Current methods for inhibiting antibiotic resistant bacteria by targeting bacterial cell metabolism and disrupting antibiotic elimination through the AcrAB-Tolc efflux pump

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ABSTRACT

Bacteria have a complex and lengthy evolutionary history of antibiotic resistance. For millions of years, bacteria have evolved a gene pool filled with multiple drug resistant genes. However, for the past 50 years, bacteria have been mutating and evolving vigorously and rapidly. Those 50 years predate to the time of the first use of antibiotic drugs in the 1940s. Since the 1940s, with the wide-spread use of the first antibiotic, penicillin, bacteria have effectively developed resistance to multiple antibiotic drugs. Bacteria develop antibiotic resistance after acquiring antibiotic resistant genes from conjugation and a horizontal transfer of those genes. Bacteria also have innate properties, structure, and functions that can increase their resistance of antibiotics. Bacteria cells can mutate its genes and block the binding of antibiotic drugs to its DNA. If the bacteria effectively impede the activity of an antibiotic through a DNA mutation, then the same mutation is shared with other bacterial cell strains through horizontal transfer. Antibiotics can be expelled from bacteria cells by efflux pumps called AcrBC-Tolc channels from the resistance-nodulation division (RND) family. Targeting the cell metabolism or the expression of efflux pumps may deter or impede the proliferation of antibiotic resistance. Researchers cultured *E. tarda* with glucose and alanine, and the uptake of kanamycin increased, eliminating approximately 3,000 times the amount of MDR bacterial cells compared to the cells only treated with kanamycin. Another researcher named Dr. Li mutated a gene of the AcrAB-Tolc binding site, forming a replacement for the highly non-polar phenylalanine amino acid residue with an alanine. His mutagenesis of the efflux pumps binding sites for AcrAB-Tolc inhibited the exit of antibiotics through the AcrAB-Tolc efflux pumps. Therefore, the review serves to discuss the new, novel, and current methods for reducing the spread of antibiotic resistant bacteria by targeting bacterial cell metabolism and its antibiotic resistant genes.

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INTRODUCTION

More bacteria are resistant to many antibiotics. The World Economic Forum Global Risks present data uncover that the greatest growing danger to our health currently is antibiotic resistance. Some bacteria have an innate response of resistance to antibiotics. Many other bacteria share mutations through conjugation or a horizontal exchange of antibiotic resistant genes. Bacterial cells can become innately resistant to antibiotics through its hereditary structure and functional properties. Bacteria have three modes of function after a horizontal gene transfer. One, bacteria can receive antibiotic resistant genes that after translation can prevent the diffusion of antibiotics through pores, causing antibiotic efflux. Secondly, mutations inserted into the genes of the targets for antibiotics, block the antibiotics binding to those sites. Three, the actual antibiotics can be targeted with hydrolysis for its complete degradation and inactivation.

Pumps called multi-drug resistance efflux pumps or MDR efflux pumps can export antibiotics out of the bacterial cells. All bacteria utilize MDR efflux pumps, yet genes translated into MDR efflux pumps can be placed on plasmids and shared among bacterial cells. Resistant Nodulation Division pumps are transported on IncH1 plasmids, isolated from the *Citrobacter freundii bacteria*, which transmits the New Delhi metallo-B-lactamase 1 or NDM1-34 for enzymatic decay of antibiotic drugs (Blair et al., 2014). Gram-negative bacteria mainly carry RND pump types for MDR efflux activity. RND pumps include the homotrimers called AcrAB-Tolc efflux pumps. The AcrAB-Tolc pump are in the inner membrane. Its structure is a triplex with a middle protein called AcrA, an outer membrane canal called the TolC, and AcrB that reside in the inner membrane part of the cell. The binding sites of these homotrimer efflux pumps can bind to and attach to many antibiotics with different sizes and chemical properties. Many genes coding for the synthesis of many of these RND pumps can be shared between bacterial cells through conjugation.

The mutations of genes needed for increased efflux activity are found in the regulatory portion of DNA sequences, which monitor the translation of the efflux-pumps. The expression of the efflux pumps can be monitored through increased mutations in an adjacent promoter site, in genes specialized for monitoring transcription factors, and in genes responsible for controlling the translation of the efflux pump genes. For example, a point mutation approximately 10 base pairs upstream of the mtrC gene of *N. gonorrhoeae* increases the amount of activity at the promoter, resulting in amplified expression of the efflux pump to remove antibiotic drugs. Bacteria can also acquire DNA from its environment, sharing antibiotic resistant genes called 'mosaic' genes. An example is *S. pneumoniae* antibiotic resistance of penicillin, which shares the mosaic penicillin-binding protein or pbp genes between bacterial cells. The pbp genes are translated into enzymes increasing resistance to penicillin.

The mosaic pbp genes originated from continued mixture and rearrangements of DNA by the bacteria called *Streptococcus mitis*. The influx of the class of antibiotics called cephalosporins has also been blocked by the penA gene, encoding for PBPs, in *N. gonorrhoeae*. If a gene mutation can cause antibiotic resistance, then it will be successfully shared through horizontal gene transfer between bacterial cells, quickly spreading that mutation among many different types of bacteria. Resistance to quinolone antibiotic drugs is propagated through the qnr

genes that are translated into pentapeptide repeat proteins or the PRPs. The PRPs attach to and inhibit the quinolones form degrading the topoisomerase Iv and the DNA gyrase of bacterial cells. When the quinolone antibiotic attaches and secretes more quinolone, the PRPs begin to bind and inhibit the quinolone bound to the topoisomerase. The PRPs remove the quinolone from the topoisomerase, allowing the topoisomerase to continue completing its activities of rebuilding dsDNA breaches and cracks causes by the quinolone. Also, many enzymes can hydrolyze and destroy antibiotics called Beta-lactams, aminoglycosides, phenicols, and macrolides. Bacteria can develop resistance to antibiotics though gene mutations or via other innate processes.

Regulating the metabolic functions of bacteria can increase the uptake of antibiotics. Metabolites in high concentrations increase bacterial antibiotic resistance. Metabolites as indole, ammonia, nitric oxide, and hydrogen sulfide can amplify antibiotic resistance. However, carbon in higher amounts eradicates more pathogenic bacteria by increasing antibiotic sensitivity. The high influx of carbon from the environment surrounding bacteria cells controls antibiotic sensitivity by raising NADH activity and increasing the proton motive force (PMF). The rise of NADH levels and the rapid activity of the proton motive force both activate the TCA cycle of bacterial carbon-dependent metabolism. Novel synthetic antibiotics as capistruin and malleilactone block bacterial cell transformation, inducing more antibiotic production from those bacterial cells. The antibiotic, bactobulin, can connect to the luxI-luxR proteins responsible for quorum sensing (Wozniak et al., 2018). The LuxI-R linkage to bactobulin stimulates a molecule that inhibits bacterial translation in the ribosome. Capistruin blocks RNA polymerase activity. Malleilactone interacts with quorum sensing molecules. *S. Aureus* has a lysine at position 164, resulting in antibiotic resistance of thailandamide resistance.

The antibiotic called thaillandamide deactivates acetyl-CoA carboxylase or the AccA. Also, Salmonella has a mutation in the gene accA. Further study is needed to design new novel therapeutic drugs that target the gene accA, targeting antibiotic resistance. Combining genetics with antibiotic development may assist with discovering more effective methods for combating the growing concern of antibiotic resistance. Also, a list of criteria and properties are needed in a database to help identify and test the environment for dangerous resistant bacterial types (Berendonk, et al., 2015). In this review, the process of bacterial antibiotic resistance, which is affected by bacterial metabolic processes, conjugation, efflux pumps, and bacterial responses to antibiotic drugs, is described. Here, the current methods to target antibiotic resistance are discussed, underlining possible alternative agents that can target bacterial cells metabolic pathways, bacterial efflux pumps, and modify bacterial antibiotic resistant genes, which can inhibit the proliferation of antibiotic resistant bacteria and its genes.

SURVEY METHODOLOGY

I ANTIBIOTIC RESISTANCE

Past antibiotic drug use needs to be considered as a part of the evolutionary history of a bacteria's becoming resistant to antibiotics. Yen and Papin (2017) cultured bacteria with three different antibiotics at three different times and phases. Through their experiment they proved

that the sequenced exposure to an antibiotic at a past time affected and altered the current phenotypical resistance and gene expression of the present bacterial strain. Antibiotic resistant bacteria have been found in fossils covered in permafrost from the tundra, in caves, and in ancient preserved human samples. Bacterial resistance to antibiotics is ancient, dating past the first use of antibiotics in the 1940s. Perry, Waglechner and Wright (2019) argue that studying the evolutionary history of antibiotic resistant bacteria is needed to determine its future evolution. Antibiotic resistance did not begin with the use of antibiotic drugs because there is a large gene pool of antibiotic resistant genes. The gene pool of antibiotic resistance has evolved for a million or more years. However, data from the study of epidemiology state that, for the past 50 years, bacteria have been rapidly evolving, becoming more resistant to multiple antibiotic drugs (Freidman et al., 2016). For the past 50 years antibiotic resistance has escalated, which is the exact time of amplified antibiotic drug use.

Alexander Fleming discovered penicillin in 1928 after he observed a contamination of mold lessening the growth of staphylococci bacteria in a petri-dish (Lobanovska and Giulia Pilla, 2017). He published his findings, showing the inhibition of bacteria to grow after exposure to penicillin. In 1940, researchers from Oxford University, described and published methods for effectively purifying and isolating penicillin in large volumes for clinicial tests (Aminov, 2017). Because of the methods from Oxford, penicillin was produced and utilized in the year 1945. Wide-spread use of antibiotics was made possible in the 1940s, however, since then the level of antibiotic resistant bacteria has exponentially increased (Landecker, 2016). Antibiotic resistance in bacteria have an immense and ancient evolutionary history. Bacterial antibiotic resistance is a prototype that models the stages of evolution through the process of natural selection. Bacteria adapt well to any environment. The addition antibiotics to its environments, forces the bacteria cells to mutate its genes, the antibiotic resistant genes, and share those genes for the survival of its species. Natural selection is the central component and the driving force of bacteria developing antibiotic resistance. After the exposure to bactericide antibiotics, the population of bacteria cells that survived are called persister cells. These persister cells not only survive but are key determinants for the initiation of antibiotic resistance (Verstraeten et al., 2016).

Gram-positive bacterial strains differ from gram-negative bacteria in that the gram-negative bacteria cell wall is fitted between its inner and outer membranes whereas gram-positive bacteria only have a single viscous cell wall, consisting of peptidoglycan. Therefore, gram-negative bacteria have a higher potential to form a resistant response to antibiotics. Knowledge of the functions that establish and proliferate antibiotic resistant bacteria is required for advancing the pharmaceutical search for targeting antibiotic resistant genes and bacteria. The bacteria called *A. baumanii* resides in hospitals and are highly contagious pathogenic bacteria. The *A. baumanii* develops into an antibiotic resistant bacterium by obtaining resistant genes from conjugation, which is mostly a horizontal transfer of those genes, and via mutations in its chromosomes. The centers for disease control account for the *A. baumanii* causation of most hospital infections, which is approximately 64% of urinary tract infections from catheter use (Eichenberg and Thaden, 2019).

The enzymes, acetyltransferase and the nucleotidyltransferases, modify the antibiotics called Aminoglycosides, forming resistant *A. baumanii* bacterial strains. Therefore, these *A. baumaii* inserted mutations into the genes gyrA and parC, which then overexpresses these enzymes to alter the antibiotic target called 16S rRNA, preventing the antibiotic, aminoglycoside, to bind and block 16S rRNA activity. The volatile fatty acid or VFA called propionate can inhibit many metabolic processes when the VFAs amass into high concentrations within a bacterial cell and can assist with eliminating antibiotic resistant bacteria (Simonte et al., 2016). However, many species of bacteria have developed processes that use the VFA, propionate, as a carbon source for generating energy. Propionate may become toxic for *E. coli* cells, as a result, *E. coli* regulate the anaerobic metabolism of propionate at a posttranscriptional level through *E. coli* cells altering mRNA after its transcription. Quinolone can effectively block the expulsion of antibiotics drugs from bacterial cells. A copy of quinolone antibiotics has been derived to inhibit the efflux pumps called NorA in *S. aureus* (Mahmood et al., 2016).

II CELL METABOLISM AND BACTERIAL ANTIBIOTIC RESISTANCE

Researchers cultured alanine and glucose with the bacteria called *E. tarda*, which is a bacterium resistant to multiple antibiotics as tetracycline, chloramphenicol, streptomycin, and sulfonamide. They examined the potential of alanine and glucose to increase the antibiotic, kanamycin, sensitivity of *E. tarda*. Their predictions and hypotheses were true, adding alanine and glucose with kanamycin to the E. tarda bacterial culture, eliminated many bacterial cells. The *E. tarda* cultured with only 1000mg of kanamycin, 40mM of alanine, and with 10mM of glucose decreased the number of bacterial cells by 101, to 3228, or to 276,000-fold, respectively (Peng et al., 2015). Therefore, they concluded that glucose elevates the uptake of antibiotics and induces the activity of PMF.

Culturing *E. tarda* with alanine and/or glucose resulted in its cells apoptosis. Pyruvate increased proportionally to elevated levels of alanine and/or glucose. The concentrations of NADH and PMF also were amplified. They concluded that alanine and glucose worked to increase and induce the absorption of antibiotics. The kanamycin inside of the *E. tarda* cells were in high concentrations after exposure to alanine and glucose. Bacterial samples without alanine or glucose had a kanamycin uptake of 9.5 ng/mL, however, the cultures with alanine-glucose raised levels of absorption of kanamycin to 65-123 ng/ml and then to 113-231 ng/ml (Peng et al., 2015). The alanine and glucose cultures of *E. tarda* overrided the activity of the multi-drug efflux pumps removal of antibiotics.

Through a Michaelis-Menten kinetics experiment, researchers found *E. coli* K12 to develop more sensitivity to the antibiotic called gentamicin when cultured in Luria-Bertani broth enhanced with glutamate and acetate (Yu Bin Shu et al., 2018). The cycle of NADH plus PMF was significantly increased after growing *E. coli* cells were cultured with glutamate. Researchers found that glucose, glutamate, and fructose all induced the absorption of antibiotics by downregulation and increasing the gene of expression of maeA and maeB, which are a part of the MAL-pyruvate pathway. The increase in the expression of maeA and maeB produced more pyruvate in the presence of high levels of glucose, which indicates that the TCA cycle could be dependent on the P-cycle. Manipulating the P-cycle or the OAA-PEP-pyruvate-AcCoACIT

pathway and the TCA cycle may be a favorable target for inhibiting antibiotic resistant genes. The P-Cycle depends on an abundance of exogenous metabolites even when genes of the P cycle are silenced, antibiotic resistance to aminoglycosides continues to decrease.

Future studies may benefit from the use of newly discovered bacteria as *V. natriegens*, which is a similar bacterium to *E. coli*. In experiments, glucose molecules were labeled with Carbon-13 to effectively measure the amounts of fluxes and changes in the metabolism of *V. natriegens*. The measures were taken during the exponential growth phase of the bacteria, *V. natriegens*. Researchers analyzed the data collected by fitting the acetate concentrations, the data of the labeling C-13 markers, the ribose of RNA, and the glucose from glycogen to a graphical model. The analysis revealed high glycolysis activity of 80 ± 0.5 and 169 ± 1 for phosphoglucose isomerase and glyceraldehyde 3-phosphate dehydrogenase, respectively. Much carboxylase activity for metabolizing pyruvate was recorded with a standard deviation of 27 ± 1. Many changes in the TCA cycle were recorded with standard deviations of 17 ± 1 for citrate synthase and 7 ± 0.4 for ketoglutarate dehydrogenase (Long et al., 2017). *V. natriegens* displayed similar yields of glycolysis to *E. coli*, equaling a 50% of energy contributed through glycolysis and 40% produced from the TCA cycle, which is like the *E. Coli* ATP metabolic production (Long et al., 2017). Both *V. natriegens* and *E. coli* utilize the energy from ATP production to transport substrates, grow cells, and maintain homeostasis.

III THE MODIFICATION OF ANTIBIOTIC RESISTANT GENES

Infections due to antibiotic resistance causes 600 deaths annually from exposure to hospital bacterial pathogens (Rahman et al., 2016). Inhibiting the gene expression of MDR efflux protein pumps, can increase bacterial sensitivity of antibiotics. Small molecules can act as inhibitors to lessen mutant strains spread and the transfer of antibiotic resistant genes. By blocking the cellular construction of the protein pumps, the transport through the protein pump channels can become reduced and impede the energy garnered through ATP hydrolysis for powering pump activity. The membrane fusion protein connects the inner membrane pump to the membrane external to the cell plasma membrane called the TolC type channel. The protein pump does not require substrates with a specific molecular weight, net charge, or surface area. Plants release cytotoxic phytonutrients, which provide immunity against foreign bacterial cells. For example, reserpine, a plant alkaloid, extracted from the roots of the petitiva plant.

Reserpine inhibits the efflux pumps of the gram-positive *B. Substilius*. The reserpine binds to the pumps, expressing three amino acids with the R-groups phenylalanine and valine. Reserpine causes a 4-fold decrease in the antibiotic resistance of MRSA caused by *S. Aureus.* Berberine has a MIC of 256 mg/L, but when the NorA pump is active, the berberine is reduced to 16 mg/L (Rahman et al., 2016). However, there are not many studies that focus on the medicinal properties and benefits of plant alkaloids. For instance, polyphenol blockers are found in green tea extractions, which can inhibit MRSA resistance. Adding 20mg/L of the polyphenols to the antibiotics norfloxacin, resulted in a 4-fold decrease in MIC (Rahman et al., 2016).

Pathogenic bacteria become resistant to antibiotics from changes in DNA sequences and conjugation that transfers antibiotic resistant genes between bacterial cells. The activities of

antibiotic resistance are destroying the antibiotics, use of efflux pumps, and shielding of targets to block the binding of antibiotics. Also, Antibiotic resistant bacteria can denature the enzymes to prevent bacterial cells interaction with antibiotics. CRISPR-Cas9 or bacteriophages can modify bacterial genomes, rendering bacteria more susceptible to antibiotics. However, Ayhan et al., (2016) constructed antisense molecules, attaching to the mRNA transcripts of antibiotic resistant genes. They designed phosphorodiamidate morpholino rings connected by a phosphodiamidate, PMO, backbone (Ayhan et al., 2016). Each ring of the PMO is bound to an antisense nucleotide base, which blocks translation of antibiotic resistant genes via steric hindrance. The site of steric hindrance is tightly adjacent to the ribosome binding sequences of the bacteria, which obstructs entrance of bacterial mRNA into ribosomes. Adding peptides to the PPMOs enhances its entrance into bacterial cells during conjugation. The phosphodiamidate, PMO, backbone impedes the nuclease activity that would disassemble the PPMO. PPMOs present a few issues, which need more research in areas of pharmacokinetics for effectively combining PPMOs with antibiotics. There are issues of mismatched base pairing, determining the position and size of the oligomer, and the effectiveness of PPMOs need further examination.

Multi-drug efflux is responsible for antibiotic resistance, which exports antibiotic drugs from bacterial cells. The antibiotics are pumped from the cytoplasm of the bacterial cells. The AcrAB-TolC efflux pumps consists of three domains. The three domains include AcrA of the mid transmembrane part of the pump, AcrB of the inner membrane, and TolC is the outer external region of the membrane. The genes for AcrAB-TolC are in an operon. However, mutations in the AcrA gene weakens the antibiotic resistance of bacteria, which become more sensitive to kanamycin and ampicillin. The change in DNA sequence of the AcrA gene decreases efflux of antibiotics. AcrS is a protein, which inhibits AcrA gene expression. The AcrS gene extends 663 base pairs and is in the AcrEF operon (Belmans et al., 2016). AcrS attaches to the promoter site of AcrA, inhibiting the efflux. Blocking expression of AcRS, increases antibiotic resistance in *E. Coli* cells. Issues are efflux pump blockers are too toxic for human cells, however a non-toxic method of linking phosphodiamidate morpholino rings can provide an antisense blockage of the mRNA for antibiotic resistant genes (Belmans et al., 2016).

The pumps classified as the resistance-nodulation division (RND) include 4 subclasses of export pumps. The four subclasses are the major facilitator superfamily (MFS), the multi-drop and TolC compound extrusion (MATE), the small multi-drug resistance pumps (SMR), and the ATP-binding cassette (ABC). The ABC pumps depend on the energy generated from ATP-hydrolysis. RND pumps remove drugs and other toxic cations. RND pumps are a triplex compound with an inner membrane region, an outer membrane channel, and a middle periplasm. The triplex releases drugs and toxins into the outer environment of the bacterial cell. After the toxins are removed, it is arduous for the antibiotics to regain re-entry into the cytosol through the pump's outer membrane. The MFS and SMR pumps are less effective. The drugs are only excreted into the middle periplasm and not into the outer membrane. However, the RND pumps operate within the periplasm to expel any antibiotics remaining after MFS and SMN exportation.

The AcrB of *E. coli* increases the MICs of antibiotics. The substrates or antibiotics bind to the AcrB segment of the AcrAB-Tolc protein pump. The non-polar and highly hydrophobic

substrates interact with the water molecules external of the bacterial cell. The antibiotics connect and form hydrogen bonds with the water molecules. The enzyme AcrAB-TolC binds the hydrophilic regions of the antibiotics, which is then hydrogen-bonded to water. The hydrophilic domains surrounding the antibiotics allow the facile removal and export of the drugs from the bacterial cells.

The Multi-drug pumps do not need specific residues or small enough binding sites for capturing and removing antibiotics. Because water molecules from H-bonds with less stable antibiotic drugs, the binding site can be as large as possible for export, and this helps the pump overcome the initial threshold of the binding free energy. Adding magnesium to AcrA rapidly increased the export of phospholipids. The AcrA connects to two vesicles to transport the phospholipids. The AcrB acts as the regulator when the amounts of antibiotics lessen the transmembrane pH gradient of the pump's channel. For example, adding streptomycin, an antibiotic, to a more acidic mid periplasm of the pump's channel, increased the frequency of pumping. Therefore, the removal of the substrates, the antibiotics, only occur in the periplasm. The rate of hydrolysis versus the V_{max} and the Km of the enzyme, AcrAB-Tolc, directly parallels with the rate of efflux. The graphical analysis of the rate of nitrocefin efflux, when compared to the periplasmic concentration of the AcrAB-Tolc pump, yielded a Michaelis-Menten curve. The Michaelis-Menten curve displayed a Vmax of 0.024 nmol/mg/s and a Km concentration of 5M, which uncovered less competition and more cooperativity (Li et al., 2015). There was more cooperativity between the rate of efflux and the pump's hydrolysis rate, including the concentration of the nitrocefin. More antibiotics within the bacterial cells leads to a higher velocity of efflux with rapid antibiotic removal.

The binding protomer is mainly hydrophobic and lies centered within the periplasm of the pump. There are three different protomer domain activities: 1) access 2) binding and 3) exportation. Each domain rotates as each alters their orientation and conformation. The conformational changes are a result of the movements of Disulfide crosslinks and of the adjacent R-groups. The protomers simultaneously rotate at least 1 or 2 protomers at a time (Li et al., 2015). Li et al (2015) performed a site directed mutagenesis to replace the non-polar phenyl alanine R-groups within the AcrAB-Tolc binding site. Without the phenylalanine, the efflux of antibiotics was blocked, and the phenylalanine was mutated to express Phe610Ala. After the mutagenesis, the substrate and antibiotic doxorubicin remained bound to the binding site because the mutated AcrB obstructed the substrates' separation from the binding site of the outer pocket. The mutation caused the binding site to eliminate expression of the glycine loop, removing the separation of the distal and the proximal binding sites.

A mutation of AcrB can replace the transport of protons by D408. After the mutation of AcrB, efflux through the AcrB pump and channel is ceased, and it lessens the intensity of virulence. The RND pumps inhibit the production of autoinducers, which are quorum sensing molecules without an accumulation of the AI molecules. As a result, quorum sensing is not initiated, and virulent gene expression is decreased. The mutation of the acrB gene is a point mutation added to the gene, which attached D408A to the translated proteins (Wang-Kan et al., 2017). The movement of protons within the pump decelerated and lessened without any efflux.

S. Typhimurim SL1344 in mice, *Galleria mellonella*, tissue cultures, and *Salmonella* all displayed deletion of efflux following the acrB gene mutation. The elimination of efflux for each organism was confirmed through RNA sequencing.

When comparing mutant forms versus the wildtype, the wildtype showed the activation of secondary molecules to continue expressing antibiotic resistant genes under stress and increased pressure. However, the mutant form was not as regulated and did not activate secondary molecules. *S. Typhimurim* passes through the M cells, leading to more systemic infection, which depends on Sp-1 and SPI-4. The SP-1 and SpI-4 is less regulated in its mutant type, which lessens the frequency of bacteria crossing into the M cells of the GIT. SPI-2 acts to fortify the bacterial cells to evade phagocytosis inside macrophages. The PhoPQ protein decreases gene expression of the IsrA CDBK, which decreases quorum sensing. The issue for quorum sensing of the *Salmonella* infection is completely eradicated after mutation of PhoPQ. When increasing the gene expression of SPI, the gene expression of Isr gradually increases.

The D408A mutant forced more regulation of gene expression for the Flagella, which is important for initiating the early stages of systemic infection. The Lux-S is monitored more, controlling the process of transcription. The upregulation of Lux-S causes metabolites to build-up in the mutated form. High concentrations of metabolites trigger the upregulation of the emrAB-MDR efflux pumps. EmrAB interacts with the Tolc of the AcrAB-Tolc pump, which removes antibiotics and toxins, and free fatty acids from bacterial cells. Therefore, inhibiting AcrAB activates EmrAB, a substitute, under pressure, and uses energy to remove toxins that accumulated.

CONCLUSION

The susceptibility of bacteria to develop more sensitivity of antibiotics depends on the bacteria's metabolism of carbon, exposure to metabolites, its reproductive activity of the horizontal transfer of antibiotic resistant genes, efflux pumps, and the bacteria's direct hydrolysis of antibiotic drugs. For example, *A. baumanii*, which is a common nosocomial bacterium within hospitals, causing urinary tract infections after catheter use, mutates its genes to increase expression nucleotidyltransferase and acetyltransferase. The over expression of these enzymes by *A. baumanii* work to block the aminoglycoside antibiotics attachment to the 16s rRNA site, amplifying the antibiotic resistant capabilities of *A. baumanii*. Therefore, the purpose for this review included making note of alternative methods that can target specific sub-cellular and molecular components, increasing the antibiotic sensitivity of multidrug resistant, MDR, bacterial cells. Altering the metabolic output of an MDR bacterial cell by exposure to glucose, increased the antibiotic sensitivity of *E. tarda*. Researchers cultured *E. tarda* with glucose and alanine, and the uptake of kanamycin increased, eliminating approximately 3,000 times the amount of MDR bacterial cells compared to the cells only treated with kanamycin. Another researcher named Dr. Li mutated a gene of the AcrAB-Tolc binding site, forming a replacement for the highly non-polar phenylalanine amino acid residue with an alanine. His mutagenesis of the efflux pumps binding sites for AcrAB-Tolc inhibited the exit of antibiotics through the AcrAB-Tolc efflux pumps. For future research, conjoining environmental data with clinical research as a possible database can inform microbiologist of the level of hazardous antibiotic

resistant bacteria in many different environments as the soil, sediment, plant, water, and fertilizer. The possibility of antibiotic resistance to spread to humans could be placed into databases.

Currently microbiologist and environmentalist now can identify and analyze the measure of antibiotic resistance in water, plant, and soil based on properties of clinical research. The main issues and challenges for more readily identifying antibiotic resistant bacteria include combining clinical and environmental databases. Fusing clinical protocol with environmental data involves the profuse necessity for forming a registry of criteria that can more systematically regulate a growing population of antibiotic resistant bacteria inhabiting the environment. Presently, there is limited knowledge and many gaps of information for testing and detecting the allocation of antibiotic resistant bacteria and antibiotic resistant genes currently disseminated throughout the environment, which lessens researchers understanding of the transfer of antibiotic resistant bacteria to humans.

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