies undergoing clinical trials to treat various bacterial infections. *[Reference: C. Ghosh, P. Sarkar, R. Issa, and J. Haldar, "Alternatives to conventional antibiotics in the era of antimicrobial resistance," Trends Microbiol., vol. 27, no. 4, pp. 323–338, 2019]*

Antibody name	***Company***	***Pathogen***	***Target***	***Condition***	***Clinical studies***
Anthim	Elusys therpaeutics	*B. anthracis*	Protective antigen	Anthrax	FDA approved
Shigamabs	Taro pharmaceuticals	Shiga-toxin-producing *Escherichia coli*	Shiga toxin (tx1 and tx2)	Shiga-toxinproducing bacterial infection	Phase II
Anti-Pseudomonas IgY	Immunsystem AB	*P. aeruginosa*	Specific target unknown	Cystic fibrosis/P. aeruginosa infections	Phase I/II
MEDI 3902	MedImmune Inc.	*P. aeruginosa*	Psl and PcrV	Noscocomial pneumonia	Phase I
Bezlotuxumab	Merck	*Clostridium difficile*	Toxin B	C. difficile-associated diarrhea CDAD	FDA approved
Valortim	Pharm-Athene	*B. anthracis*	Protective antigen	Anthrax	Phase I
Aerumab (AR-101)	Aridis Pharmaceuticals	*P. aeruginosa*	LPSa	Pneumonia/ ventilator-associated pneumoni	Phase I/Phase II
MEDI 4893	MedImmune Inc.	*S. aureus*	a-toxin	Hospital-acquired pneumonia	Phase II

Aerucin	Aridis Pharmaceuticals	*P. aeruginosa*	Alginate on cell surface	Pneumonia	Phase II
Salvecin (AR 301)	Aridis Pharmaceuticals	*Staphylococcus aureus*	a-toxin	Sepsis Pneumonia	Phase II Phase I
Raxibacumab	GlaxoSmith Kline	*Bacillus anthracis*	Protective antigen	Anthrax	FDA approved
Pagibaximab	Biosynexus	*Staphylococci*	Lipoteichoic acid	Staphylococcal sepsis	Phase III
514G3	XBiotech	*S. aureus*	Immunomodulator	Bacteremia	Phase II
Aurexis	Bristol-Meyrs Squibb	*Staphylococci*	Clumping factor A	Staphylococcal infections	Phase II
Pseudomonas aeruginosa immune globulin (MEPIGIV)	National Center for Research Resources	*Pseudomonas aeruginosa*	Specific target unknown	Cystic fibrosis	Phase II

7.7.2 Synthetically Designed Strategies

(i) Synthetic Mimics of Antimicrobial Peptides (SMAMPs)

There are many attempts made by scientists to produce molecules, which act as antimicrobial peptides. These are called SMAMPs (synthetic mimics of antimicrobial peptides). Mainly three approaches were used to produce these molecules; use of polymers to mimic AMPs, use of peptidomimetic oligomers and use of small molecules [83]. These strategies were used to remove problems like toxicity, liability and high manufacturing cost, which are limiting factors in utilising AMPs.

Overcoming protease stability is the earliest attempts made by scientists and they modified peptide backbone of AMPs by keeping the peptide design steady by charge. Molecules as oligoacyl- lysines, peptoids, oligoureas, b-peptides etc. were produced which are oligomeric in nature and can produce secondary structures with antimicrobial properties.

Antimicrobial polymers were produced by adding cationic domain and hydrophobic domain into polymeric peptides. These polymers can be classified into categories like segregated monomers, same-centred polymers, and facially-amphiphilic polymers. These polymers show

moderate antimicrobial properties. These polymers also show positive results in resistant bacteria to make them sensitive towards antibiotics [84].

(ii) Innate Defence Regulatory Peptides

AMPs have another function during invasion by pathogenic bacteria. These peptides stimulate host immune system to act against pathogen. In this context, scientists produced some peptides, which do not have any antimicrobial properties but can act against endotoxins and modulate immune system of host [85]. These peptides are called innate defence regulatory (IDR) peptides. Study of these peptides in mice showed that these peptides could help in removing many severe pathogenic microbes and malarial infection from host body.

(iii) Antibacterial Oligonucleotides

A new approach to treat many diseases including infectious diseases is gene silencing. In this context, an oligonucleotide which is antisense to the target mRNA is used to silence the genes responsible for resistance or any other gene which is essential for bacteria to survive. This method is very effective during treatment of MDR pathogen. These oligonucleotides were modified prior to use to make them resistance against nucleases present in bacterial cytoplasm. These modifications include morpholino and phosphorodiamidate groups. In addition, cell-penetrating peptides (PPMOs) are added with these oligonucleotides for easy transport by negatively charged cell membrane. Some PPMOs show antimicrobial activity against vrious kinds of bacteria like *S. enterica,* and *A. baumannii* [86].

(iv) Inhibitors of Bacterial Virulence

Bacteria produce various molecules, which can be extracellular or can attach to surface of bacterial cell. These factors help bacteria to produce virulence in host organism. Another strategy, which can be utilised as alternative to antibiotics, is inhibiting expression of these virulence factors by bacteria. In this strategy, we are not targeting bacterial cellular processes directly, there are less chance for bacteria to generate resistant against these methods. Scientists are doing trial with numerous antibodies, which can interfere with bacterial virulence. Recently, a new virulence inhibitor that is based on liposome called CAL-02, was introduced against *Pneumococcal pneumonia*. Clinical trials for this drug is being done to see how effective the treatment is against the infection. It has also been found that this drug can also inhibits virulence factors like toxins produced by bacteria like *S. pneumoniae, P. aeruginosa, S. aureus* etc.

8. Discussion

Discovery of antibiotics gave a hope to humankind against infectious diseases. However, the development of resistance in the bacteria pose a major threat towards health of people as well as other organisms. Bacteria are developing resistance by different mechanisms and the effect of antibiotic towards bacteria is getting weaker. On top of that, bacteria developed different mechanisms to transfer those resistance mechanisms to one another, which is leading to spread of resistance very rapidly in a bacterial species. There are some bacteria that are resistant to almost all the antibiotics known to us until now like Methicillin resistant *staphylococcus aureus* strain (MRSA).

Overuse of antibiotics is the main reason for development of resistance in the bacteria. Most resistance is generated against cheaper antibiotics. Hospital waste are main source of antibiotics in different types of environment like water and soil, which is leading hospital, acquired infection. In developing countries where disposal system is not developed are facing a major threat by antimicrobial resistance.

Non-effectiveness of antibiotics and continuous development and proliferation of resistance against different antibiotics is leading to the development of other technologies that can be used as potential treatment against resistant bacteria. Resistance generated by genetic mutation can be treated by CRISPR Cas 9 technology. Bacteriophage can also be act as alternative to antibiotics. These methods can be used individually or as combination with antibiotics. Combinations of different antibiotics can also be used for treatment.

Every country in the world is facing the problem of antimicrobial resistance. In some places, it has more impact than other does. We know the obvious reasons of development and proliferation of resistant is “overuse”. We are in a dire need of alternative technologies to prevent the spread of resistance and to treat diseases caused by resistance bacteria. Elimination of resistance from bacteria can take some time but we can take proper measures to prevent further development of resistance.

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