Chemographic Analysis of Resistance Prune Antibiotics

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Thesis for degree of Bachelor of Science

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HÁSKÓLI ÍSLANDS

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Abstract

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Introduction: It is now becoming evident that pathogenic bacteria once again pose a serious threat to health worldwide as the number of antimicrobial-resistant bacteria continues to grow. Bacteria are living organisms that are able to adapt in order to be better fit to survive, this ability has allowed them to develop different mechanisms to withstand the actions of antibiotics. At least one mechanism of resistance is known against every class of antibiotics and many bacteria are resistant against a number of antibiotics. Bacteria can acquire resistance through modifications of the antimicrobial molecule, prevention to reach the antibiotic target, changes and/or bypass of target sites. The purpose of this study was to find out if there are areas of chemical property space related to a higher risk of resistance development and if the specific properties in these areas can be specified. Also if there is a difference in the properties of antibiotics that are prune to initiate resistance development in gram-positive as compared to gram-negative bacteria.

Methods: Available literature was reviewed in an attempt to gather information about some common antibiotics or families of antibiotics and bacteria that are known to be resistant to them. Principal component prediction (PCA-prediction) was subsequently performed on the antibiotics within ChemGPS-NP, a chemical global positioning system.

Results: A final set of 44 antibiotics where analyzed with ChemGPS-NP and visualized in three of the eight dimensions where the axes represent size, shape, polarizability (PC1), aromaticity and conjugation related properties (PC2) and lipophilicity including polarity and H-bond donor capacity (PC3). From this data four graphs were created, the first one representing the placement of the antibiotics in chemical space, in the second one the antibiotics were labeled based on their sensitivity to resistance development, in the third one they were labeled based on the resistant bacteria being gram-positive or gram-negative and in the fourth one they were divided by their mechanism of action.

Discussion: There are neighborhoods in chemical space which are related to a higher risk of resistance development, as indicated by the antibiotics in this study – most of which seem quite susceptible to resistance development. There also seem to be areas of chemical space more related to resistance of gram-negative bacteria. It is not unlikely that resistance development has a stronger association to the use of antibiotics rather than chemical properties, but antibiotics that have a broad spectrum of activity and few serious side effects have been used extensively and that seems to have contributed to the development of resistance. It does seem that none of the antibiotics studied is immune to resistance development. Chemographics could be a useful tool in the search for novel antibiotics, and I believe that the only way for us to continue to be able to fight off bacteria is to use available antibiotic agents wisely and continue to devote research to find novel agents.

Ágrip

Chemographic Analysis of Resistance Prune Antibiotics

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Inngangur: Það er nú orðið ljóst að bakteríur eru aftur að fara valda verulegri ógn þegar kemur að heilsu fólks á heimsvísu þegar fjöldi baktería sem eru ónæmar gegn verkun sýklalyfja heldur áfram að aukast. Bakteríur eru lifandi verur sem hafa einstaka hæfileika til þess að aðlaga sig að umhverfi sínu til þess að verða betur hæfar til þess að komast af. Þessir eiginleikar hafa gert þeim kleift að þróa ónæmi gegn öllum fjölskyldum sýklalyfja sem eru í notkun og þónokkur fjöldi baktería er ónæmur gegn mörgum fjölskyldum sýklalyfja, svokallaðar fjölónæmar bakteríur. Bakteríur geta öðlast ónæmi með því að breyta sýklalyfinu sjálfu, bakteríur geta komið í veg fyrir að sýklalyfið komist að skotmarki sínu og þær geta breytt skotmarki sýklalyfjanna. Markmið þessarar rannsóknar var að greina hvort það væru einhver svæði í hinum kemíska geimi (e. Chemical Space) sem eru tengd áhættu á myndun ónæmis og hvort hægt væri að tilgreina eiginleika efna sem þar eru. Eins ef það er munur á eiginleikum sýklalyfja sem eru frekar tengd ónæmi hjá gram-jákvæðum eða gram-neikvæðum bakteríum.

Aðferðir: Upplýsingum um nokkur sýklalyf og fjölskyldur af sýklalyfjum og bakteríur sem eru ónæmar gegn þeim var safnað með því að fara í gegnum brot af þeim gögnum sem til er um efnið. Ekki er því um að ræða tæmandi lista. Principal component analysis var síðan framkvæmd á sýklalyfjunum með ChemGPS-NP sem staðsetti þau í átta víddum í hinum kemíska geimi (e. chemical space).

Niðurstöður: Samansafn af 44 sýklalyfjum voru greind með ChemGPS-NP og staðsett í þrjár víddir þar sem ásarnir táknuðu Principal Component 1 (PC1) (stærð, lögun, skautunarhæfni), PC2 (aromaticity, conjugation related properties) og PC3 (fitusækni, skautun, geta til að gefa frá sér vetnisatóm). Búin voru til fjögur gröf. Á því fyrsta mátti sjá staðsetningu sýklalyfjanna í þessum þremur víddum, í því næsta voru sýklalyfin merkt eftir því hversu viðkvæm þau eru fyrir ónæmismyndun, í því þriðja voru sýklalyfin merkt eftir því hvort ónæmu bakteríurnar eru gram-jákvæðar eða gram-neikvæðar og í því fjórða var sýklalyfjunum skipt upp eftir verkun þeirra.

Umræður: Það virðast vera svæði í hinum kemíska geimi sem eru tengd mikilli myndun ónæmis eins og sést á sýklalyfjunum í þessari rannsókn sem flest eru frekar viðkvæm fyrir myndun ónæmis. Það virðast einnig vera svæði sem eru tengd myndun ónæmis hjá gram-neikvæðum bakteríum. Það er ekki ólíklegt að myndun ónæmis hafi sterkari tengsl við notkun sýklalyfja heldur en efnafræðilega eiginleika sýklalyfjanna en þau sýklalyf sem hafa breitt virknisvið og fáar alvarlegar aukaverkanir hafa verið mikið notuð og það líklega ýtt verulega undir ónæmismyndun gegn þeim. Það virðist vera að ekkert sýklalyf komist undan myndun ónæmis. Chemographics gætu verið nytsamlegt tól í leit að nýjum sýklalyfjum og ég held að eina leiðin fyrir okkur að geta varist áfram bakteríum er að nota skynsamlega þau sýklalyf sem til eru og halda áfram að rannsaka leiðir til að finna ný sýklalyf.

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Abbreviations

AME Aminoglycoside modifying enzymes

ATP Adenosine triphosphate

DNA Deoxyribonucleic acid

ED Euclidean distance

MDR Multi drug resistant

MRSA Methicillin-resistant *Staphylococcus aureus*

PABA Para-aminobenzoic acid

PC Principal component

PCA Principal component analysis

PBP Penicillin binding proteins

RNA Ribonucleic acid

1. Introduction

One of the most pressing issues that we are faced with currently concernig public health is the emergence of antibiotic resistant bacteria. It is very alarming that decades after the introduction of antibiotics, that infections are again becoming a threat. This crisis of antibiotic resistance has been linked to the misuse and overuse of available antibiotics. The lack of new drug development in the last years is also believed to play a vital role (1). It seems very important to both gain a better undarstanding of current mechanisms of resistance that bacteria possess but also to analyze the current antibiotics available in order to facilitate in the development of new agents.

Bacteria are unicellular, microscopic living organisms. When observed under the microscope they can be rod shaped, ball shaped or spiral. Most bacteria are not pathogenic, rather necessary for normal physiology, aid in food preperation and digestion, provide vitamins to the body and so forth. There are however bacteria of different types that are capable of producing and releasing chemicals and toxins and can thereby cause bacterial infections (2). Microbial fossils have been found that are older than oldest known eukaryotes and current evidence suggests that bacteria were present before the oxygen concentrations in the atmosphere and surface oceans first rose, which facilitated the emergence of eukaryotes. There are 10^{30} bacteria estimated to populate the Earth. On average, they divide every 1 to 10 days, which enables them to relatively rapidly acquire new phenotypic traits. They can be caused by new genes, new combinations of genetic material, new regulatory circuits, and new metabolic networks (3). Bacteria strive to adapt to their environment in order to be fit to survive and one aspect of this adaptation is the development of distinctive mechanisms of resistance to all antibiotical agents available (4).

Infectious diseases were the leading cause of death worldwide at the beginning of the 20th century. It was due to the success of vaccination and the discovery of antibiotics that this changed (5). One of the hallmarks of 20th century medicine was the introduction of antibiotics for the treatment of infectious diseases (6, 7). Antibiotics are used for both preventive and curative measures, are essential in fighting infectious diseases and also enable modern medical procedures, such as surgery and chemotherapy, that would otherwise be unthinkable (8-10).

Antibiotics is a term used to describe antibacterial agents that suppress the growth of microorganisms that may lead to their destruction (11). Antibiotics can be either bacteriostatic, they inhibit the growth of microorganisms, or bacteriocidal i.e. they kill the microorganism (12). Antibiotics can be substances that are produced by various microorganisms like bacteria, fungi, actinomycetes, or synthetic and semisynthetic substances (11).

It was in 1929 that Alexander Fleming reported his observation that penicillium mould had grown on culture medium and produced a substance that caused the death of certain bacteria. It wasn't until 1940 that the isolation of this active substance was achieved (7).

The first available systemic antibiotics, sulfonamides and penicillin, were not accessible for the general public immediately. They were scarce and very expensive and initially almost exclusively used by the military during World War II. Manufacturing processes were simplified as more antibiotics were discovered and by that the public gained more access and antibiotic use became widespread (4). The noteworthy success of penicillin in treatment for bacterial infections rapidly created interest of researchers and different laboratories began intensive search for other antibiotics produced by moulds isolated from soil samples. This search quickly yielded results and since this time hundreds of antibiotics have been identified (11).

1.1 Gram-positive and Gram-negative bacteria

One of the primary ways to distinguish bacteria is the gram staining. It is a method that distinguishes bacteria based on the composition of their cell walls. Gram-positive bacteria, which turn purple, trap the stain in the peptidoglycan layer, which is a thick, cross-linked, meshlike structure. Gram-negative bacteria have a thin peptidoglycan layer that does not retain the crystal violet stain, they are counterstained with safranin and turn red (13). Mycobacteria cannot be classified with the gram stain because they have a waxy outer shell. Neither can Mycoplasmas which contain no peptidoglycan. Mycobacteria have a peptidoglycan layer that is intertwined with and covalently attached to an arabinogalactan polymer and surrounded by a waxlike lipid coat of mycolic acid, cord factor, wax D, and sulfolipids. The coat is the cause of mycobacteria's virulence and it is antiphagocytic (13).

Gram-positive bacteria surround themselves with a thick cell wall. It is essential to cell survival and growth, and is a major target of antibiotics. The main component of the cell wall is the peptidoglycan (14). Peptidoglycan, also known as murein, is found on the outside of the cytoplasmic membrane of almost all bacteria. It is not found in Mycoplasmas, Planctomyces and Rickettsia. This layer of peptidoglycan preserves cell integrity by withstanding the turgor. It also serves as a scaffold for anchoring other cell envelope components. Any degradation or inhibition of its biosynthesis during cell growth will result in the cell's lysis, therefore this layer has been a target of antibiotics (15). The peptidoglycan layer contains pores to allow diffusion of metabolites to the plasma membrane. The cell wall of gram-positive bacteria may also contain proteins, teichoic and lipoteichoic acids, and complex polysaccharides. The peptidoglycan can be degraded by lysozymes, they cleave the glycan backbone which causes the bacteria to succumb to the large osmotic pressure difference across the cytoplasmic membrane, and lyse (13).

Gram-negative bacteria have a more complex cell wall. They have an outer cell layer that consist of an outer membrane, a thin layer of peptidoglycan and a periplasmic space ostensibly situated between these two. The outer membrane is a bilayer structure made out of lipopolysaccharide and phospoholipids, with proteins embedded in the membrane (16). Lipopolysaccharides are composed of three covalently-linked regions, the lipid A, the central core polysaccharide, and the outer O-polysaccharide side chain. This forms a highly controlled crystalline structure that has low fluidity. Lipid A is a phosphorylated glucosamine disacharride unit and it has fatty acids attached to it. It is hydrophobic and the structure is quite similar between Gram-negative bacteria. The core polysaccharide is a complex oligosaccharide linked to the lipid A by 3-deoxy-D-manno-2-octulosonate. The O-side chain is formed of numerous repeating units of oligosaccharides. It is very diverse between bacterial species and is used for identification of subspecies. Lipopolysaccharides are non-covalently cross-linked. They are kept in position at the outer membrane surface by divalent cations (16). The strong interactions between the lipopolysaccharides in the outer membrane with the enrichment of fully saturated phospholipids in the inner leaflet of the outer membrane, greatly reduces the fluidity of the outer membrane (17).

The periplasmic space contains components of transport systems for iron, proteins, sugars and other metabolites, and a variety of hydrolytic enzymes important for the breakdown of macromolecules for metabolism. The gram-negative cell wall does neither contain teichoic nor lipoteichoic acids like the gram-positive cell wall does. The outer membrane contains proteins that traverse the entire lipid bilayer, a group of these proteins is known as porins. These porins form pores that enable the diffusion of small, hydrophilic molecules, like antimicrobials (13). Porins are hydrophilic channels and they can be either non-specific and allow general diffusion or they can be substrate specific and mediate the entry of particular solutes (16). Porins take part in the exchange of nutrients across the outer membrane of gram-negative bacteria and they are also involved in pathogenesis (17).

Gram-negative bacteria have an extra layer of protection provided by the outer membrane without compromising the exchange of material required for sustaining life. It serves as a selective barrier with its pore-forming proteins. The permeability properties of this barrier has a great impact on the susceptibility of the microorganism to antibiotics, since most antibiotics target intracellular processes (18). The outer membrane is the first line of defense against toxic compounds and plays a big role in the impermeability of gram-negative bacteria which are impermeable to large, charged molecules (17).

The permeability properties of the outer membrane of gram-negative bacteria have a great impact on the susceptibility of the microorganism to antibiotics, which are to great extent intracellularly targeted. The emergence of multidrug resistant strains of gram-negative clinical pathogens, like *Escherichia coli, Salmonella* spp*., Klebsiella* spp*., Enterobacter* spp*., Campylobacter* spp*., Acinetobacter* spp*.* and *Pseudomonas* spp*.* is in some part due to modifications in the lipid or protein composition of the outer membrane barrier. Resistance generally emerges when a decrease in the rate of entry of these compounds is generated (17).

When it comes to antibiotics gaining intracellular access to gram-negative bacteria, aminoglycosides, macrolides, rifamycins, novobiocin and fusidic acid are all hydrophobic antibiotics that are able to permeate through the outer membrane bilayer (18).

β-lactams, tetracyclines, chloramphenicol and fluoroquinolones are all small hydrophilic antibiotics that are able to go through the porins to enter the bacterial cell. Two major porin-based mechanisms for antibiotic resistances have been reported; alterations of outer membrane profiles and altered function due to specific mutations reducing permeability (18).

1.2 Antibiotics

There are more than 15 classes of antibiotics available. They all target essential physiological or metabolic functions of the bacterial cell. These mechanisms of action include inhibition of cell wall synthesis (e.g. β-lactams), inhibition of protein synthesis (e.g. tetracyclines, macrolides), inhibition of DNA synthesis (e.g. fluoroquinolones), competitive inhibition of folic acid synthesis (e.g. sulfonamides, trimethoprim), inhibition of RNA synthesis (e.g. rifampin), cell membrane disorganizing (e.g. polymyxins), and other mechanisms (e.g. metronidazole) (4, 19).

1.2.1 β-lactams

β-lactams are a major class of antibiotics and according to the European Center for Disease Control and Prevention they are the most frequently used antibiotic class, both in the community and hospital sector in all EU/EEA countries (20).

β-lactams can be divided into penicillins and non-penicillins. The group of penicillins contains two naturally occuring penicillins, penicillin G and penicillin V. Other members of the group, cloxacillin, dicloxacillin, flucloxacillin, nafcillin, amoxicillin, ampicillin, piperacillin, and ticarcillin are synthetic penicillins. The non-penicillin group contains cephalosporins, carbapenems, and monobactams (21).

Penicillins, as well as all β-lactams, kill susceptible bacteria by inhibiting the transpeptidase that cross-links cell wall peptidoglycan (22). The naturally occuring penicillins have limited activity against gram-negative bacteria and are readily hydrolyzed by β-lactamases. The semi synthetic penicillins have enhanced antimicrobial properties like activity against gram-negative bacteria (11).

Less then a year after the introduction of penicillin, four penicillin-resistant strains of staphylococci were isolated from patients. This did not become a serious problem immediately as this initial resistance to penicillin could be overcome with an elevated dosage of the drug. During the next few years the majority of hospital isolates of *Staphylococcus aureus* were resistant to penicillin G and six other common antimicrobials including streptomycin, tetracycline and erythromycin. This was the first encounter of multiple-resistant pathogens. This created the incentive to search for novel antimicrobials and for a short period of time, the introduction of new antimicrobials managed to keep ahead in the race against antibiotic resistance. This was done by making slight changes in the structure of the antibiotics. The semi-synthetic methicillin was able to withstand the action of β-lactamases and could temporarily replace penicillin where it had become useless. Ampicillin, another semi-synthetic β-lactam, was able to inhibit a wide range of gram-negative organisms (5).

In the 1980s almost all effort was taken away from developing new agents but bacteria kept on developing resistance (5). During the past 20 years only two new classes of antibiotics, lipopeptides and oxazolidinones, have been introduced. Both these classes are effective against gram-positive bacteria. The last antibiotics discovered against gram-negative bacteria were the quinolones in 1962 (23).

Cephalosporins were first isolated from fungi. They have the same mechanism of action as penicillins. Modern cephalosporins are variations on the prototypic molecule produced by *Cephalosporin acremonium.* The variations are in side chain substitutions at R1 (C7) and R2 (C3) of the cephalosporin nucleus. Cephalosporins are classified into generation based on their spectrum of activity. First generation cephalosporins are active against aerobic gram-positive cocci including *Staphylococcus aureus.* Second generation agents are more active against gram-negative bacteria and third generation cephalosporins even more. Fourth generation cephalosporins have a broad spectrum of activity against both gram-negative and gram-positive organisms (24). Resistance to cephalosporins is somewhat due to the spread of CTX-M type extended-spectrum β-lactamases (25).

Carbapenems function as other β-lactams do and they are active against many aerobic and anaerobic gram-positive and gram-negative organisms. Resistance to them has been increasing on account of β-lactamases that are able to hydrolyze them (21). The carbapenem backbone consists of a carbon at the 1st position, substituents at C-2, a C-6 ethoxy, and sp^2 – hybridized C-3. The first carbapenem, Imipenem, was a more stable form of thienamycin, which was a 4:5 fused ring lactam of penicillins with a double bond between C-2 and C-3 with the substitution of carbon for sulfur at C-1 and hydroxyethyl side chain. Thienamycin displayed potent broad-spectrum antibacterial and β-lactamase inhibitory activity. Imipenem has high affinity for PBPs and is stable against β-lactamases, it is however susceptible to deactivation by dehydropeptidase I (DHP-I), found in the human renal brush border so coadministration with an inhibitor is necessary. As a class of β-lactams, carbapenems do not diffuse easily through the bacterial cell wall and generally enter gram-negative bacteria through porins (26).

Some species have intrinsic resistance to carbapenems although it is not common among clinically important bacteria. An example is *Stenotrophomonas maltophilia* which possesses the endogenous metallo-beta-lactamase L1. Gram-positive bacteria can acquire resistance to carbapenem and other β-lactams through mutation-derived changes of their penicillin-binding proteins (PBP). Gram-negative bacteria have further mechanisms. Some species can prevent carbapenems from reaching their PBP by decreasing the permeability of their outer membrane, e.g. by changing their porins or decreasing their expression. Another mechanism is the active expulsion of carbapenems out of the periplasmic space by efflux pump systems. The overexpression of efflux pumps that expel carbapenems may lead to carbapenem resistance associated with multidrug resistance, since many efflux pumps are not substrate specific. Certain bacteria are also able to produce β-lactamases that can inactivate carbapenems, along with other β-lactams (27).

Monobactams, unlike most other β-lactams, have a β-lactam ring that is not fused to another ring. The most common monobactam, aztreonam, has a large R group attached to the β-lactam ring that inhibits its hydrolysis by Toho-1 β-lactamase (28). Monobactams are resistant to most β-lactamases but they are only active against gram-negative aerobic bacilli like *Pseudomonas* species, *Neisseria meningitidis* and *Haemophilus influenzae* (21).

1.2.2 Quinolones

Quinolones target the bacterial type II topoisomerases, gyrase and topoisomerase IV. These enzymes play essential roles in most nucleic acid processes, help control levels of DNA under-winding and over-winding, and remove knots and tangles from the bacterial chromosome. Quinolones bind non-covalently at the enzyme-DNA interface in the cleavage-ligation active site. Nalidixic acid was the first quinolone introduced, used in treatment of urinary tract infections caused by enteric bacteria. Followed by the introduction of fluoroquinolones, a group of antibiotics that inhibit the activities of DNA gyrase which in turn inhibits bacterial DNA replication and transcription causing cell death (29). Fluoroquinolones have a broad-spectrum of activity against infections of the urinary tract, respiratory tract, skin and soft tissues, and sexually transmitted diseases. Both gram-negative and gram-positive bacteria are susceptible (21).

Quinolone resistance is becoming a prevalent clinical issue, it is most often associated with specific mutations in gyrase and/or topoisomerase IV. Plasmids that carry quinolone resistance genes have been identified and they generally cause low-level resistance and can be transmitted horizontally and vertically between bacteria. These genes are known to cause decreased binding of gyrase and topoisomerase IV to DNA, they also bind to gyrase and topoisomerase IV and inhibit quinolones from entering cleavage complexes formed by enzymes. These plasmids can also code for efflux pumps. There are also chromosome-encoded efflux pumps known, as well as chromosomally-encoded downregulation of porin channels, necessary to enter gram-negative bacteria (30).

1.2.3 Macrolides

Macrolides are a family of antibiotics produced by members of the *Streptomyces* species and some bacteria such as *Arthrobacter* spp. Their antibacterial mechanism involves binding to the 50S ribosomal subunit. That causes inhibition of the biosynthesis on ribosomal protein level. They are effective against both gram-negative and gram-positive bacteria. Their construction is based on the large macrocyclic lacton ring, whose activity is based on the presence of macrolide ring containing one or more deoxy sugar (31).

Bacterial resistance to macrolides has been spreading and two common mechanisms in bacterial pathogens include modification of the bacterial ribosome, either by methylation or mutation, and extrusion of the drugs from the bacterial cell by an efflux pump. (32)

1.2.4 Tetracycline

Tetracycline is a family of antibiotics that inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor site. They have a broad-spectrum of activity, including many gram-positive and gram-negative bacteria. Tetracycline structure is based on a linear fused tetracyclic nucleus to which a variety of functional groups are attached. Tetracyclines enter gram-negative bacteria through the OmpF and OmpC porin channels as positively charged cation-tetracycline coordination complexes. They accumulate in the periplasm, the metal ion-tetracycline complex dissociates, and the uncharged tetracycline is able to diffuse through the lipid bilayer of the inner membrane. The favorable antimicrobial properties and lack of serious side effects have caused tetracycline to be very widely used, which has contributed to the widespread emergence of resistant strains. Many resistance genes are known and they encode for efflux-pumps, ribosomal protection proteins and enzymatic mechanisms of resistance (33).

1.2.5 Sulfonamides

Sulfonamides compete with para-aminobenzoic acid (PABA) for the enzyme dihydropteroate synthetase. PABA is an essential precursor in the synthesis of folic acid, required for the synthesis of DNA and RNA in bacteria. Many sulfonamides have been developed since their discovery in the 1930s but their importance has declined in the face of growing resistance (21). Sulfonamides tend to persist in the environment and that contributes to bacteria becoming resistant to them (34).

1.2.6 Trimethoprim

Trimethoprim is bacteriostatic and inhibits bacterial growth by blocking the production of tetrahydrofolate, the active form of folic acid in susceptible pathogens. Bacterial cell walls are impermeable to exogenous folate, and are therefore dependent on this endogenous production (35). Trimethoprim is active against most common bacterial pathogens as well as protozoa, and is used to treat various urinary, pulmonary and other infections (21). Several mechanisms of bacterial resistance have been described. Intrinsic resistance can occur when cell wall permeability factors exclude the drug from binding to dihydrofolate reductase, as occurs with *Pseudomonas aeruginosa*. The presence of a dihydrofolate reductase less susceptible to trimethoprim inhibition has also been noted in e.g. *Neisseria meningitidis.* Acquired resistance mainly stems from a chromosomal mutation that results in the production of a dihydrofolate reductase which is less vulnerable to trimethoprim inhibition (35).

1.2.7 Rifampin

Rifampin inhibits DNA-dependent RNA polymerase in prokaryotic cells by binding to it. It is one of the most active antituberculosis agents known, and is also effective against leprosy and most gram-positive bacteria as well as some gram-negative species. Resistance can develop rapidly in a one-step process in which a chromosomal mutation changes its target site on microbial DNA-dependent RNA polymerase and therefore rifampin should never be used in monotherapy (21).

1.2.8 Chloramphenicol

Chloramphenicol inhibits bacterial protein synthesis in susceptible organisms by binding reversibly to the 50S subunit of the 70S ribosome which in turn inhibits the mitochondrial enzyme peptidyl transferase, which is necessary for peptide bond formation. Chloramphenicol has a broad spectrum of bacteriostatic activity against most gram-positive, gram-negative, aerobic, and anaerobic pathogens (36).

1.2.9 Aminoglycosides

Aminoglycosides are a family of antibiotics, highly potent and broad-spectrum. They act by impairing bacterial protein synthesis through binding to prokaryotic ribosomes. They enter gram-negative bacteria by a self-promoted uptake process involving the drug-induced disruption of Mg^{2+} bridges between adjacent lipopolysaccharide molecules. Aminoglycosides are unlikely to be able to penetrate through porin channels because of their large size. Transport across the inner membrane is dependent upon electron transport. The resistance mechanisms that primarily affect aminoglycosides are a decreased uptake and/or accumulation of the drug in bacteria and the bacterial expression of enzymes which modify the antibiotic and thereby inactivate it (37).

1.2.10 Lincosamide

The lincosamides are a small but important class of antibiotics. They are produced by several *Streptomyces* species. Their chemical structure consists of an amino acid moiety and a sugar moiety. They are mostly active against gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus,* and against selected gram-negative anaerobes and protozoa. Clindamycin is the only semisynthetic lincosamide that is in clinical use (38).

1.2.11 Fusidic Acid

Fusidic acid belongs to the fusidanes family of antibiotics. The molecule has a steroid-like structure but does not possess any steroid activity. Its antibacterial activity is specifically potent against common skin pathogens, including *Staphylococcus aureus* (39). Fusidic acid inhibits protein synthesis by preventing the turnover of elongation factor G from the ribosome. The utility of fusidic acid depends on the rate of resistance, which differs between countries. Resistance usually occurs through point mutation(s) in the chromosomal gene encoding EF-G which typically confers high-level resistance (40).

1.2.12 Novobiocin

Novobiocin is produced by *Streptomyces spheroides* and *Streptomyces niveus* strains. It is bacteriostatic through inhibiting the function of ATP-dependent gyrase (41). Novobiocin has much greater effect against gram-positive bacteria since many gram-negative bacteria have either intrinsic resistance because of their double outer membrane or possess efflux pumps that prevent the drug from reaching its minimal inhibitory concentration within the bacteria (42).

1.2.13 Glycylcycline

Tigecycline is the first drug in the glycylcycline class of antibiotics. It has a broad spectrum of activity, including activity against drug-resistant gram-positive organisms. Tigecycline binds to the bacterial 30S ribosome, blocking the entry of transfer RNA which inhibits protein synthesis by halting the incorporation of amino acids into peptide chains and thus limits bacterial growth (43). Resistance to tigecycline can be mediated by efflux pumps (44).

1.2.14 Vancomycin

Vancomycin is a glycopeptide antimicrobial and inhibits bacterial cell wall biosynthesis by binding to the D-alanyl-D-alanine terminus of lipid-attached peptidoglycan precursors, blocking formation of the mature peptidoglycan that gives the cell wall its rigidity (45). Vancomycin was first isolated from *Amycolatopsis orientalis.* It has a broad spectrum of activity against gram-positive bacteria and was fully active until recently to methicillin-resistant *Staphylococcus aureus.* Recently cases of vancomycin-resistant enterococci have raised concerns over widespread vancomycin use (46). The first clinical isolates of vancomycin-resistant enterococcal strains emerged in the mid 1980s and occured because of the replacement of the D-Ala-D-Ala dipeptide terminus of cell wall peptidoglycan precursors by D-alanyl-D-lactate, which greatly reduces the affinity of vancomycin binding (45).

1.3 Antimicrobial resistance

Antimicrobial resistance can be thought of as the inability of a given antibiotic to reach its target at a sufficient concentration to inhibit the target's activity (47). Emergence of antibiotic resistance has been a constant threat since the beginning of the antibiotic era (48). However resistance to antibiotics was largely a problem of hospitalized patients during the first 25 years following the introduction of antibiotics. These bacteria were not only resistant but also able to survive in the hospital environment and then infect the most vulnerable population (4). Bacteria were also typically only resistant to a single antibiotic or class of antibiotics. This changed during the late 1950s with the phenomenon of multi-resistance which was initiallly observed in enterobacteria. Multidrug-resistance has been spreading ever since and the situation has become so dire that some microorganisms are resistant to most antibacterial agents available (49).

The World Health Organization has named antibiotic resistance as one of the three most important public health threats of the 21st century (10). There is ancient origin and widespread presence of diverse resistance genes and the modern evolution of resistance that has led to many resistant bacteria that have genotypes and phenotypes resisting the actions of antibiotics. This phenomenon is caused by the natural selection process in microorganisms and promotion by human activities during the last 70 years of the antibiotic era (50).

At least one mechanism of resistance is described for each class of commonly used antibiotics and many bacterial species show multi- or pan-resistant phenotypes (6). Organisms that have been recognized as a major threat have been called ESKAPEE and include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp. and *Escherichia coli* (51). Bacteria can acquire resistance through several biochemical routes. It can be through modifications of the antimicrobial molecule, prevention to reach the antibiotic target, changes and/or bypass of target sites, and resistance due to global cell adaptive processes (10). Frequently, bacteria can possess several elements of resistance with different mechanisms that impact a single class of antibiotics (8).

1.3.1 Modifications of the antimicrobial molecule

Modifications of the antimicrobial molecule is one of the most successful ways that bacteria have to fight the presence of antibiotics. This can be accomplished by producing enzymes that inactivate the antibiotic by adding specific chemical moieties to the compound or that destroy the molecule itself. This makes the antibiotic unable to interact with its target, the bacteria (10).

Both gram-positive and gram-negative bacteria are known to be able to produce enzymes that chemically change the antimicrobial. An example of this mechanism are aminoglycoside modifying enzymes (AME) that covalently modify the hydroxyl or amino groups of the aminoglycoside molecule and render it useless. AME are often plasmid encoded but also associated with transposable elements. Plasmid exchange and dissemination of transposons spreads the drug-resistant phenotype both within a given species and among a large variety of bacterial species (37).

Resistance to β-lactam antibiotics is mainly due to β-lactamases, enzymes that destroy the amide bond of the β-lactam ring which makes the antibiotic ineffective. It was in the early 1940s that these enzymes were first described, one year before penicillin was introduced to the market. Clinically, it was observed that some infections caused by *Staphylococcus aureus* could not be treated with penicillin. The mechanism of resistance was a plasmid-encoded penicillinase, readily transmitted between strains of *S. aureus*. To overcome this problem, β-lactam compounds with a wider spectrum of activity and less susceptibility to penicillinases were manufactured, for example ampicillin. It was only shortly after their introduction that a new plasmid-encoded β-lactamase was found among gram-negative bacteria that were able to hydrolyze ampicillin. Throughout, the development of newer generations of β-lactams has been followed systematically by the appearance of enzymes capable of destroying them and to date more than 1000 β-lactamases have been described (5, 10).

1.3.2 Preventing the antibiotic from reaching its target

Another mechanism of resisting antimicrobial activity is preventing the antibiotic from reaching its target**.** Most antibiotic targets are intracellular or periplasmic so they have to be able to penetrate the bacterial cell wall to reach their target. Antibiotics that are hydrophilic, like tetracyclines, β-lactams and some fluoroquinolones, are especially vulnerable to changes in permeability of the outer membrane of gram-negative bacteria, since they cannot diffuse through but often use diffusion channels, known as porins, to penetrate. Therefore many bacteria have a natural barrier to antibiotics, like all gram-negative bacteria can resist the actions of vancomycin, a glycopeptide, which is not able to penetrate the outer membrane. Porin-mediated resistance can be achieved by a shift in the type of porins expressed, a change in the level of porin expression or impairment of the porin function. These types of changes generally result in low-level resistance. An example of porin-mediated resistance is the alteration of the porins of *Pseudomonas aeruginosa* that take up certain amino acids and carbapenems. Mutations in the gene encoding for these types of porins has resulted in *Pseudomonas aeruginosa* strains resistant to carbapenem (10).

Carbapenems have the broadest spectrum of activity out of all the β-lactams and like other β-lactams do not diffuse through the bacterial cell wall. They enter gram-negative bacteria through porins. They exert their antimicrobial effects by permanently acylating the PBP, the enzymes that catalyze the formation of peptidoglycan in the cell wall of bacteria. An important characteristic of carbapenems is the ability to bind to multiple different PBP. The binding to PBP causes the peptidoglycan of the bacterial cell wall to weaken and the cell bursts due to osmotic pressure (26). Carbapenems have a high resistance to the action of β-lactamases and have been very effective against *Pseudomonas aeruginosa* which has little susceptibility to most antibiotics. This low susceptibility is caused by decreased outer membrane permeability and an efficient drug efflux system. *P. aeruginosa* acquires its nutrients through dedicated specific porins, so the outer membrane has very few general diffusion porins that antibiotics could use to enter the bacteria. Carbapenems have been able to enter through specific porins that are used for the uptake of basic amino acids and peptides, which share structural similarity with the carbapenem molecule (18).

1.3.3 Efflux pumps

Efflux pumps are a very effective method for bacteria to pump out antibiotics and thereby prevent them from reaching their target. Bacterial multidrug efflux pumps are present in all microorganisms and are, with a few exceptions, chromosomally encoded and present a conserved organization both at the genetic and protein levels (52). Efflux pump expression is usually down regulated. Their expression can be increased by means of mutations in the elements regulating their expression. It can also be triggered in the presence of their effectors or under some specific growing conditions. Efflux pumps can therefore cause antibiotic resistance at three different levels. First of all they can be involved in intrinsic resistance when they are preset at a basal level of condition. Second of all they can contribute to acquired resistance when mutants achieving high-level of expression of the efflux pumps are selected. At last they can contribute to transient, non-inheritable, phenotypic resistance when bacteria are growing in the presence of an efflux pump effector or the growing conditions trigger their overexpression (53).

Many classes of efflux pumps have been described in both gram-negative and gram-positive bacteria. They can be either substrate-specific and pump out a particular antibiotic or have broad spectrum specificity as is the case with multidrug-resistant bacteria. Many antimicrobial classes are affected by efflux pumps, including protein synthesis inhibitors, fluoroquinolones, β-lactams, carbapenems and polymyxins (10).

Efflux pumps are divided into five families based on their structure; the resistance nodulation division (RND), the small multidrug resistance (SMR), the multi antimicrobial extrusion (MATE), the major facilitator superfamily (MFS), and the ATP-binding cassette (ABC) superfamilies. Mainly in the case of gram-positive bacteria, the efflux pumps can work independently of any other protein. However with gram-negative organisms, the efflux pumps form tripartite complexes capable to traverse both bacterial membranes. These complexes are made out of the inner-membrane efflux pump, a membrane fusion protein and an outer membrane protein (2, 53).

Bacterial multidrug efflux pump systems of the MFS and RND superfamilies represent common mechanisms for bacterial resistance to antimicrobial agents (2). Active efflux of antibiotics in bacteria contributes greatly to both intrinsic and acquired multidrug resistance of clinical relevance. Many multidrug efflux pumps have been characterized in numerous bacterial species that are highly clinically relevant and of public health concern, e.g. in the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp) (50).

1.3.4 Changes in target sites

Changes in target sites is another common method for bacteria to acquire antimicrobial resistance. This can be achieved by target protection or modification of the target site. Tetracycline, fluoroquinolones and fusidic acid are known to be vulnerable to actions of target protection. Modifications to the target site affect almost all families of antimicrobial compounds. These changes to the target can be point mutations in the genes encoding the target site, enzymatic alterations of the binding site, or replacement or bypass of the original target. All of these changes result in a decrease in the affinity of the antibiotic for the target site (10).

1.3.5 Global cell adaptive changes

Bacteria have, through years of evolution, developed advanced mechanisms in order to withstand environmental stressors and pressures in order to survive in hostile environments, like the human body. Bacterial organisms are under attack from the host‘s immune system so it is crucial for them to adapt if they are to survive. Bacterial pathogens have developed complex mechanisms in order to maintain necessary cellular processes like cell wall synthesis and membrane homeostasis. Some bacteria have utilized these mechanisms in order to withstand the action of certain antibiotics, the most clinically relevant examples are resistance against daptomycin and vancomycin (10).

1.4 Chemical space

Chemical space is a concept used to describe multidimensional descriptor space, a region defined by the descriptors chosen to describe a set of chemicals. Chemical space is essentially infinite and contains all possible molecules. The charting of compounds is useful for the discovery of new active compounds for future therapies since it provides an efficient mapping device for selection of high-probability hits and prediction of their properties and activities (54).

Thousands of different molecular descriptors exist, and any combination of these descriptors can be used to produce a property space with tens to hundreds of different dimensions, from which a chemical space map can be derived (55). ChemGPS is a chemical global positioning system based on medicinal chemistry drug-like molecules and ChemGPS-NP has been adapted to natural products and attempts to better represent the entire biologically relevant chemical space. ChemGPS-NP places structures in chemical space via principal component analysis (PCA) score prediction. There are 35 descriptors that contribute to the PCA score in ChemCPS-NP and include aspects of size, shape, lipophilicity, polarity, polarizability, flexibility, rigidity, and hydrogen bond capacity (54). Molecules close to each other in the ChemGPS-NP space present similar physicochemical profiles (56).

1.5 This Study

With the present issues regarding antibiotic resistance and the growing concern that the “age of antibiotics” may have passed, this study aims to review and chemographically analyse some known antibiotics in an attempt to probe their resilience against resistance mechanisms. Are there places in Chemical Space that are related to a higher risk of resistance development? Can their physical chemical properties be specified? Is there a difference in the properties of antibiotics that are connected to resistance development of gram-positive and gram-negative bacteria?

2. Methods

ChemGPS-NP was designed to represent the entire biologically relevant chemical space. The space map coordinates are *t*-scores from principal component analysis (PCA) that uses a subset of 35 descriptors. These descriptors were chosen by certain inclusion criteria that stated that a descriptor should:

1. Have a comprehensible physical meaning.
2. Reveal loading in at least one component of the principal component analysis model when terminated with statistical cross-validation criterion.
3. Be able to distinguish between the compounds.
4. Encode relevant aspects of molecular complexity.
5. Describe at least any of the following intuitively important molecular properties: lipophilicity, polarity, size/shape, hydrogen bond capacity, polarizability, flexibility, and rigidity.

The final set of descriptors are listed in Table 1 (54).

Principal component analysis is a widely used multivariate statistical technique. It analyzes a data table representing observations described by several dependent variables and aims to extract the important information from the data and express it as a set of new orthogonal variables called principal components (57). PCA is a tool that can be used to visualize the similarities between the biological samples and to filter noise (58).

PCA can be used to map out certain compounds and it is expressed in terms of scores and loadings, where the scores are related to the objects and the loadings are related to the variables. The results can be viewed in score and loading plots, where the relative distance between compounds in chemical space is the measure of their similarity with respect to the particular set of descriptors considered (54).

ChemGPS-NP has eight principal components (PC) that can be considered as dimension. They describe physico-chemical properties for a reference set of compounds (54). PC1 represents size, shape, polarizability, PC2 represents aromaticity and conjugation related properties, PC3 represents lipophilicity, polarity and H-bond donor capacity, PC4 represents flexibility and rigidity, PC5 represents electronegativity and number of nitrogens, halogens and amides, PC6 represents number of rings, rotational bonds and amids and hydroxyl groups, PC7 represents number of double-bonds, oxygen and nitrogen atoms and PC8 represents aromatic and aliphatic hydroxyl groups, molecular saturation and Lipinski alert index (56).

Compounds that have similar physicochemical properties reside close to each other in the chemical property space described by the ChemGPS-NP coordinates. Therefore, the metrics that define the closeness of compounds in ChemGPS-NP could quantitatively provide a measure of molecular similarity. The Euclidean Distance (ED) based on the eight ChemGPS-NP coordinates is used to measure the relative distance between molecules. Low ED reveals proximity between molecules in the ChemGPS-NP map (56).

$$ED = \sqrt{\sum_{i=1}^{8} (p_i - q_i)^2}$$

Antibiotics included in the study were chosen after review of existing literature in an attempt to gather information about some common antibiotics or families of antibiotics and bacteria that are known to be resistant to them. The list is in no way a complete representation of bacterial resistance but should give insight to the problem.

The position in all eight dimensions was calculated using ChemGPS-NP for a final set of 44 antibiotics in order to analyze their properties. They were then plotted using Grapher 2.5 with the x-axis (green) representing PC1, y-axis (yellow) representing PC2 and z-axis (red) representing PC3.

Table 1: List of descriptors used for PCA

Number	Descriptor
1	Molecular weight
2	Sum of atomic van der Waals volumes
3	Sum of atomic Sanderson electronegativities
4	Sum of atomic polarizabilities
5	Mean atomic van der Waals volume
6	Mean atomic Sanderson electronegativity
7	Number of atoms
8	Number of non-hydrogen atoms
9	Number of bonds
10	Number of non-hydrogen bonds
11	Number of multiple bonds
12	Aromatic ratio
13	Number of rings
14	Number of rotable bonds
15	Rotable bond fraction
16	Number of double bonds
17	Number of aromatic bonds
18	Number of carbon atoms
19	Number of nitrogen atoms
20	Number of oxygen atoms
21	Number of halogens
22	Number of benzene-like rings
23	Number of aromatic carbon atoms (sp^2)
24	Number of amides
25	Number of aliphatic hydroxy groups
26	Number of aromatic hydroxy groups
27	Number of donor atoms for hydrogen bonds (N and O)
28	Number of acceptor atoms for hydrogen bonds (N, O, and F)
29	Unsaturation index
30	Hydrophilic factor
31	Ghose-Crippen molar refractivity
32	Topological polar surface area using N and O
33	Topological polar surface area using N, O, S, and P
34	Ghose-Crippen octanol-water partition coefficient
35	Lipinski alert index (drug-like index)

3. Results

The antibiotics and the resistant bacteria that were gathered and analyzed are outlined here below in Table 2.

Table 2: List of collected antibiotics and resistant bacteria

Antibiotic	Resistant bacteria
Aminoglycosides	*Acinetobacter baumannii* (49) *Burkholderia pseudomallei* (49) *Enterobacter cloacae* (49) *Escherichia coli* (49) *Pseudomonas aeruginosa* (49) *Stenotrophomonas maltohilia* (49) *Vibrio cholerae* (49) *Vibrio parahaemolyticus* (49)
β-lactams	*Aeuromonas hydrophila* (49) *Enterobacter cloacae* (49) *Escherichia coli* (49) *Mycobacterium tuberculosis* (49) *Neisseria gonorrhoeae* (49) *Pseudomonas aeruginosa* (49) *Salmonella typhimurium* (49) *Serratia marcescens* (49) *Staphylococcus aureus* (49) *Stenotrophomonas maltophila* (49)
Penicillin	*Staphylococcus* sp. (1) *Streptococcus pneumoniae* (1)
Ampicillin	*Campylobacter jejuni (49)* *Proteus mirabilis (49)* *Salmonella enterica (50)* *Streptococcus pneumoniae* (59)
Carbapenems	*Acinetobacter baumannii (49)* *Enterobacter aerogens (1, 49)* *Enterobacter cloacae (1, 49)* *Klebsiella oxytoca (49)* *Klebsiella pneumoniae (49)* *Salmonella enterica (49)* *Streptococcus pneumoniae* (59)
Cephalosporins	*Enterobacter aerogenes* (1, 49) *Klebsiella pneumoniae (49)*

	Salmonella enterica (49)
	Staphylococcus sp. (1)
	Streptococcus pneumoniae (59)
	Vibrio cholerae (49)
Cefotaxime	*Campylobacter jejuni* (49)
Macrolides	*Burkholderia pseudomallei* (49)
	Enterococcus faecium (49)
	Escherichia coli (2, 49)
	Neisseria gonorrhoeae (49)
	Pseudomonas aeruginosa (49)
	Stenotrophomonas maltophilia (49)
	Streptococcus pneumoniae (2)
	Streptococcus pyogenes (49)
Erythromycin	*Acinetobacter baumannii* (2, 49)
	Aeuromonas hydrophila (49)
	Camphylobacter jejuni (49)
	Clostridium difficile (2, 49)
	Enterobacter aerogens (49)
	Enterobacter cloacae (49)
	Escherichia coli (49)
	Haemophilus influenzae (49)
	Mycobacteria tuberculosis (49)
	Salmonella typhirium (49)
	Staphylococcus aureus (6)
	Staphylococcus epidermidis (49)
	Streptococcus sp.*(1)*
	Vibrio cholerae (49)
Chloramphenicol	*Acinetobacter baumannii (2, 49)*
	Bordatella bronchiseptica (49)
	Burkholderia cenocepacia (49)
	Burkholderia pseudomallei (49)
	Campylobacter jejuni (49)
	Enterobacter aerogens (2, 49)
	Enterobacter cloacae (49)
	Escherichia coli (2, 49, 50)
	Klebsiella pneumoniae (49)
	Proteus mirabilis (49)
	Pseudomonas aeruginosa (49)
	Salmonella enterica (49, 50)
	Salmonella typhimurium (49)

	Serratia marcescens (49)
	Staphylococcus aureus (49)
	Vibrio cholerae (49)
Fluoroquinolones	*Acinetobacter baumanii (49)*
	Burkholderia cenocepacia (49)
	Camphylobacter jejuni (49)
	Enterobacter aerogens (2, 49)
	Enterobacter cloacae (49)
	Enterococcus faecalis (49)
	Enterococcus faecium (2, 49)
	Escherichia coli (2, 49)
	Klebsiella pneumoniae (49)
	Listeria monocytogenes (2, 49)
	Mycobacterium smegmatis (49)
	Mycobacterium tuberculosis (49)
	Neisseria gonorrhoeae (49)
	Pseudomonas aeruginosa (49)
	Salmonella typhimurium (49)
	Serratia marcescens (49)
	Staphylococcus aureus (6, 49)
	Stenotrophomonas maltophila (49)
	Streptococcus pneumoniae (49)
	Vibrio cholerae (49)
Ciprafloxacin	*Proteus mirabilis (49)*
	Vibrio parahaemolyticus (49)
Norfloxacin	*Vibrio parahaemolyticus (49)*
Tetracycline	*Acinetobacter baumanii (49)*
	Aeromonas hydrophila (49)
	Burkholderia pseudomallei (49)
	Campylobacter jejuni (49)
	Enterobacter aerogens (49)
	Enterobacter cloacae (49)
	Escherichia coli (49)
	Enterococcus faecalis (49)
	Mycobacterium tuberculosis (49)
	Neisseria gonorrhoeae (49)
	Pseudomonas aeruginosa (49)
	Salmonella enterica (50)
	Salmonella typhimurium (49)
	Staphylococcus aureus (6, 49)

	Stenotrophomonas maltophila (49) *Serratia marcescens (49)* *Shigella* sp. (1) *Streptococcus pyogenes (2)* *Vibrio cholerae (49)*
Vancomycin	*Enterococcus* sp.(1) *Mycobacterium tuberculosis (49)* *Staphylococcus* sp. (1)
Rifampin	*Acinetobacter baumanii (49)* *Escherichia coli (49)* *Haemophilus influenzae (49)* *Mycobacterium tuberculosis (49)* *Neisseria gonorrhoeae (49)* *Salmonella typhimurium (49)*
Trimetoprim	*Acinetobacter baumanii (49)* *Aeromonas hydrophila (49)* *Burkholderia cenocepacia (49)* *Enterobacter cloacae (49)* *Escherichia coli (2, 50)* *Pseudomonas aeruginosa (49)* *Proteus mirabilis (49)* *Staphylococcus aureus (6, 49)* *Vibrio cholerae (49)*
Sulfamethoxazole	*Acinetobacter baumanii (49)* *Campylobacter jejuni (60)* *Helicobacter pylori (60)* *Neisseria meningitidis (60)* *Staphylococcus aureus (60)* *Staphylococcus haemolyticus (60)*
Clindamycin	*Acinetobacter baumanii (49)* *Enterococcus faecalis (61)* *Enterococcus faecium (61)* *Staphylococcus aureus (61)* *Streptococcus pneumoniae (61)*
Fusidic acid	*Acinetobacter baumanii (49)* *Aeromonas hydrophila (49)* *Escherichia coli (49)* *Staphylococcus aureus (6, 49)*

Nalidixic acid	*Escherichia coli (49)* *Salmonella typhimurium (49)* *Vibrio cholerae (49)*
Novobiocin	*Acinetobacter baumanii (49)* *Enterobacter aerogens (49)* *Escherichia coli (49)* *Haemophilus influenzae (49)* *Mycobacterium tuberculosis (49)* *Pseudomonas aeruginosa (49)* *Staphylococcus aureus (49)* *Serratia marcescens (49)* *Streptococcus pneumoniae (49)* *Salmonella typhimurium (49)* *Vibrio cholerae (49)*
Tigecycline	*Acinetobacter baumanii (49)* *Enterobacter cloacae (49)* *Pseudomonas aeruginosa (49)* *Proteus mirabilis (49)* *Staphylococcus aureus (6, 49)*

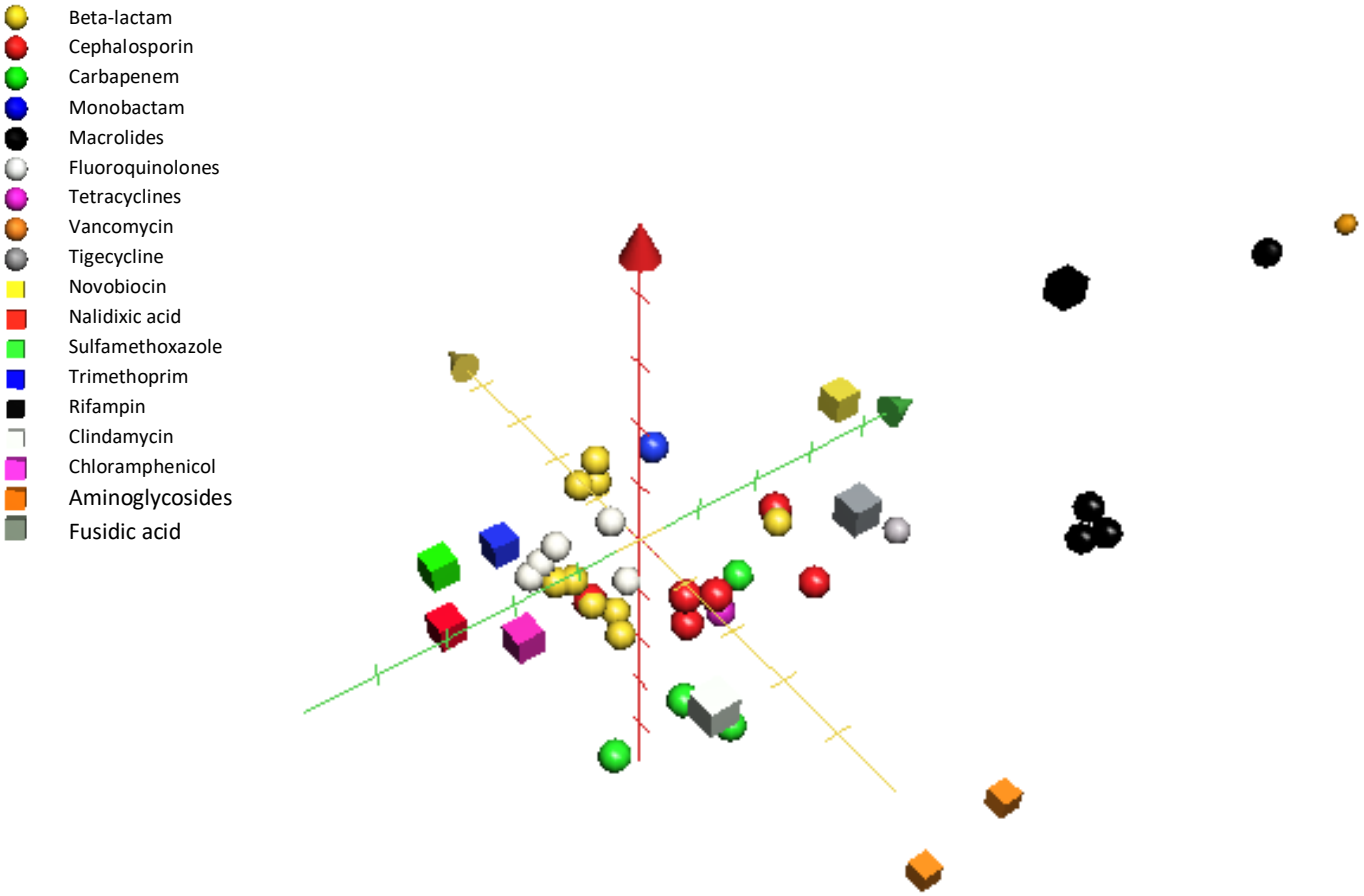

Figure 1: ChemGPS-NP analysis of the selected antibiotics positioned in ChemGPS-NP physical-chemical property space. The first three components are shown, PC1 representing primarily size-dependent properties is the green axis, PC2 describing aromaticity and conjugation related properties is the yellow axis and PC3 which shows lipophilicity, polarity and H-bond donor capacity is the red axis. In this plot different chemical groups of antibiotics are displayed.

In *Figure 1* all antibiotics included in the study have been positioned in ChemGPS-NP physical-chemical property space. The first three principal components are shown, PC1 representing primarily size-dependent properties is the green axis, PC2 describing aromaticity and conjugation related properties is the yellow axis and PC3 which shows lipophilicity, polarity and H-bond donor capacity is the red axis. In this plot different chemical groups of antibiotics are displayed. The penicillins are represented with yellow spheres, cephalosporins with red spheres, carbapenems with green spheres, monobactam with a blue sphere, macrolides with black spheres, fluoroquinolones with white spheres, tetracyclines with purple spheres, vancomycin with an orange sphere, tigecycline with a grey sphere, novobiocin with a yellow square, nalidixic acid with a red square, sulfamethoxazole with a green square, trimethoprim with a blue square, rifampin with a black square, clindamycin with a white square, chloramphenicol with a purple square, aminoglycosides with orange squares and fusidic acid with a grey square.

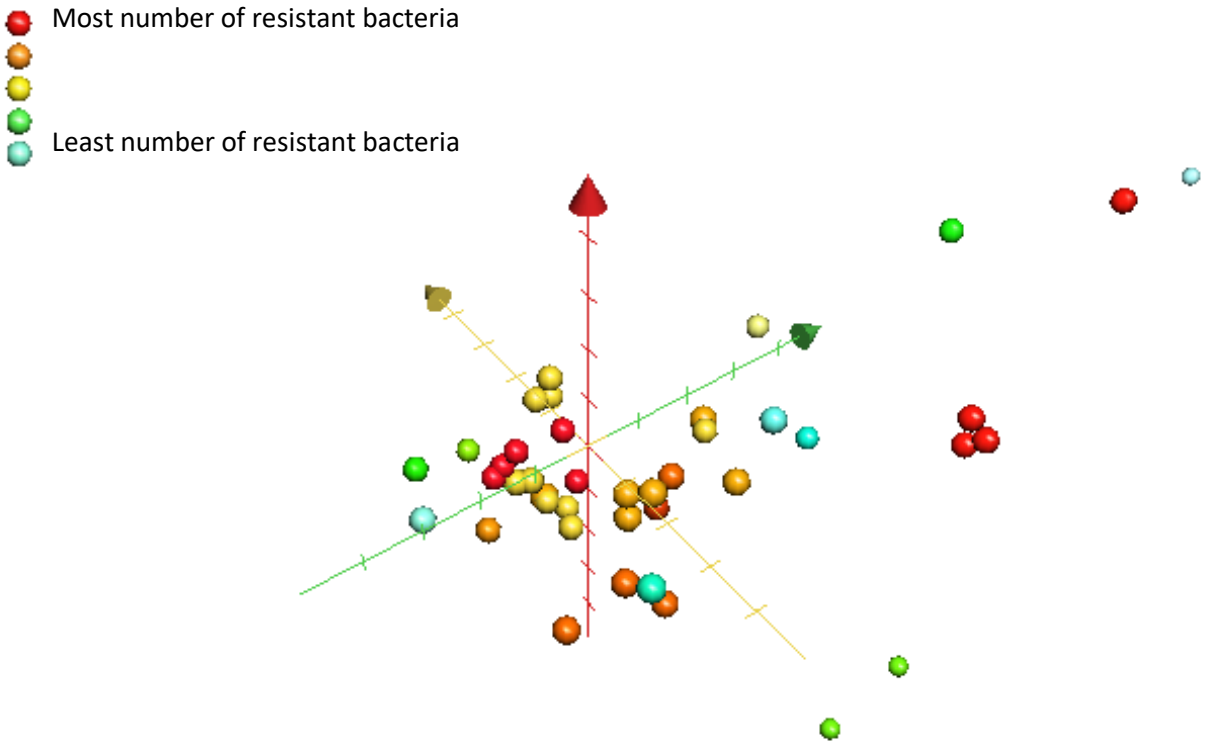

Figure 2: ChemGPS-NP analysis of the selected antibiotics positioned in ChemGPS-NP physical-chemical property space. The first three components are shown, PC1 representing primarily size-dependent properties is the green axis, PC2 describing aromaticity and conjugation related properties is the yellow axis and PC3 which shows lipophilicity, polarity and H-bond donor capacity is the red axis. The antibiotics have been labeled based on their sensitivity to resistance development according to the information gathered in this study. The fluoroquinolones are the most sensitive and represented with dark red spheres, followed by the macrolides represented by slightly lighter red color. The color continues to lighten and changes to orange, then yellow, then green and at last to blue as the sensitivity seems to decrease. After the macrolides come the tetracyclines, carbapenems, chloramphenicol, cephalosporins, penicillins, novobiocin, trimethoprim, aminoglycosides, rifampin, sulfamethoxazole, clindamycin, tigecycline, fusidic acid, nalidixic acid and vancomycin.

In *Figure 2* the selected antibiotics have been labeled based on the number of bacteria resistant to them. The antibiotics with most resistant bacteria are represented with dark red spheres. As the number of resistant bacteria decreases, the red color becomes lighter, then becomes orange, yellow, green and at last blue. From the information gathered in this study it is the fluoroquinolones that have the greatest number of resistant bacteria, followed by macrolides, tetracycline, carbapenems, chloramphenicol, cepalosporins, penicillins, novobiocin, trimethoprim, aminoglycosides, rifampin, sulfamethoxazole, clindamycin, tigecycline, fusidic acid, nalidixic acid and vancomycin.

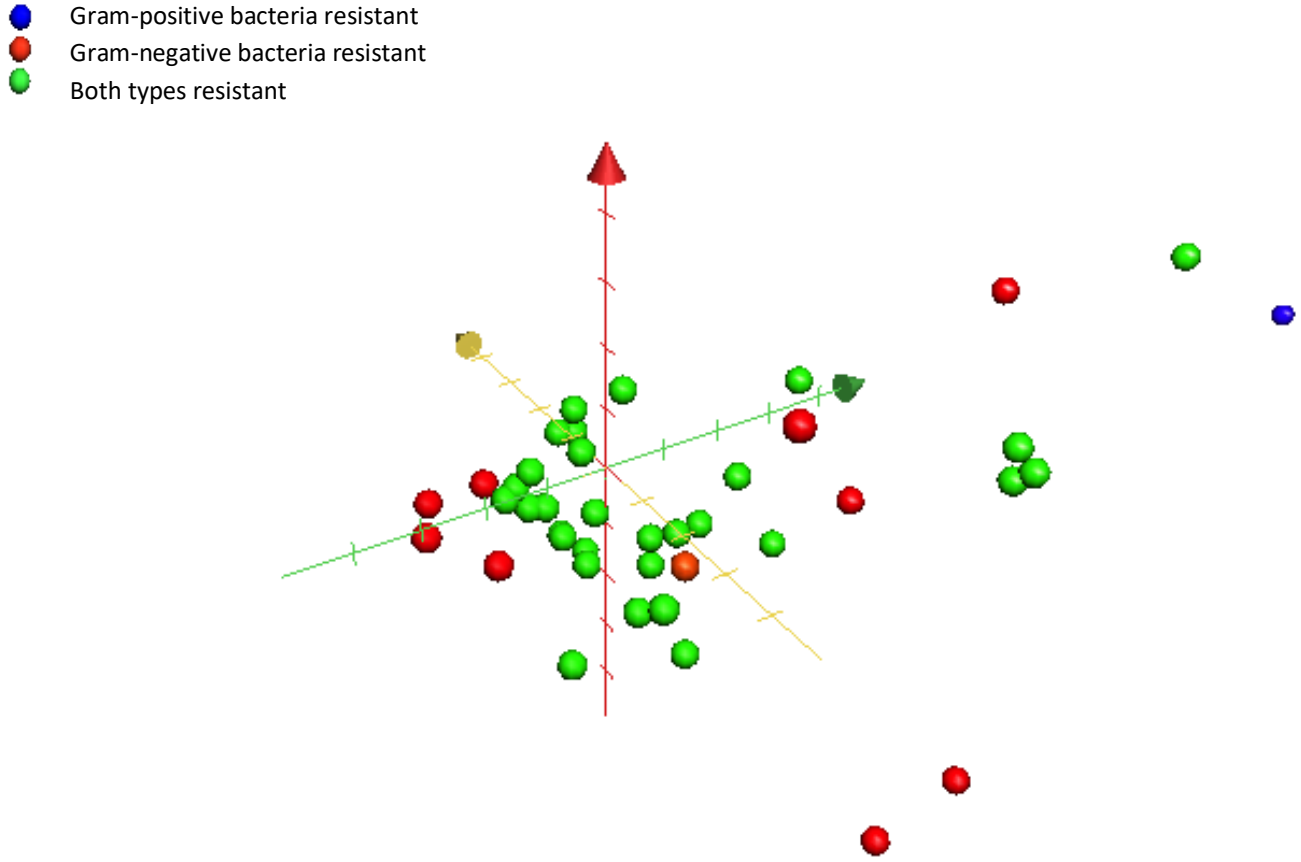

Figure 3: ChemGPS-NP analysis of the selected antibiotics positioned in ChemGPS-NP physical-chemical property space. The first three components are shown, PC1 representing primarily size-dependent properties is the green axis, PC2 describing aromaticity and conjugation related properties is the yellow axis and PC3 which shows lipophilicity, polarity and H-bond donor capacity is the red axis. Here the antibiotics have been divided based on the type of bacteria being resistant to them. The blue sphere represents vancomycin which only has gram-positive bacteria develop resistance, the red spheres represent antibiotics that have mainly gram-negative bacteria resistant. These antibiotics are aminoglycosides, rifampin, nalidixic acid, chloramphenicol, tetracycline, tigecycline, trimethoprim, sulfamethoxazole and fusidic acid. The green spheres represent antibiotics that have both gram-positive and gram-negative bacteria resistant. These are the β-lactams, macrolides, fluoroquinolones, clindamycin and novobiocin.

In *Figure 3* the antibiotics studied have been colored based on the type of resistant bacteria being gram-positive or gram-negative. The sole antibiotic in this study for which all known bacteria are gram-positive, vancomycin, is labeled with a blue sphere. The antibiotics that have primarily gram-negative bacteria resistant to them are labeled with red spheres. These antibiotics are aminoglycosides, rifampin, nalidixic acid, chloramphenicol, tetracycline, tigecycline, trimethoprim, sulfamethoxazole and fusidic acid. Some of these antibiotics are also ineffective against one single gram-positive bacterium i.e. *Staphylococcus aureus*. However, for the purpose of this study, it was decided to regard them as primarily associated with gram-negative bacteria resistance. The antibiotics that show resistance development in both gram-positive and gram-negative pathogens are labeled with green spheres. These are all the β-lactams, macrolides, fluoroquinolones, clindamycin and novobiocin. In this study the case of *Staphylococcus aureus* exhibits a unique pattern, the reason for which is as of yet unclear.

- Inhibit protein synthesis
- Inhibit cell wall synthesis
- Inhibit nucleic acid synthesis

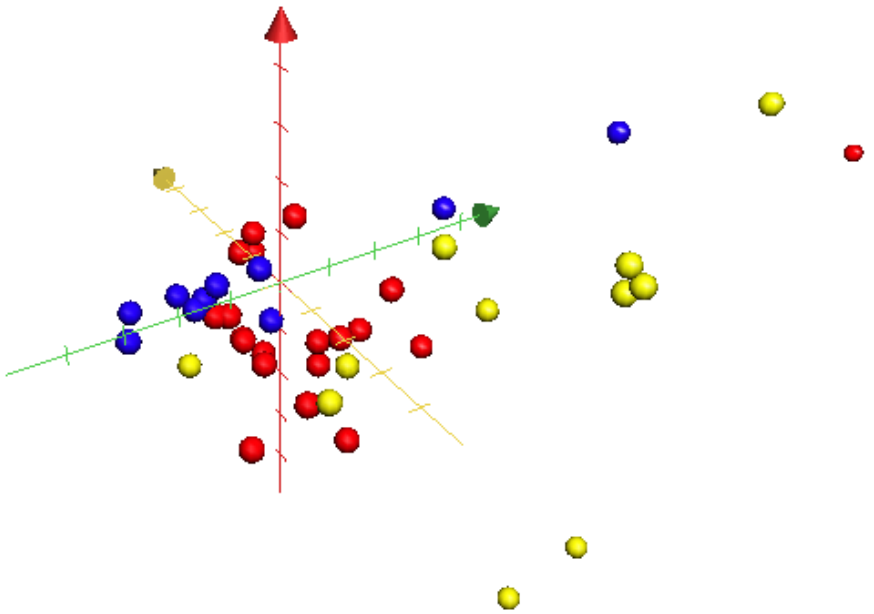

Figure 4: ChemGPS-NP analysis of the selected antibiotics positioned in ChemGPS-NP physical-chemical property space. The first three components are shown, PC1 representing primarily size-dependent properties is the green axis, PC2 describing aromaticity and conjugation related properties is the yellow axis and PC3 which shows lipophilicity, polarity and H-bond donor capacity is the red axis. The antibiotics have been labeled based on their mechanism of action. The antibiotics that inhibit protein synthesis, clindamycin, tigecycline, macrolides, tetracycline, chloramphenicol and aminoglycosides, are represented with yellow spheres. The antibiotics that inhibit nucleic acid synthesis, fluoroquinolones, novobiocin, trimethoprim, rifampin, sulfamethoxazole and nalidixic acid, are represented with blue spheres. The antibiotics that inhibit cell wall synthesis, the β-lactams and vancomycin, are represented with red spheres.

In *Figure 4* the antibiotics under study are labeled based on their mechanism of action. The antibiotics that inhibit protein synthesis are labeled with yellow spheres. These are clindamycin, tigecycline, macrolides, tetracycline, chloramphenicol and aminoglycosides. The antibiotics that inhibit nucleic acid synthesis are labeled with blue spheres and are fluoroquinolones, novobiocin, trimethoprim, rifampin, sulfamethoxazole and nalidixic acid. The ones that inhibit cell wall synthesis are labeled with red spheres and include all the β-lactams and vancomycin. With the exception for vancomycin, which is always pronouncedly offset due to its large molecular size, the remaining antibiotics show differences in their distribution pattern. All those related to cell wall synthesis are forming a tight cluster of red spheres. The blue spheres, representing antibiotics that inhibit nucleic acid synthesis, have very little distribution in PC2, the yellow spheres much more so.

4. Discussion

If we look at the chemographic analysis of the selected antibiotics, *Figure 1,* we can see that members of the same family reside close to each other in chemical space. This is because they have similar structural and chemical properties. It is therefore understandable that if a pathogen acquires resistance to a member of the family that it often renders other members of the same family useless against said pathogen.

Vancomycin, the orange sphere, is very far out on the x-axis which represents size, which is understandable since vancomycin is a very large molecule. The macrolides, black spheres, are also quite far out on the same axis.

The β-lactams reside quite close to the crossing of the three axis. The cephalosporins seem to be less aromatic than the other families and the penicillins seem to be spread out a bit over principal component 3, which is lipohphilicity, polarity and H-bond donor capacity. One yellow sphere, that represents piperacillin, is further along the x-axis. Piperacillin is a semisynthetic, broad-spectrum antibiotic and is larger than the other penicillins.

As can be seen in *Figure 2*, the fluoroquinolones are quite susceptible to bacteria developing resistance against their actions. This can propably to some extent be explained by their widespread use. Fluoroquinolones are effective against a wide range of pathogens and have a very favorable profile of side effects. This has led to their overuse which has most likely contributed greatly to the development of these resistance mechanisms. Macrolides, β-lactams and tetracyclines are also quite susceptible to resistance mechanism. These antibiotics, like fluoroquinolones, have a wide range of activity against both gram-positive and gram-negative bacteria. They also don‘t display serious side effects and have been extensively used which has most likely contributed greatly to the spread of resistant bacteria. On the other hand, vancomycin seems less susceptible to resistance mechanism. Vancomycin is only effective against gram-positive bacteria and has some undesirable side effects. It has therefore not been used as a first line of treatment but only when necessary.

The issue of resistance is great among gram-negative bacteria, which is propably due in some part to the innate resistance they possess to many antibiotics as well as how challenging it is to penetrate them because of their extra layer of protection. *Figure 3* displayes the types of bacteria that are resistant to the selected antibiotics. The macrolides, β-lactams, fluoroquinolones, clindamycin and novobiocin are ineffective against a number of gram-positive and gram-negative bacteria due to their development of resistance. Vancomycin is only effective against gram-positive bacteria and therefore only gram-positive bacteria have developed resistance. There are quite a number of antibiotics that have mainly gram-negative bacteria that are resistant to them, like aminoglycosides, rifampin, nalidixic acid, chloramphenicol, tetracycline, tigecycline, trimethoprim and sulfamethoxazole. *Staphylococcus aureus* is also resistant to some of these.

Staphylococcus aureus is one of the major bacterial pathogens, capable of causing life-threatening infections. The pathogen has shown remarkable diversity of resistance mechanisms toward antimicrobial agents. Methicillin-resistant *S.aureus* (MRSA) is of special concern, these strains are resistant to all β-lactam antibiotics (6). *S. aureus* is naturally susceptible to virtually every antibiotic that has ever been developed. Penicillin resistant strains were first observed in the community by the early 1950s. It was in 1961 that the first strain resistant to methicillin were reported and the methicillin resistance, unlike the penicillinase-mediated resistance which was narrow spectrum, it was broad and also caused resistance against penicillins, cephalosporins and carbapenems. The increasing incidence of MRSA caused more usage of vancomycin which was the last remaining antibiotic to which MRSA strains were reliably susceptible. Under this intensive selective pressure, vancomycin resistant strainst of *S.aureus* emerged and the first one was reported in 2002 (62).

The mechanisms of action of the selected antibiotics can be roughly divided into antibiotics that inhibit cell wall synthesis, antibiotics that inhibit nucleic acid synthesis, and antibiotics that inhibit protein synthesis. In *Figure 4* the antibiotics have been plotted in chemical space and labeled based on their mechanism of action. It can be noted that the antibiotics that inhibit nucleic acid synthesis are similarly placed according to PC2 and PC3 but are scattered along the x-axis which represents PC1. It can therefore be concluded that they differ in size-related properties but are similar when it comes to aromaticity, conjugation-related properties, lipophilicity, polarity and H-bond donor capacity. The antibiotics that inhibit cell wall synthesis, with the exception of vancomycin which is far along the x-axis due to its large molecular size, are similarly placed according to PC1 and PC2 but are spread along the z-axis representing PC3.

If we look at the number of resistant bacteria based on mechanism we can see that antibiotics sensitive to resistance belong to all of the groups, fluoroquinolones inhibit nucleic acid synthesis, macrolides and tetracyclines inhibit protein synthesis and the β-lactams inhibit cell wall synthesis.

If the figures are analyzed it can be deduced that there are places in chemical space that are related to a higher risk of resistance development, mainly the areas occupied by the antibiotics in this study as they are almost all quite susceptible. Their physical chemical properties can be specified by further analyzing the results of the Principal Component Analysis. There does seem to be areas of chemical space more related to resistance of gram-negative bacteria, they also seem to be tougher to penetrate and pose a more serious threat when it comes to available treatment options.

It is not unlikely that resistance development has a stronger association to the use of antibiotics rather than chemical properties, but antibiotics that have a broad spectrum of activity and few serious side effects have been used extensively and seem to have triggered the spread of resistance.

I think that chemographics could be a useful tool in the search for novel antibiotics, and that the only way for us to continue to be able to fight off bacteria is to use available antibiotic agents wisely and continue to devote research to find novel agents as no antibiotic seems to be immune to resistance development.

This study could be further extended to include more antibiotics and a more complete list of resistant bacteria. The amount of literature available is very vast and it was beyond the scope of this small project to analyze all available literature to gather a complete list. It is also debatable when a bacterium should be considered resistant to a certain antibiotic. Is it enough that a resistant strain has been found in experimental setting, is it when a resistant strain has been diagnosed in the clinic or should the resistance have spread to a certain level.

This study does provide a novel approach to the issue of antibiotic resistance. A lot of energy and resources have been spent on trying to better understand the resistance mechanisms that bacteria possess. The fact is that, as has been outlined in the introduction, that bacteria have great abilities when it comes to adapting and surviving in challenging environments, as in the presence of antibiotics. Therefore, it seems to be wishful thinking to be able to find an antibiotic that no bacteria will ever develop resistance against. I think that it is more promising to find more effective ways of developing new antibiotics, to try to be ahead of the resistance development that the bacteria will almost definitely go through. I think that chemographic analysis, as is performed here in this study, could become a useful tool in the search for novel antibiotics. Further research could be done on the chemographics of existing antibiotics and that information could be used to further screen natural products in order to find new antibiotic agents, that could relieve the antibiotics that are being used today but are becoming less effective.

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