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Discussion Paper

Combatting Antimicrobial Resistance

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WHO Report [1]

• Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi.

• AMR is an increasingly serious threat to global public health that requires action across all government sectors and society.

• Without effective antibiotics, the success of major surgery and cancer chemotherapy would be compromised.

• The cost of health care for patients with resistant infections is higher than care for patients with non-resistant infections due to longer duration of illness, additional tests and use of more expensive drugs.

• In 2016, 490 000 people developed multi-drug resistant TB globally, and drug resistance is starting to complicate the fight against HIV and malaria, as well.

Antimicrobial resistance happens when microorganisms (such as bacteria, fungi, viruses, and parasites) change when they are exposed to antimicrobial drugs (such as antibiotics, antifungals, antivirals, antimalarials, and anthelmintics). Microorganisms that develop antimicrobial resistance are sometimes referred to as "superbugs."

As a result, the medicines become ineffective, and infections persist in the body, increasing the risk of spread to others.

Opening Words

The human health problem that annoys the population of earth these days due to the resistance and character-change of many bacteria strands due to horizontal gene transfer is threatening public health, The nosocomial infections situation is alarming, one of 25 entering a healthcare facility gets infected and one of nine dies from the infection. Antibiotic resistance is a growing public health problem. The United Nations recently acknowledged this as "one of the biggest threats to modern medicine," dedicating a high-level meeting to the issue at the 2016 General Assembly [2]. The infectors are now everywhere in the public domain, but mainly in lavatories, restaurants, recreation parks for example, everywhere, and this overflow of infectors is the next even more alarming stage of the pandemic. There is an urgent need to harness forces for the combat with the microbes, with the hope to win this battle. Hand wash may help, but more thorough hygiene is needed, even in the soil. The current regulatory environment is a further factor constricting research and development of antibacterial agents by large and small pharmaceutical companies. Regulatory authorities are less prepared to accept adverse side-effects with antibiotics than other classes of therapeutic agents and demonstration of superiority is required for new antimicrobials. Both these factors are likely to contribute to a further decline in pharmaceutical industry involvement in the discovery and development of new antibacterial and antiseptic agents.

Challenge

One major world problem, the microbe's pandemic, should be addressed biblically. Erase the old and look for new, state of the art antimicrobial [3] and antiseptic agent.

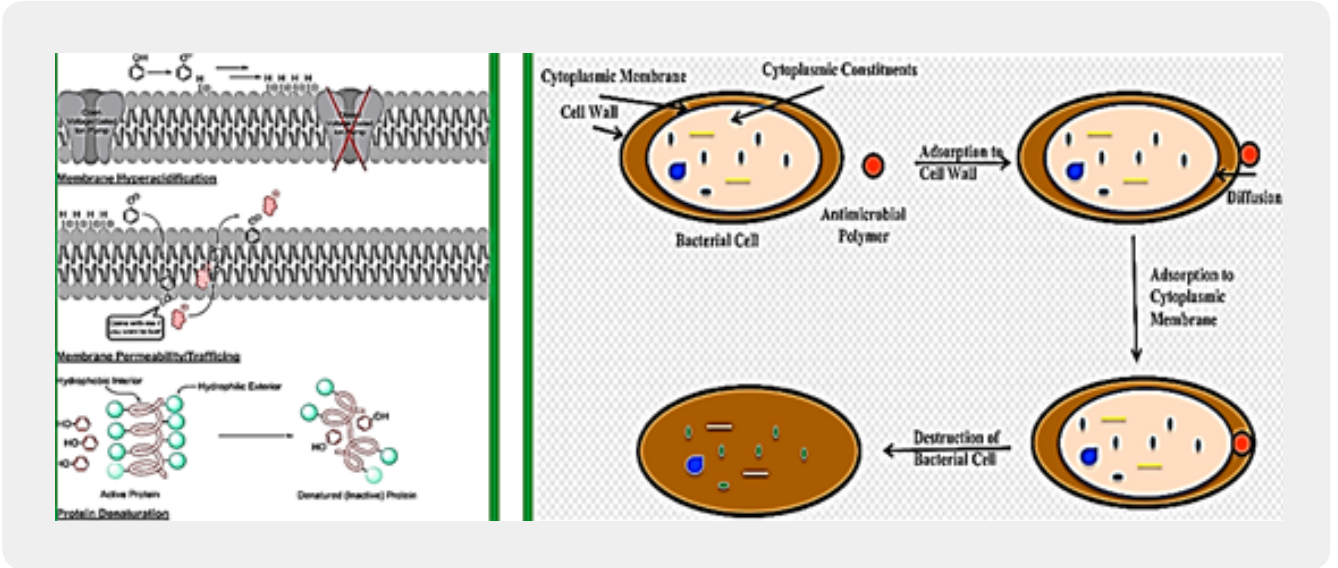


Figure 1: *General pathway to eradicate bacteria with an antimicrobial agent*

We, humans, have adapted to live in harmony with different microorganisms throughout evolution - the microbiome [4]; this balanced symbiotic relationship can sometimes shift and allow pathogenic bacteria to blossom and cause infections. In the struggle for survival, a complex mechanism involving many key components assists in the elimination of these infectious agents.

The problem's focuses are many [5], here we mention three target areas: Health (Hospital Acquired Infections, HAI), Food sterilization and Agriculture [6] (Livestock, soil sterilization).

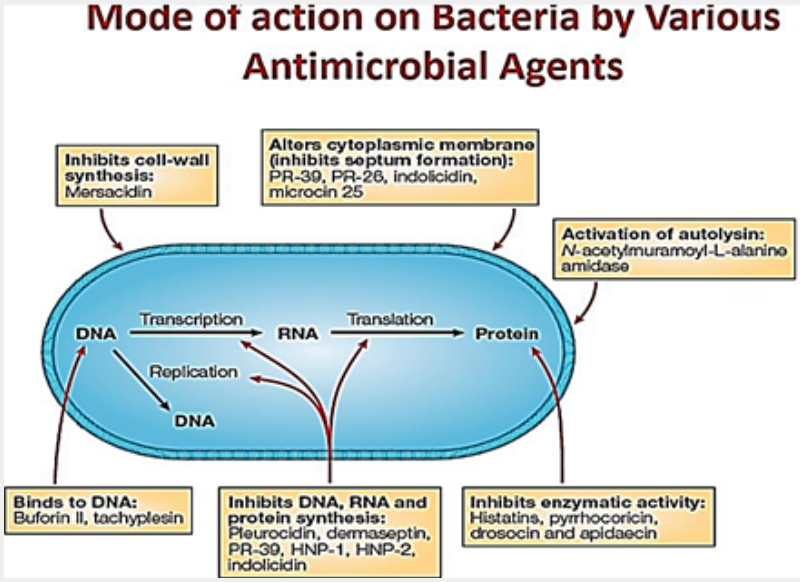
Introduction

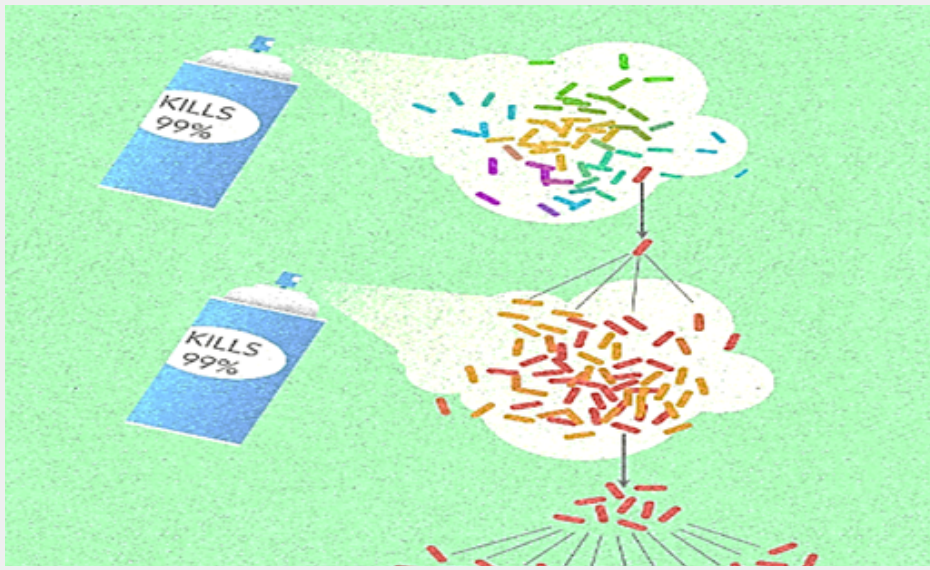
Healthcare-associated infections (HAIs)-infections patients can get while receiving medical treatment in a healthcare facility-are a major, yet often preventable, threat to patient safety. Together with health care and public health partners, CDC is working to bring increased attention to HAIs and prevention.

The advantage of antimicrobial peptides is the generality of their mechanism of action, which involves either compromising the bacterial membrane integrity or disrupting essential components inside the cells. This differs from the specific receptors targeted by conventional antibiotics which allow the pathogenic bacteria to develop resistance more rapidly. Furthermore, antimicrobial peptides are fast-acting and biodegradable, which alleviates the current concern over residual antibiotics in the environment. In addition to their direct microbicidal activities, these host defense peptides are particularly attractive because of the multiple activities that are associated with many members of this family. These include the regulation of the innate and adaptive immune systems, inflammation and wound healing, and additional anti-infective activities such as being antifungal, antiviral, antiparasitic or anticancerous.

However, This perception of the bioactivity of Amp and presumably their surrogates mat be too over estimated. The case with Gramicidin may contradict it.

Antimicrobial agents have changed human and animal health systematism by revolutionizing our weapons in the war against infectious disease, resulting in improved survivability. There was a time where the perfect antimicrobial drug "The magic bullet a search for the perfect drug" was considered a n achievable target [7]. For both human and animals. However, this health triumph has been tempered by the subsequent realization that bacterial populations can quickly modify them to resist [8].





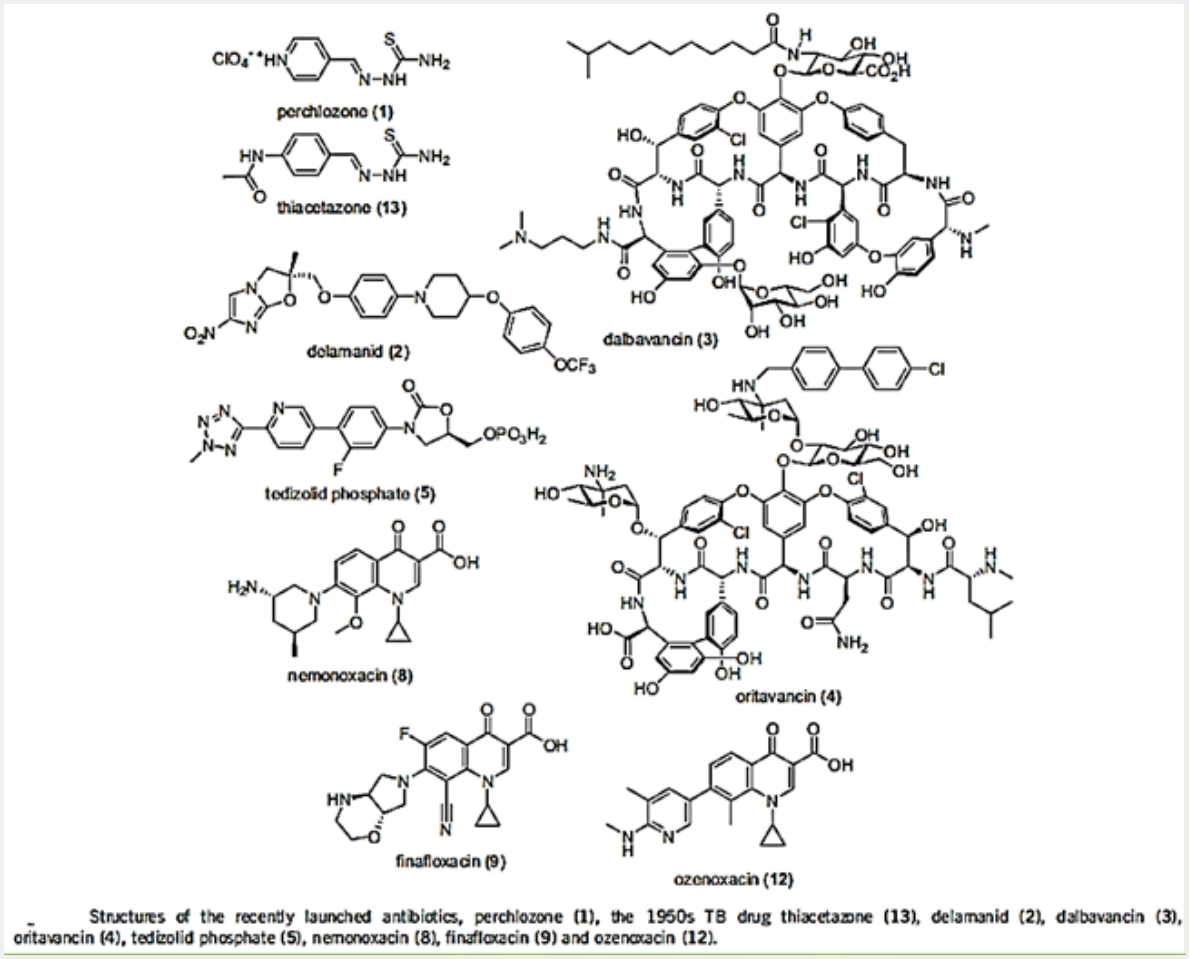
Frequent antibiotic use over long periods of time puts selective pressure on bacteria and causes resistance to spread. When an antibiotic is used to treat a typical bacterial infection, most bacteria are killed. Sometimes, however, a bacterium with an advantage life. This bacterium can then reproduce and pass its advantage on, creating many more antibiotic-resistant bacteria.

Table 1 Antibiotics and β-lactamase inhibitor combinations launched from 2000 to 2015, their antibiotic class, activity spectra, country of first approval, lead source and NP lead source if applicable

Year approved	Drug name[a,b]	Class	Bacteria type	Country of first approval	Lead source	NP lead source
2000	Linezolid	Oxazolidinone	G+ve	USA	S	
2001	Telithromycin	Macrolide	G+ve/G−ve	Germany	NP-derived	Actinomycete
2002	Biapenem	Carbapenem	G+ve/G−ve	Japan	NP-derived	Actinomycete
2002	Ertapenem	Carbapenem	G+ve/G−ve	USA	NP-derived	Actinomycete
2002	Prulifloxacin	Fluoroquinolone	G+ve/G−ve	Japan	S	
2002	Pazufloxacin	Fluoroquinolone	G+ve/G−ve	Japan	S	
2002	Balofloxacin	Fluoroquinolone	G+ve/G−ve	South Korea	S	
2003	Daptomycin[b]	Lipopeptide	G+ve	USA	NP	Actinomycete
2004	Gemifloxacin	Fluoroquinolone	G+ve/G−ve	USA	S	
2005	Doripenem	Carbapenem	G+ve/G−ve	Japan	NP-derived	Actinomycete
2005	Tigecycline	Tetracycline	G+ve/G−ve	USA	NP-derived	Actinomycete
2007	Retapamulin[b,c,d]	Pleuromutilin	G+ve	USA	NP-derived	Fungus
2007	Garenoxacin	Quinolone	G+ve/G−ve	Japan	S	
2008	Ceftobiprole medocaril	Cephalosporin	G+ve/G−ve	Canada	NP-derived	Fungus
2008	Sitafloxacin	Fluoroquinolone	G+ve/G−ve	Japan	S	
2009	Tebipenem pivoxil	Carbapenem	G+ve/G−ve	Japan	NP-derived	Actinomycete
2009	Telavancin	Glycopeptide	G+ve	USA	NP-derived	Actinomycete
2009	Antofloxacin	Fluoroquinolone	G+ve/G−ve	China	S	
2009	Besifloxacin[d]	Fluoroquinolone	G+ve/G−ve	USA	S	
2010	Ceftaroline fosamil	Cephalosporin	G+ve/G−ve	USA	NP-derived	Fungus
2011	Fidaxomicin[b]	Tiacumicin	G+ve	USA	NP	Actinomycete
2012	Bedaquiline[b]	Diarylquinoline	G+ve (TB)	USA	S	
2012	Perchlozone (1)	Thiosemicarbazone	G+ve (TB)	Russia	S	
2014	Delamanid (2)	Nitroimidazole	G+ve (TB)	Europe	S	
2014	Dalbavancin (3)	Glycopeptide	G+ve	USA	NP-derived	Actinomycete
2014	Oritavancin (4)	Glycopeptide	G+ve	USA	NP-derived	Actinomycete
2014	Tedizolid phosphate (5)	Oxazolidinone	G+ve/G−ve	USA	S	
2014	Ceftolozane (6)+tazobactam[e] (7)	β-Lactam+β-lactamase inhibitor	G−ve	USA	NP-derived/NP-derived	Fungus/actinomycete
2014	Nemonoxacin (8)	Quinolone	G+ve/G−ve	Taiwan	S	
2014	Finafloxacin[d] (9)	Fluoroquinolone	G+ve/G−ve	USA	S	
2015	Ceftazidime[e] (10)+avibactam[b] (11)	β-Lactam+DBO β-lactamase inhibitor	G−ve	USA	NP-derived/S	Fungus
2015	Ozenoxacin (12)[d]	Quinolone	G+ve	Japan	S	

Abbreviations: G−ve, Gram negative; G+ve, Gram positive; NP, natural product; S, synthetic; TB, tuberculosis, USA, United States of America.
[a]The structures of the antibiotics approved from 2000 to 2012 can be found in our previous reviews.[2,3]
[b]First member of a new antibiotic or β-lactamase inhibitor class approved for human therapeutic use.
[c]Pleuromutilin derivatives have been previously used in animal health.
[d]Approved for topical use.
[e]Tazobactam and ceftazidime were first launched in 1992 and 1983, respectively.

The short list in the table above represents some of the newest antibiotic drugs approved for antiinfection treatment. Please notice the poor yields in antibacterial agents in the last decades, only a score of novel drugs was found and approved [9], do not provide weapons to fight infections in healthcare installations (nosocomial infections) and give only little hope to the millions suffering from the fact6 that they do not provide remedy to those that are infected with the resistant microbes and in most cases, must die while treated in hospitals.

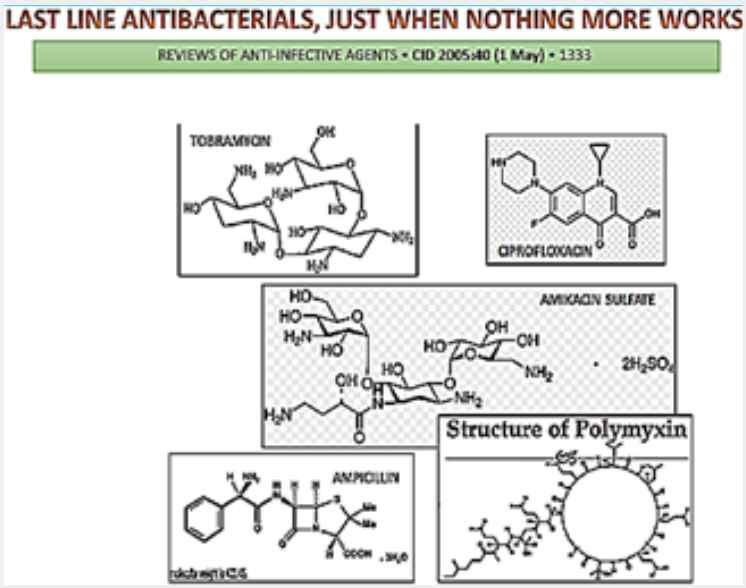


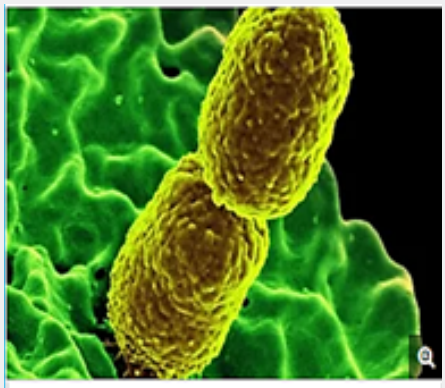
Structures of the recently launched antibiotics, perchlozone (1), the 1950s TB drug thiacetazone (13), delamanid (2), dalbavancin (3), oritavancin (4), tedizolid phosphate (5), nemonoxacin (8), finafloxacin (9) and ozenoxacin (12).

One of the reasons is that even the most modern findings in the area are impotent in the killing of bacteria when resistant strands are the matter.

CRE are a type of bacteria that are highly resistant to many antibiotics. They have earned the name "killer bacteria" and "nightmare bacteria" for good reason. The death rate for those infected with CRE, or carbapenem-resistant Enterobacteriaceae, (The Enterobacteriaceae are a large family of Gram-negative bacteria) is greater than 40 percent, according to the California Department of Public Health (CDPH), and could be as high as 50 percent, according to the Centers for Disease Control (CDC).

Infections usually occur in hospitals, nursing homes and other health care settings, the CDC said. Members of the general population are generally not at risk.

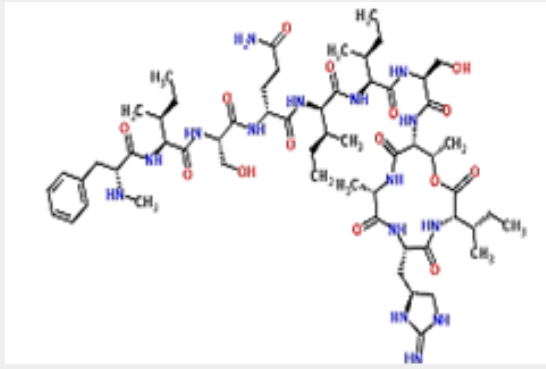




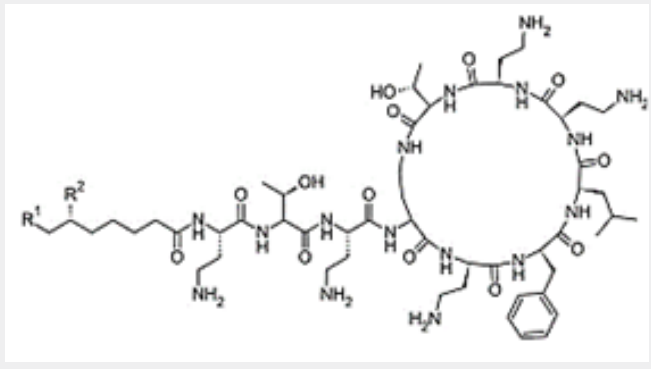
This image shows two rod-shaped bacteria called Klebsiella pneumoniae that are resistant to treatment with the "last resort" antibiotic drug called carbapenem. In this image, the mustard-colored bacteria are interacting with the green-colored human white blood cells.

The emergence of drug-resistant strains of major pathogenic bacteria such as Staphylococcus aureus is an increasingly serious public health concern [10]. To evade bacterial drug resistance mechanisms, new effective chemotherapeutic agents, which have novel mechanisms of action as well as different cellular targets compared with conventional antibiotics, need to be developed [2].

The shortage of new antibiotics to combat multidrug-resistant (MDR) strains has led to a renewed interest in polymyxins [11]. Polymyxins are active against MDR Gram-negative bacteria such as Pseudomonas aeruginosa, Klebsiella pneumonia, Acinetobacter baumannii, and Enterobacter species [12]. Early reports described high incidences of nephrotoxicity and neurotoxicity during polymyxin therapy [9], and the use of polymyxins was replaced in the 1970s by antibiotics considered to be less toxic. However, recent studies showed that polymyxins have acceptable effectiveness and are considerably less toxic than originally reported. Cationic am Colistin (polymyxin B) was re-introduced as "last resort" for treating patients with acute infection based on drug-resistant bacteria, However established that the cyclic peptide is very harmful to central organs like heart, liver, kidney brain and is a lethal substance in about 60% of its use causes mortality Polymyxins, a group of polypeptide antibiotics that consists of5 chemically different in 1947. Only polymyxin B and polymyxin E (Colistin) have been used extensively worldwide in Colistin was discovered in 1949 and was non-ribosomally synthesized by Ba5]. Coli of colistimethate sodium in 1959. However, them due to multidrug-resistant (MDR), gram-negative bacteria in patients with cystic fibrosis. However, the emergence of bacteria resistant to most classes of commercially available antibiotics and the shortage of new antimicrobial agents with activity against gram-negative microorganisms have led to the reconsideration of polymyxins as a valuable therapeutic option.



Teixobactin



Polymyxin B (Colistin)

The novel antimicrobial peptide-based Teixobactin identified [14,15]. Recently, Scientists have discovered an antibiotic capable of fighting infections that kill hundreds of thousands of people each year, a breakthrough that could lead to the field's first major new drug in more than a quarter-century. The new agent that was isolated, identified and synthesized, gives great hope to medicine regarding the combat withy resistant bacteria. Teixobactin and some of its analogs were synthesized by a number of research groups. This awakes the hopes that are centered around the following:

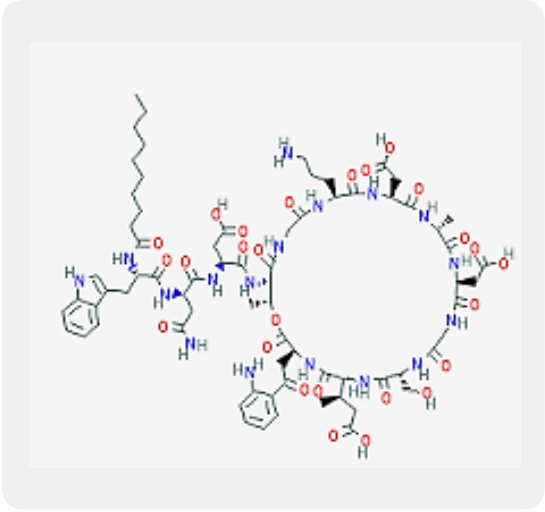
1. Selectivity preferred eradication, Gram+ or Gram-.

2. Effective eradication is of all bacteria regular and resistant.

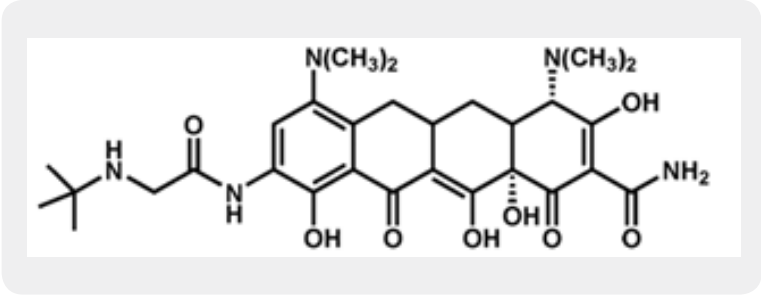
3. Low toxicity to humans.

Not only one target is attacked by Teixobactin, but multiple targets, and they are all lethal. For bacteria, it will be very hard to modify this target, especially this part of the molecule that's bound by Teixobactin.

Until the discovery of Teixobactin, the following were the top antibiotics in the clinic:



Daptomycin



Tigecycline

The last major new antibiotic, daptomycin, was discovered in the 1980s by Eli Lilly & Co. After being abandoned in early testing, Antibiotic-resistant bacteria kill at least 700,000 people a year, per a U.K. government review. Unchecked, those infections could lead to 10 million more deaths a year by 2050, the report found.

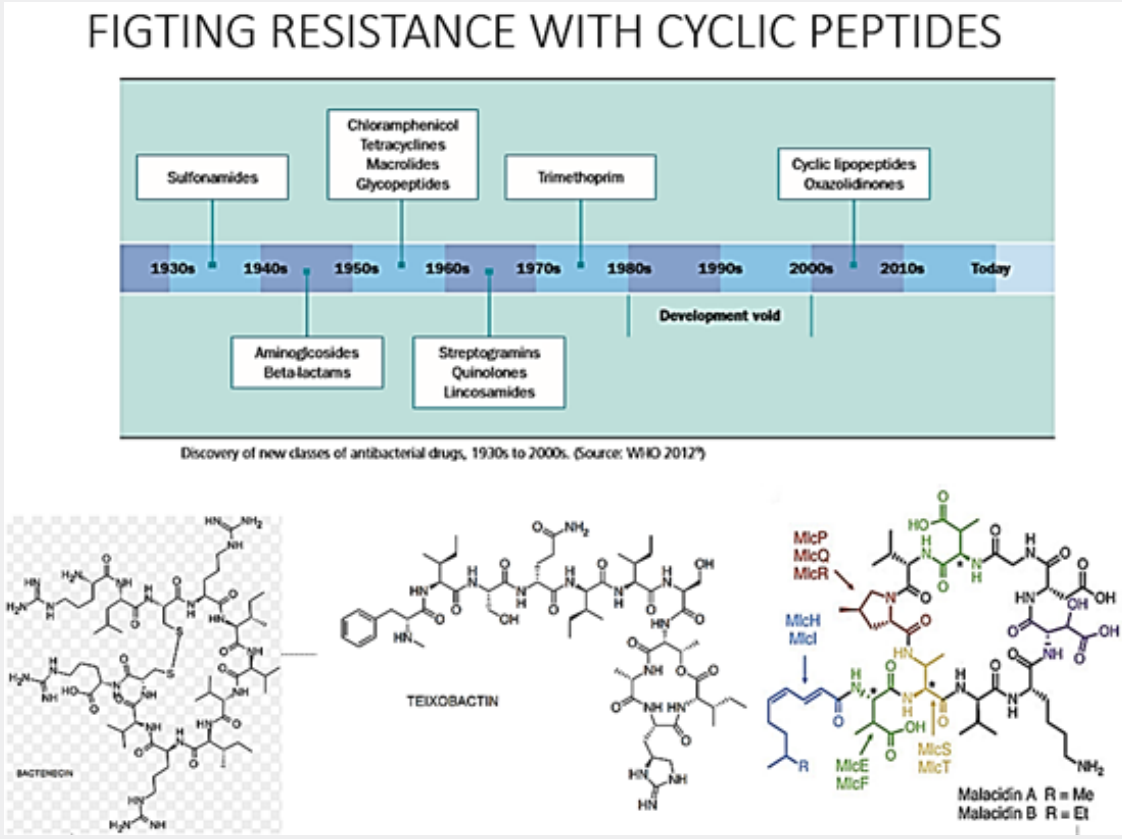
Tigecycline [16] is the one of newest antibiotic to have been released. It has been on the market for years. It This includes activity against gram-positives, gram-negatives except for P. Aeruginosa, many anaerobes and many atypical pathogens as well as several resistant pathogens. Because it is not vulnerable to many of the resistance mechanisms that affect the beta-lactam antibiotics, it has potential utility in those types of situations. It has been approved for use in complicated skin and skin structure infections, as well as for treatment of complicated intra-abdominal infections due to susceptible pathogens. These are the only 2 indications that are approved right now. It has very interesting pharmacokinetics and a long half-life. It will be dosed, after a load, twice a day, and it has a rather low serum concentration, but very good tissue distribution. Tigecycline is available as an intravenous solution. The primary side effects or adverse reactions that have been seen in clinical trials have been gastrointestinal symptoms, usually nausea.

The hunt for new antibiotics is benefiting from recent technological advances which make it easier to rapidly comb the DNA of different soil organisms, which are otherwise hard to raise in a petri dish [17].

The soil is a good place to look for new organisms because it is here that bacteria naturally compete for resources and use a range of exotic chemical compounds to kill each other.

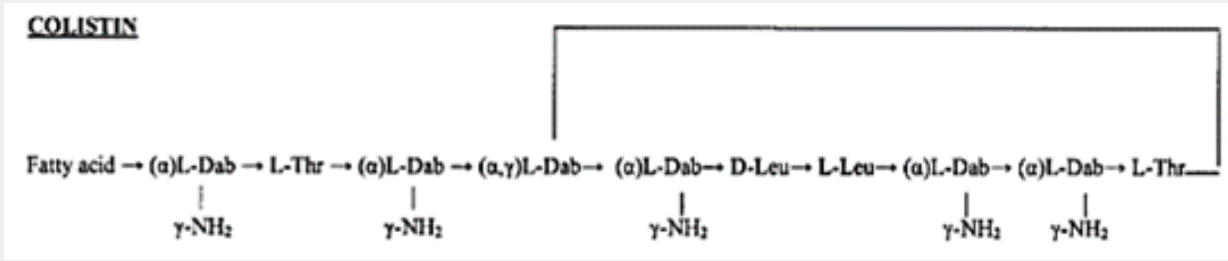
The paladin chemical, identified by Dr Sean Brady and his laboratory at Rockefeller University, New York, works by attacking a fundamental step in bacterial growth, essentially interfering with a major building block that the bacteria use to build and repair their outer membrane.

In their study, published as a letter in the journal Nature Microbiology, the authors cautioned that it is only effective against one group of bacteria - the gram positives, which include MRSA



Cyclic peptides are leading the current anti-resistant antimicrobials. Bactenecin, Teixobactin [18], and the Milacainides [19] are the most active that eradicate resistant bacteria strands.

The finding new antibiotics to treat gram-positive infections like MRSA was good news but could not address the most pressing need. Our concern is the so-called gram-negative bacteria which are difficult to treat and where resistance is on the increase.



Colistin [20]: The Revival of Polymyxins for the Management of Multidrug-Resistant Gram-Negative Bacterial Infections [21].

Gram-negative bacteria cause pneumonia, blood sepsis [22] and urinary tract infections as skin infections. Twenty-four patients (mean age 44.3 years, mean Acute Physiology and Chronic Health Evaluation II score 20.6) received 26 courses of colistin. Clinical response was observed for 73% of the treatments. Survival at 30 days was 57.7%. Deterioration in renal [23] function was observed in 14.3% of 21 patients who were not already receiving renal [24] replacement therapy, but in only one case did this deterioration have serious clinical consequences. Colistin was discovered in 1949 and was nonribosomally synthesized by *Bacillus polymyxa* subspecies *colistinus* Koyama [25]. Colistin was initially used therapeutically in Japan and in Europe during the 1950s and in the United States in the form of colistimethate sodium in 1959 [6]. However, the intravenous formulations of colistin and polymyxin B were gradually abandoned in most parts of the world in the early 1980s because of the reported high incidence of nephrotoxicity. Subsequently, the intravenous use of colistin was mainly restricted during the past 2 decades for the treatment of lung infections due to multidrug-resistant (MDR), gram-negative bacteria in patients with cystic fibrosis [10-12]. However, the emergence of bacteria resistant to most classes of commercially available antibiotics and the shortage of new antimicrobial agents with activity against gram-negative microorganisms have led to the reconsideration of polymyxins as a valuable therapeutic option. Currently applied Fosfomycin or Colistin may cause renal damages, it is therefore demands less damaging antibiotic agents. It seems that we need new antibiotics to treat this class.

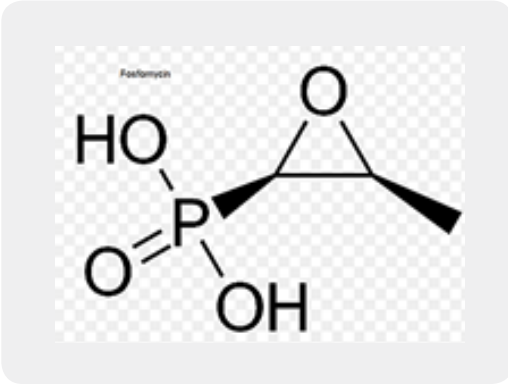
Carbapenem-Resistant Enterobacteriaceae (CRE) Infection

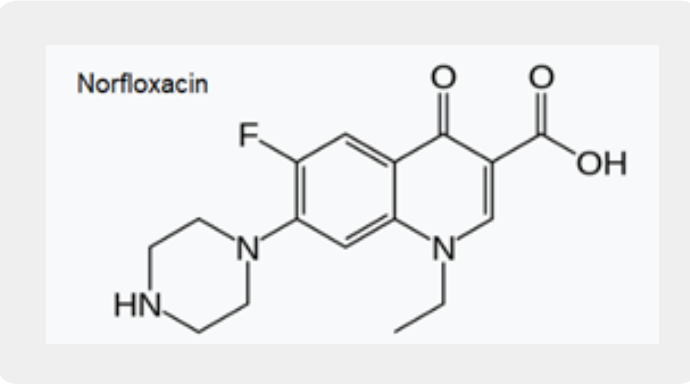
CRE infections [26] are most commonly seen in people with exposure to healthcare settings like hospitals and long-term care facilities, such as skilled nursing facilities, and long-term acute care hospitals. In healthcare settings, CRE infections occur among sick patients who are receiving treatment for other conditions. Patients whose care requires devices like ventilators (breathing machines), urinary (bladder) catheters, or intravenous (vein) catheters, and patients who are taking long courses of certain antibiotics are among those at risk for CRE infections.

In recent years, carbapenem resistance among Enterobacteriaceae has dramatically increased and represents an important threat to global health.

Some CRE bacteria have become resistant to almost all available antibiotics and can be deadly-one report cites they can contribute to death in up to 50% of patients who become infected. The Therapeutic options for carbapenem-resistant Enterobacteriaceae infections are limited.

Some reports have investigated the clinical efficacy of intravenous Fosfomycin [27] in patients with CRE infections. Michalopoulos *et al.* reported 11 cases of ICU patients having CRKP infections who were treated with intravenous fosfomycin in combination with other antibiotics; the clinical and microbiological outcome was good with an all-cause hospital mortality of 18.2%, and no adverse events were reported. More recently, Pontikis *et al.* investigated clinical outcome of 48 fosfomycin-treated (mainly in combination with colistin or tigecycline) ICU patients having XDR fosfomycin-susceptible P. aeruginosa (n D 17) and K. pneumoniae (n D 41) carbapenemase-producing isolates; the authors reported a survival rate of 54.2%, evidence of bacterial eradication in 56.3% of cases, and development of fosfomycin-resistance in 3 cases.



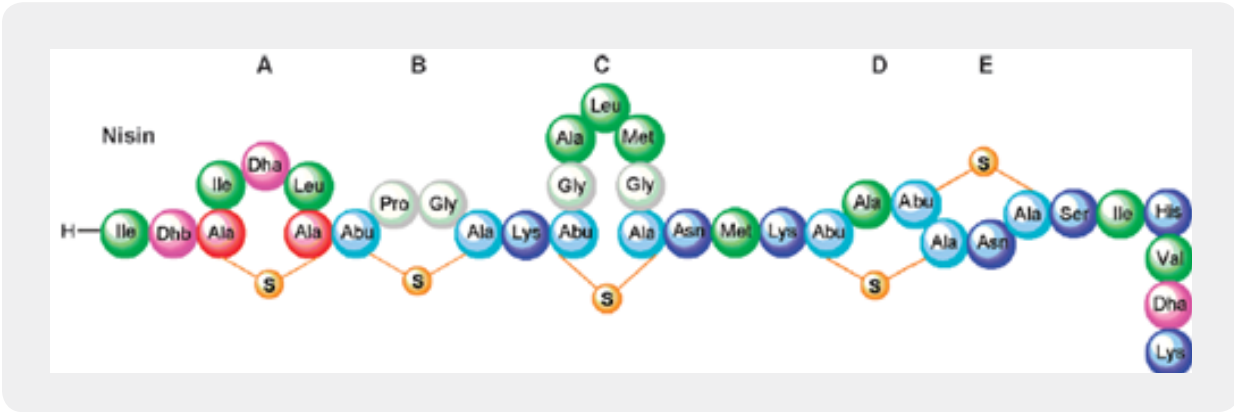


Fosfomycin (also known as phosphomycin or phosphonomycin and the trade names Monurol and Monuril) is a broad-spectrum antibiotic produced by certain *Streptomyces* species, although it can now be made by chemical synthesis.

As a single dose, fosfomycin is more convenient than multiple-dose therapy Norfloxacin, for the same antibacterial efficacy.

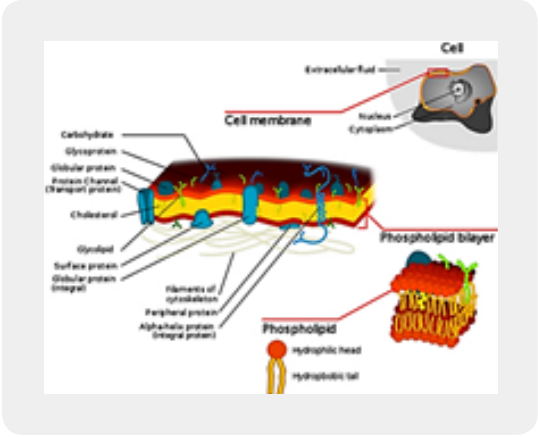
Bioactive Macrocyclic Peptides and Peptide Mimics [28a-c], AIB -based peptide backbone as scaffolds for helical peptide mimics, Antibiotics like Nisin and other peptides and mimics were tested as potent agents for resistant bacteria.

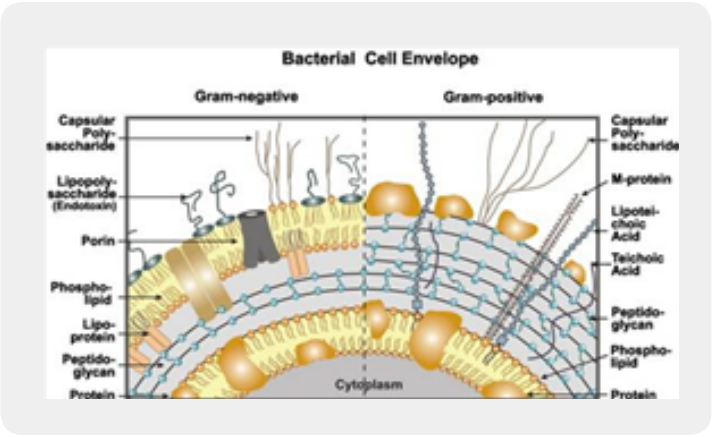
Nisin is a bacteriocin produced by Lactococcus lactis subsp. Latics that exhibits a broad inhibitory spectrum against gram-positive bacteria, including bacterial endospores [29a,b]. Recent studies have shown that the spectrum of nisin activity can be extended to include gram-negative bacteria. Application of nisin in combination with the chelating agent EDTA results in inhibition of Salmonella species and other gram-negative bacteria.



One of the approaches is to learn about the potential application of short peptide mimics like β-turn mimics, on the recognition with perspective to apply this if future drug design. The appearance of β-turns in protein interaction is by far more common than that of other, like γ-turns. Non-covalent [30,31] interactions between the β-turn-mimics and some receptors on the cell wall of bacteria may supply enough energy differences that may allow differentiation between various bacterial transmembrane cell wall receptors [32] due to the receptor and β-turn mimic interactions. The interactions of some β-turn mimics with many classes of proteins which vary in their secondary structure (β-sheets, globular) have been found to rely on the interaction between β-turn mimics and the proteins.

Bacteria Cell Wall and Proteins [33]





Many variants of β-turn mimic, one count nine β-turn types [34], have been applied so far in this area of research. It has been reported that Surrogate at the β-turn domain of Gramicidin S increase the biological activity [35]. One can read about benzodiazepines, β-turn mimic Hot═Tap for example [36].

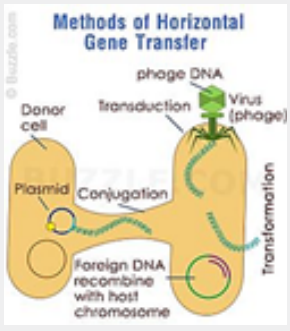
The lack of production and introduction of the newer and effective antibiotic/antibacterial drugs in clinical practice in the post-antibiotic golden age [37] has seen an increase in the emergence of the resistant pathogenic bacterial infections creating a significant problem in the global health of humankind.

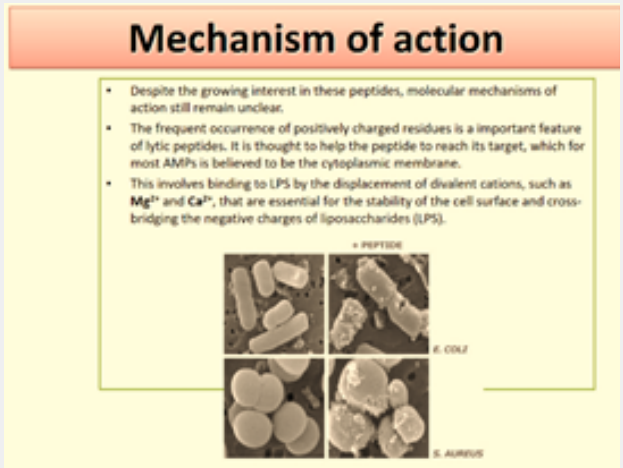
The situation today is that In 2011, an estimated 722,000 [38] patients contracted an infection during a stay in an acute care hospital in the US; 205 Americans die from hospital-acquired infections (hospital-acquired infections HAI, nosocomial infections [39]) every day.

Compounding the problem of antimicrobial-drug resistance is the immediate threat of a reduction in the discovery and development of new antibiotics [40,41].

It is now known that many different Gram-positive and Gram-negative pathogens communicate via the production and sensing of small, diffusible signal molecules, to coordinate virulence determinant production [42]. Horizontal genes transfer enables the transfer of resistance from one sort of bacteria to another either by Transformation, involves uptake of short fragments of naked DNA by naturally transformable bacteria. Transduction, involves transfer of DNA from one bacterium into another via bacteriophages. Conjugation which involves transfer of DNA via sexual pilus and requires cell-to-cell contact [43] (see cartoon below). Hunting the nightmare bacteria [44], Therefore, this event, now termed quorum sensing, represents a novel therapeutic target offering the opportunity to attenuate virulence, and thus control infection, by blocking cell-to-cell communication.

HAI Estimates Occurring in US Acute Care Hospitals, 2011	
Major Site of Infection	**Estimated No.**
Pneumonia	157,500
Gastrointestinal Illness	123,100
Urinary Tract Infections	93,300
Primary Bloodstream Infections	71,900
Surgical site infections from any inpatient surgery	157,500
Other types of infections	118,500
Estimated total number of infections in hospitals	**721,800**





Frequency of aerobic bacteria from postoperative wound infection at Ayder Teaching and Referral Hospital, Mekelle, Ethiopia (January to June 2012)

No.	Gram	Microorganism	Frequency N (%
1	G+	*S. aureus*	40 (34.2)
2	G-	*Klebsiella spp.*	29 (24.8)
3	G+	CoNS[a]	18 (15.4)
4	G-	*Proteus spp.*	15 (12.8)
5	G-	*P. aeruginosa*	11 (9.4)
6	G-	*E. coli*	6 (5.1)
7		*Citrobacter spp.*	4 (3.4)
		Total	123 (100)

The frequency of aerobic bacteria from postoperative wound infection at Ayder Teaching and Referral Hospital, Mekelle, Ethiopia (January to June 2012) [45].

Wall [46] teichoic acids are found only in certain Gram-positive bacteria (such as staphylococci, streptococci, lactobacilli, and Bacillus spp); so far, they have not been found in gram-negative organisms. Teichoic acids are polyol phosphate polymers, with either ribitol or glycerol linked by phosphodiester bonds; their structures are illustrated in Figure 2-9. Substituent groups on the polyol chains can include D-alanine (ester linked), N-acetylglucosamine, N-acetylglucosamine, and glucose; the substituent is characteristic for the teichoic acid from a bacterial species and can act as a specific antigenic determinant. Teichoic acids are covalently linked to the peptidoglycan. These highly negatively charged polymers of the bacterial wall can serve as a cation-sequestering [47].

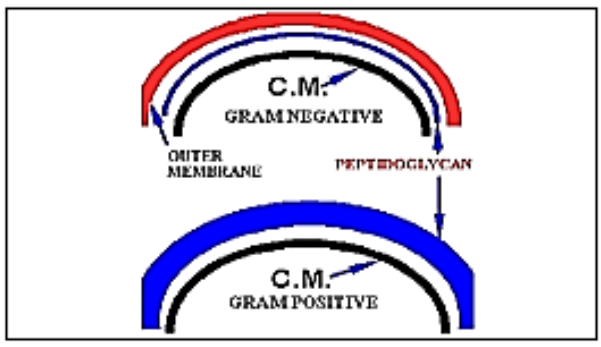
Assessing Compound Permeability in Gram-Negative Bacteria to Enable Rational Drug Design

In Gram-negative bacteria, the envelope is a sophisticated barrier protecting the cell against external toxic compounds. Membrane transporters, e.g., porins or efflux pumps, are main filters regulating the internal accumulation of various hydrophilic molecules. Regarding bacterial susceptibility towards antibacterial agents, membrane permeability is part of the early bacterial defense. The bacterium manages the translocation process, influx and efflux, to control the intracellular concentration of various molecules. Antibiotics and biocides are substrates of these mechanisms and the continuing emergence of multidrug resistant isolates is a growing worldwide health concern. Different strategies could be proposed to bypass the bacterial membrane barrier, comprising influx and efflux mechanisms, in order to restore the activity of antibiotics [48].

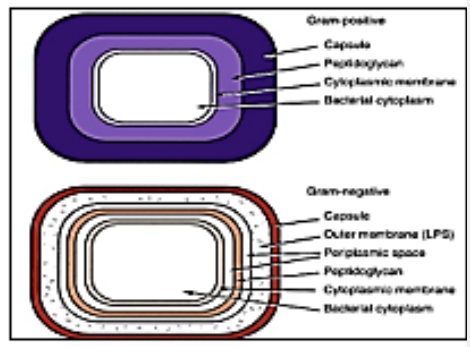
Accessing drug targets in the cytoplasm of Gram-negative pathogens is difficult because of the permeability barrier that limits the accumulation of promising antibiotics to effective levels within the cell.

The ability to measure compound permeation and accumulation in Gram-negative bacteria is potentially an enabling component of structure-activity-relationships which guide rational drug design and optimization. Strategies addressing these challenges and the development of enabling technologies will be discussed.

While antibiotic resistance is increasing rapidly, drug discovery has proven to be extremely difficult. Antibiotic resistance transforms some bacterial infections into deadly medical conditions. A significant challenge in antibiotic discovery is designing potent molecules that enter Gram-negative bacteria and also avoid active efflux mechanisms. Critical analysis in rational drug design has been hindered by the lack of effective analytical tools to analyze the bacterial membrane permeability of small molecules. We design, fabricate, and characterize the nanofluidic device that actively loads more than 200 single bacterial cells in a nanochannel array. We demonstrate a gigaohm seal between the nanochannel walls and the loaded bacteria, restricting small molecule transport to only occur through the bacterial cell envelope. Quantitation of clindamycin translocation through wild-type and efflux-deficient (ΔtolC) Escherichia coli strains via nanofluidic-interfaced liquid chromatography-mass spectrometry shows higher levels of translocation for wild-type E. coli than for an efflux-deficient strain. We believe that the assessment of compound permeability in Gram-negative bacteria via the nanofluidic analysis platform will be an impactful tool for compound permeation and efflux studies in bacteria to assist rational antibiotic design.

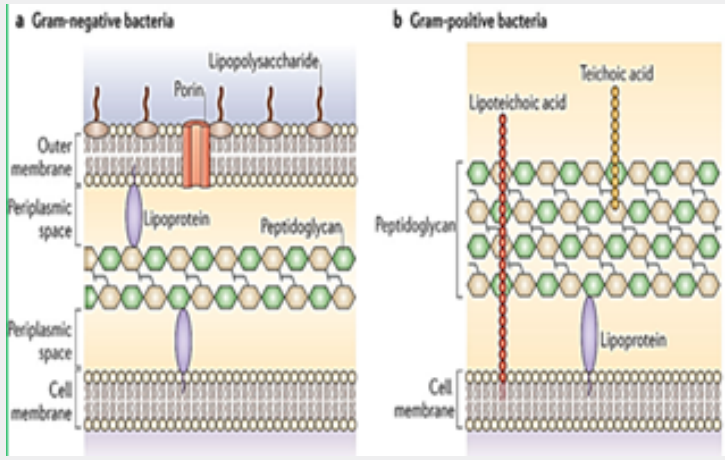


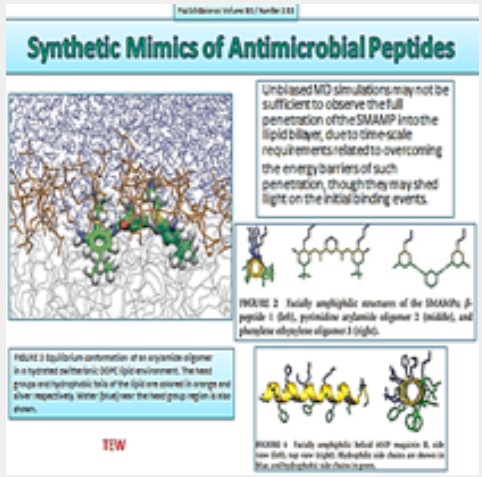
Cartoon illustrating the relative structure of gram negative (top) and gram positive (bottom) cell walls. The major differences lie in the thickness of the rigid peptidoglycan layer and in the presence of an outer membrane in gram negative cells. In gram negative cells the peptidoglycan layer is very thin, being only a few molecules thick, whereas in gram positive



Structures of gram-positive and gram-negative bacterial cell walls.
Note the larger glycan layer in the gram-positive cell wall. The outer membrane of the gram-negative cell wall contains lipopolysaccharide (endotoxin).

The eradication of a Gram-positive cell is demanding the crossing of one cell envelope, the outer membrane Blue in diagram) whereas the Gram-negative cell eradication demands the damaging of both the outer and inner membranes.





The transport of solutes through to the inner or cytoplasmic membrane of bacteria usually takes place through specific active transport systems that require energy [49]. The transport of the agents from the on the outer membrane organized carpet to its destination in the inner cell wall is different regarding the two sorts of bacteria. We presume that the penetration of the molecule into the membrane of the bacteria is different in the two types: Whereas penetration [50] of the peptide mimic to the Gram-negative bacteria needs a great energetic [51] effort due to the crowded situation used by stacking it with membrane proteins and lipo-proteins [52], demanding a high energy track for both types of the surrogates where the slight change in energy demand is too small compared to the overall penetration energy [53].

Recently [54], evidence was provided that killing takes place only when bacterial cell membranes are completely saturated with AMPs. This condition is achieved for all bacteria. However, Since the in Gram-negative bacteria the outer membrane are crowded, packed with various proteins (up to 50% of the total membrane weight [55]), compared of only 15% in the S-layer (surface layer) [46] in gram-positive, it demands more energy for saturation in gram-negative than in gram-positive.

The introduction of the N-CH3 unit to the Penta peptides surrogates stiffens the structure thereby causes an increase in energetic demand for saturation. The fraction of this energy in Gram-negative is smaller than in Gram-positive due to membrane packing composition. It is easier for the N-CH3 to penetrate the outer membrane in gram-positive bacteria and in Gram-negative. Since saturation is achieved in gram-positive with fewer energy demands, the Gram-positive compared to gram-negative are eradicated in preference (through "snorkeling" [56] for example).

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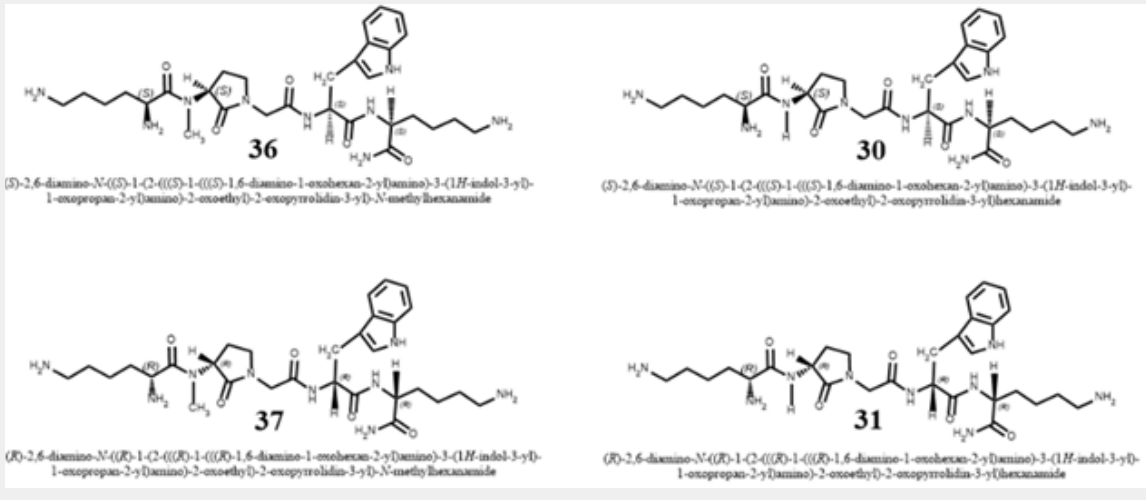
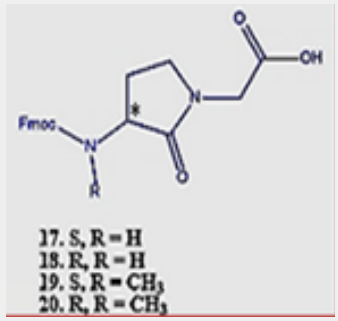


The minimal inhibitory concentration (MIC) refers to the lowest coincentration of the antibacterial that stops visible growth

compound number	COMPOUND SEQUENCE	*MIC (μg/ml)*		HEMOLYSIS %
		S.Aureu	E.Coli	
30	S-Lys-S-Trp-17-S-Lys	200	100	<3
31	R-Lys-R-Trp-18-R-Lys	200	125	<3
36	S-Lys-S-Trp-19-S-Lys	75	125	<3
37	R-Lys-R-Trp-20-R-Lys	75	135	11

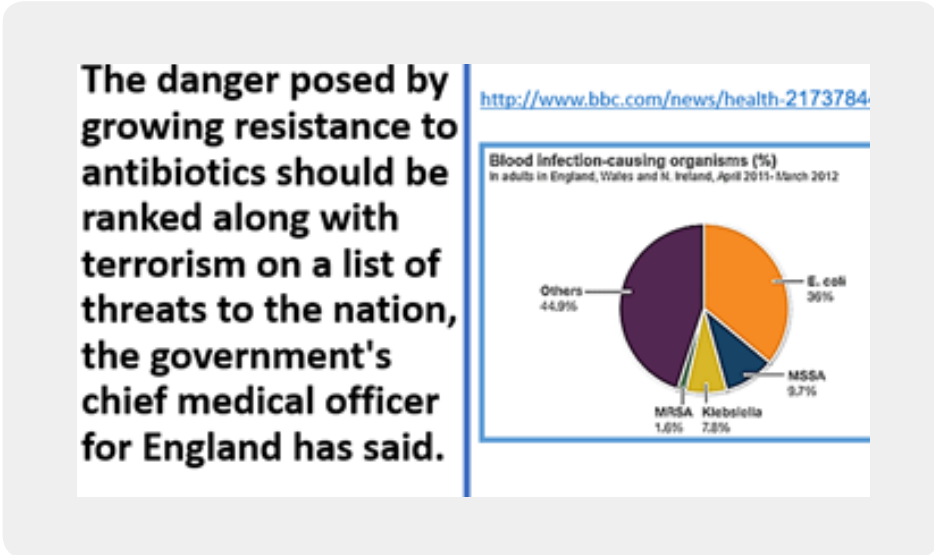


Conclusion from the experiment of N-methylation

The Gram-positive bacteria are easier to penetrate [57] since the outer membrane is poor in membrane proteins. In such circumstance, small energy changes can become significant for the travel of the agent into the outer membrane. The N-CH3 are less flexible and therefore penetrates easier to the membrane. Finally, after the surrogates settle in the inner part of the outer membrane, the Lys ε-amine unit can snorkel [58] out and disintegrate the membranes of both Gram-positive and Gram-negative bacteria. The interactions of an AMP with the membrane cannot be explained by a sequential amino-acid pattern or motif; rather, they originate from a combination of physicochemical and structural features [59] including size, residue composition, overall charge, secondary structure, hydrophobicity and amphiphilic character [60,61].

The interactions of an AMPs surrogates with the membrane cannot be explained only by a particular sequential amino-acid pattern or motif; rather, they originate from a combination of physicochemical and structural features [59] including size, residue composition, overall charge, secondary structure, hydrophobicity and amphiphilic character [60,61]. Also, interactions with the many components that furnish the architecture of the membranes are crucial. From our experiment, we conclude that the venerability of bacteria may depend on small structural variation in the composition of the biocide [8].

Among the gram-positive organisms, methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus faecium (VRE) represent the greatest therapeutic challenges.



New Drugs Needed

Human medicine has reaped the bene fits of antibiotics for the past 80 years, but without urgent action, the utility of these agents will be drastically minimized. To limit the spread of resistance, physicians must use antibiotics judiciously and apply infection-control procedures consistently. These measures alone, however, will not be sufficient. New antibiotics are desperately needed. To address this emerging crisis, the medical community, governments, pharmaceutical companies, and public health agencies must all work together. Only a coordinated and committed response can slow the rising tide of multidrug-resistant bacteria.

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