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Tetracycline Resistance on Protein Synthesis: A Brief Review

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ABSTRACT

Tetracycline is an antibiotic with powerful antibacterial activities against a wide variety if microorganisms. It is a potent antibacterial antibiotic that inhibits protein synthesis to work and it is also a good medication because of its low toxicity and adverse reaction, oral absorption, and effectiveness against disease-causing germs. Its major mechanism of action is based on inhibition of protein synthesis. The relatively low toxicity & allergic reaction, effective oral absorption, and wide range of effectiveness against disease causing microorganisms are reasons why tetracycline is regarded as a good medication. Tetracycline mechanism of action is mainly its inhibitory ability of protein synthesis. It inhibits aminoacyl-tRNA from binding to the ribosome's A site and thus prevent any further amino acid addition to the developing polypeptide chain. It inhibits protein synthesis at once and disrupt several enzymatic activities essential to propagation and survival. This inhibitory ability is less apparent in surrounding human/animal cells during treatment, which is due to inability of such cell to pump tetracycline into their cytoplasm against concentration gradient unlike bacteria cells. In addition, its ability to disrupt cellular membrane causes organelles o leak from the cell and thus impedes propagation or multiplication. However, tetracycline resistance was observed over time. Tetracycline resistance has been observed to be caused by the action of intrinsic enzymes synthesized to inactivates or degrade it. Also, the excretion and efflux of tetracycline from the cytoplasm, thus reducing cytoplasmic concentration and ultimately, its efficacy. Such microorganism possesses membrane proteins or transporters that can export tetracycline at a rate equal to or greater than its influx rate. The transporters could be tetracycline specific or a multidrug transporter. Another mechanism of tetracycline resistance is known as ribosome's protection. Although the mechanism is not well known, ribosome protective resistance protein (TetM) binds to the ribosome's binding site. This molecule has similarities to elongation factor, and it allows the elongation of polypeptide chain while reducing the affinity of the binding site to tetracycline. This ultimately makes tetracycline ineffective against the microorganisms. This mechanism can also be used in conjunction with efflux mechanism of tetracycline resistance.

1. Introduction

In the 1950s when tetracycline was discovered, its antibacterial spectrum exceeded that of any other antibiotic. Tetracyclines are renowned for their powerful antibacterial action against a wide variety of bacteria, including Gram-positive and Gram-negative strains, rickettsia, spirochetes, and even large viruses such as those belonging to the lymphogranuloma group. Tetracyclines are commonly prescribed for a variety of bacterial infections, including those caused by *Escherichia coli* and *Haemophilus influenzae*, bile duct infections, bacterial respiratory disorders (such as bronchitis prophylaxis), mixed infections of the mouth, pharynx, or intestines, brucellosis, tularemia, plague and other pasteurellosis, leptospirosis, and lymphogranuloma. Tetracycline drugs, however, have lost potency due to bacterial resistance. Antibiotics that were formerly the treatment of choice for bacterial illnesses such as staphylococcus, streptococcus, and pneumococcus are now regarded as inferior (Aljedani, 2022; Cholera & Neutropenia, 2022). Tetracyclines are antibiotics that inhibit bacterial growth by interfering with protein synthesis or by causing membrane damage. Increasingly diverse bacteria are acquiring resistance to the bacteriostasis-inhibiting actions of tetracycline (Begum et al., 2021).

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The efflux pump mechanism and the EF-G-like protein offering ribosome protection are both prevalent bacterial resistance mechanisms, although neither degrades tetracycline. In a few species, oxygen degrades tetracycline. Tetracycline resistance in bacteria is the result of many efflux transporters, including multidrug-resistance pumps and tetracycline-specific exporters. The mechanism of tetracycline transport is starting to take form because of the discovery of single amino acids that are crucial for tetracycline transport and substrate selectivity in these carrier proteins (Kyriakidis et al., 2021).

There are several reasons why tetracycline is such a good medication:

- They are effective against most bacteria and viruses.
- The evidence shows that oral absorption is effective.
- Their toxicity is rather low.
- Few individuals are allergic to them.
- Cost-effectiveness is an important selling feature (Terreni et al., 2021)

2. Materials and Methods

In the last 48 years, tetracyclines have been widely made use of for the treatment and prevention of bacterial infections in humans and animals. Due to their broad use, tetracyclines' manufacturing volume has increased the most among antibiotics (Low et al., 2021). Like the development of bacterial resistance to all antimicrobial medications, the emergence of tetracycline-resistant bacteria has led to major restrictions on its usage.

Consequently, one of the most significant future goals in the treatment of infectious diseases is the development of methods to prevent bacterial resistance. Such advancement would be facilitated by a greater understanding of the mechanisms behind antibiotic action and resistance (Keet & Rip, 2021; Tshibangu-Kabamba & Yamaoka, 2021). Following a short introduction of the antibacterial action and absorption of tetracycline, this research focuses on the molecular characteristics of the two most prevalent types of tetracycline resistance. This study will also assess the present level of knowledge about the mechanism of action of tetracycline, tetracycline resistance mechanisms, and the regulation of resistance genes. A further purpose is to collect information on plasmids and other gene-transfer elements, particularly those with the ability to transmit tetracycline-resistance genes.

Structure and Action Mechanism of Tetracycline

Figure 1 shows structure of tetracycline.

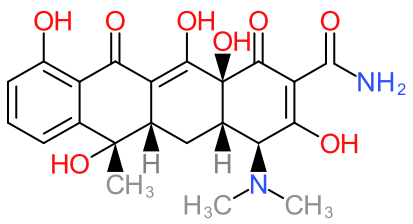

Fig. 1. Tetracycline structure

Apart from dactyclocylines that are novel tetracycline derivatives produced from Dactyclosporangium spp, all other tetracycline derivatives have been successfully used to treat human diseases (Sharma et al., 2018; Vasudevan et al., 2020). According to research, dactylocyclines are different as they are glycosides of tetracycline, and their application is restricted to bacteria devoid of tetracycline resistance genes of class K. In contrast, dactylocyclines are ineffective against microorganisms sensitive to tetracycline. This is presumably due to the inability of gram-negative and some gram-positive bacteria to absorb dactylocyclines, which would normally inhibit their growth (Fitzgerald, 2012; Wang et al., 2019)

Like tetracycline, dactylocyclines inhibit protein synthesis in the linked transcription-translation system (Wang et al., 2019). These results are encouraging because they imply that other tetracycline compounds that are effective against recognized resistance mechanisms may be identified. Success in the development of lactamase-resistant, lactam antibiotics and new quinolones has provided renewed optimism for the creation of novel tetracycline derivatives that are effective against tetracycline-resistant bacteria. For the creation of such drugs, a thorough understanding of resistance mechanisms is important (Kartalidis et al., 2021; Lima et al., 2020).

Tetracycline's Mechanisms of Action

Tetracyclines inhibit aminoacyl-tRNA from binding to the ribosome's A site, hence inhibiting protein synthesis. Tetracycline suppresses bacterial growth by attaching to the 30S component of the ribosome. This prevents any further amino acids from being added to the developing peptide chain. In most instances, the impact is inhibitive and subsides when the medication is discontinued. Tetracycline binds to the small ribosomal subunit of both prokaryotes and eukaryotes, however its effects on mammalian cells are less apparent (30S and 40S, respectively). Unlike human cells, bacteria pump tetracycline forcefully into their cytoplasm against a concentration gradient. This explains why tetracycline has such a low off-target effect on human cells (Bunick et al., 2021; Dmitriev et al., 2020).

Tetracyclines impede several enzymatic activities required for bacterial cells to carry out their essential tasks. Protein synthesis is the most readily inhibited biological process. Tetracycline facilitates the leakage of nucleotides and other substances from cells due to its ability to break the cytoplasmic membrane. In other words, this does not kill the bacteria but stops its multiplication (Bhattacharjee, 2022; Dalhoff, 2021).

Tetracycline Resistance Mechanisms

There are three different ways for bacteria to gain tetracycline resistance:

- Inhibiting tetracycline's access to ribosomes,
- Altering the ribosome to lower the binding affinity of tetracycline, and
- Generating enzymes that degrade tetracycline

In clinical isolates, all three types of resistance were seen. In recent years, several tetracycline resistance genes have been identified, necessitating a classification scheme for them (Bunick et al., 2021; Rusu & Buta, 2021).

Protection of Ribosomes from Tetracycline

Consumption falls: Tetracycline can only inhibit protein synthesis if it enters the cell of the bacteria and binds to the ribosome. Tetracycline build-up in the cytoplasm of tetracycline-susceptible bacteria cannot be explained only by diffusion (Hörömpöli et al., 2021; Ramachanderan & Schaefer, 2021; Sanganyado & Gwenzi, 2019).

Tetracycline exists in two forms: the protonated (TH2) form and the magnesium (Mg) chelated form (THMg). Unlike the THMg form, which cannot diffuse into phospholipid bilayers, the TH2 form diffuses readily. As the internal pH of the bacteria is larger than its outside pH, tetracycline stays trapped as THMg inside the bacteria (Sanganyado & Gwenzi, 2019). Tetracycline-resistant bacteria would have a tough time growing if the medication could only be taken up by diffusion across the cytoplasmic membrane. Not surprisingly, no such resistance has emerged (Michael-Kordatou et al., 2018). Modification of porin proteins (such as OmpF) to inhibit the diffusion of tetracycline into the periplasm is a possible mechanism of resistance in gram-negative bacteria. Multiple gram-negative bacteria possess this resistance mechanism, which may diminish sensitivity by a ratio of 6 to 18 (Prajapati et al., 2021). Antibiotics such as 3-lactams and fluoroquinolones are no longer effective against these resistant bacteria. Recent research indicates that variations in OmpF and other outer membrane proteins contribute to the diverse antibiotic resistances of some E. coli strains (Bello & Dingle, 2018; Ropponen et al., 2021).

- Tetracycline (T) may decrease protein synthesis in sensitive bacteria by binding to their ribosomes after the medication has accumulated to a sufficient level inside the bacterium (Salyers et al., 1990).

- Tetracycline is efficiently exported from the cell when bacteria with an efflux-type resistance gene produce a cytoplasmic membrane protein. This keeps an intracellular concentration low enough for protein synthesis to commence (Sharkey & O'Neill, 2018; Shen et al., 2020).

- In response to high intracellular concentrations of the drug, bacteria with a ribosome protection-type resistance gene produce a 72-kDa cytoplasmic protein that binds to the ribosomes and allows them to continue protein synthesis. Although one of the ribosomal subunits obviously darkens when the 72-kDa protein is active, it is still unknown if the resistance protein interacts with the ribosome.

- Tetracycline-resistant bacteria produce a 44-kilodalton enzyme that converts tetracycline (T) to its inactive (t) form, enabling the medication to escape the cell unimpeded. Oxygen and NADPH are essential to the enzymatic reaction (Salyers et al., 1990).

3. Results and Discussion

Excretion of Tetracycline

Tetracycline's access to ribosomes may also be restricted by pumping the antibiotic out of the cell at a rate equal to or greater than its absorption. It is reasonable to conclude that tetracycline efflux is the most studied and well-known antibiotic resistance mechanism in this class (Lakemeyer et al., 2018).

The tetracycline transporter protein expressed by the resistance gene is a cytoplasmic membrane protein whose optimal functioning needs energy. The mechanisms by which this efflux protein eliminates tetracycline from the cell and offers cell protection remain unknown. The efflux of tetracycline clearly reduces its buildup within sensitive cells, but the intracellular concentration of tetracycline is still quite high compared to the levels that inhibit protein synthesis. One explanation is that tetracycline may exist in several ionic forms. Perhaps one ribosome form fits better than the other (Hong, 2018; Tripathi, 2020).

Protein synthesis may need the maintenance of high intracellular concentrations of tetracycline through preferential pumping of the active form if this is the case. It is also likely that the co-induced repressor protein and Tet protein are responsible for the high internal levels of tetracycline (Henderson et al., 2021). It is also possible that ribosomes in living cells are more resistant to tetracycline than those in in vitro systems.

There are currently eight distinct kinds of tetracycline efflux genes. Classes A through E are used to categorize the family Enterobacteriaceae, which includes the genera Haemophilus, Vibrio, Aeromonas, and Moraxella (Sony et al., 2021; Tshibangu-Kabamba & Yamaoka, 2021). The class P gene, which encodes tetracycline efflux-type resistance, has recently been discovered to be exclusive to Clostridium species (Hendriksen et al., 2019; Korry et al., 2020). Only gram-positive bacteria contain the K and L classes. At first sight, it seemed that *Staphylococcus spp*. were more likely to include class K than Streptococcus and Enterococcus spp (Weiner-Lastinger et al., 2020). On the other hand, recent findings of gram-positive cocci and Bacillus spp. strains carrying both class K and class L resistance genes have cast doubt on the existence of such a clear division (Sánchez-López et al., 2020).

Although sensitive to the lipophilic analog minocycline has been used to classify efflux resistance genes in the past, DNA-DNA hybridization is currently the preferred technique. It has been shown that bacteria having resistance genes from Efflux Classes A, B, E, and K are more resistant to minocycline than bacteria harboring resistance genes from other efflux classes, with Class B exhibiting much higher resistance than Classes A, E, and K. The reason for this disparity is unknown (in minocycline susceptibility), but it implies there may be two functional classes of efflux-based resistance (Taiariol et al., 2021).

Ives and Bott showed that a region of the B. Subtilis chromosome near to the replication beginning may provide tetracycline resistance when the copy number is increased (Ives & Bott, 1990). Tetracycline resistance was supplied by several tandem duplications of this chromosomal region, while the initial strain bearing a single copy of this area was sensitive to the antibiotic. The same tetracycline resistance was seen when the region was cloned into a plasmid with several copies (Li et al., 2015). Clearly, a single copy of the gene is insufficient to provide resistance owing to inadequate expression. The sequencing of the gene revealed that it belonged to class L (Ives & Bott, 1990).

Not all Bacillus subtilis strains include the cryptic tetracycline efflux gene (Ogawara, 2019; Wu et al., 2019). Although *B. subtilis* is not a pathogen that affects humans, the discovery of a hidden resistance gene in this strain suggests that other potentially harmful organisms may also harbor cryptic resistance genes. There is a high degree of

DNA sequence similarity across the different categories of structural efflux resistance genes. Classes A and C share 74% of their genetic makeup, but classes B and C share just 45% of their genetic makeup (Bahram et al., 2018; Sawa et al., 2020).

Similarly, tet(K) and tet(L), two gram-positive efflux structural genes, share 69% of their DNA sequence (Breijyeh et al., 2020; Sun et al., 2019). The phylogenetic research of classes A–C and classes K–L has indicated that these two groupings are likely descended from a common ancestor through diverging lines of ancestry (Koonin et al., 2020). *Streptomyces nimosus*, which has a resistance gene, may represent a third bacterial branch. The amino-terminal regions of tetracycline efflux proteins resemble those of other proton-dependent transport proteins, including sugar transporters. The efflux-type resistance genes may have descended from transport genes as a result (Bianchi et al., 2019; Du et al., 2018; Osman et al., 2019).

Safeguarding Ribosomes

The most well-known type of tetracycline resistance is tetracycline efflux, while ribosome protection is less well-known. Despite its relative unfamiliarity, this mechanism is probably more widespread than tetracycline efflux (Ashhurst-Smith, 2012). On sodium dodecyl sulphate polyacrylamide gel electrophoresis, the resistance gene product migrates as a 68-kDa protein; however, DNA sequence analysis reveals that the actual size of the protein is 72 kDa. This cytoplasmic protein interacts with the ribosome, making it resistant to tetracycline inhibition (Niu et al., 2021; Sträter et al., 2021). It is unknown precisely how the resistance protein interacts with the ribosomes. It has been shown that one of the ribosome protective resistance proteins (TetM) binds to ribosomes.

TetM has no effect on the binding of tetracycline to ribosomes, as shown by Manavathu et al. in 1990. TetM does not appear to catalyze the covalent modification of a ribosomal component, unlike erythromycin (Bhattacharjee, 2022). The specific function of the ribosome protection protein is still up for dispute until its binding sites on the ribosome and its role in protein synthesis are established. Tet(M), Tet(O), and Tet(Q), three distinct families of ribosome protective resistance genes, have been discovered through characterization and sequencing research (Sharkey & O'Neill, 2018).

In 1986, Burdett and others postulated the presence of a second class of ribosome protection genes in streptococci, which they referred to as class N (Burdett, 1986). However, further research has disproven this notion. Tet(M) was first discovered in gram-positive cocci, but it has now been recognized in many additional bacterial species. Included in this category are Neisseria, Haemophilus, Mycoplasma, Ureaplasma, Streptococcus, Staphylococcus, Peptostreptococcus, Bacteroides, Kingella, and Bacillus spp. The amino acid sequence of ribosome protection resistance proteins is very similar to that of elongation factor G (Breijyeh et al., 2020; Hayes et al., 2020; Price et al., 2018).

In the GTP-binding site region of elongation factor G, homology is greatest. TetM has ribosome dependent GTPase activity, like elongation factor G (Burdett, 1991). This indicates that genes producing bacterial elongation factors may have been the origins of ribosome protection genes. Combinations of efflux and ribosome protection resistance genes have been found in bacterial strains (Berglund et al., 2020; Roberts, 2019). It was discovered in 1990 that many Staphylococcus aureus strains carry both tet(K) and tet(O) and that some isolates have all three (M). Also, Roberts observed that some strains of Streptococcus spp. and Peptostreptococcus spp. It would be intriguing to see if the overall impact of these several forms of resistance is greater than the effect of any one type of resistance alone (Roberts, 2019).

4. Conclusion

Tetracyclines are great candidates for reintroduction into medicine due to their numerous favorable characteristics. The recent discovery of a new family of tetracycline derivatives, the dactylocyclines, implies that further classes of tetracycline may be found in the future. After studies revealed that certain tetracycline derivatives may not hinder protein synthesis, there is reason to anticipate that new tetracycline derivatives may be on the horizon. Bacteria exposed to tetracycline derivatives that do not target the ribosome are unlikely to acquire tetracycline-resistant genes that protect the ribosome. To elucidate the mechanisms of tetracycline efflux and ribosome protection, further study is required.

List of Abbreviations

EF-G - Elongation Factor G

t-RNA - Transfer RNA

TH2 - T helper type 2

THMg - T helper cells with magnesium

OmpF - Outer Membrane Protein F

kDa - Kilodalton

NADPH - Nicotinamide Adenine Dinucleotide Phosphate

DNA - Deoxyribonucleic acid

RNA - Ribonucleic acid

Competing Interests

The authors have declared that no competing interests exist.

References

Aljedani, D. M. (2022). Antibiotic treatment (Tetracycline) effect on bio-efficiency of the larvae honey bee (Apis mellifera jemenatica). Saudi Journal of Biological Sciences, 29(3), 1477-1486. **https://doi.org/10.1016/j.sjbs.2021.11.024**

Ashhurst-Smith, C., Hall, S. T., Stuart, J., Burns, C. J., Liet, E., Walker, P. J., ... & Blackwell, C. C. (2012). Alloiococcus otitidis: an emerging pathogen in otitis media. Journal of Infection, 64(2), 233-235. **https://doi.org/10.1016/j.jinf.2011.11.008**

Bahram, M., Hildebrand, F., Forslund, S. K., Anderson, J. L., Soudzilovskaia, N. A., Bodegom, P. M., ... & Bork, P. (2018). Structure and function of the global topsoil

microbiome. Nature, 560(7717), 233-237. **https://doi.org/10.1038/s41586-018-0386-6**

Begum, S., Begum, T., Rahman, N., & Khan, R. A. (2021). A review on antibiotic resistance and way of combating antimicrobial resistance. GSC Biological and Pharmaceutical Sciences, 14(2), 087-097. **https://doi.org/10.30574/gscbps.2021.14.2.0037**

Bello, A., & Dingle, T. C. (2018). What's that resistance mechanism? Understanding genetic determinants of gram-negative bacterial resistance. Clinical Microbiology Newsletter, 40(20), 165-174. **https://doi.org/10.1016/j.clinmicnews.2018.10.001**

Berglund, F., Böhm, M. E., Martinsson, A., Ebmeyer, S., Österlund, T., Johnning, A., ... & Kristiansson, E. (2020). Comprehensive screening of genomic and metagenomic data reveals a large diversity of tetracycline resistance genes. Microbial genomics, 6(11). **https://doi.org/10.1099%2Fmgen.0.000455**

Bhattacharjee, M. K. (2022). Antibiotics that inhibit protein synthesis. In Chemistry of Antibiotics and Related Drugs (pp. 149-177). Cham: Springer International Publishing. **https://doi.org/10.1007/978-3-031-07582-7_6**

Bianchi, F., van't Klooster, J. S., Ruiz, S. J., & Poolman, B. (2019). Regulation of amino acid transport in Saccharomyces cerevisiae. Microbiology and Molecular Biology Reviews, 83(4), e00024-19. **https://doi.org/10.1128/MMBR.00024-19**

Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules, 25(6), 1340. **https://doi.org/10.3390/molecules25061340**

Bunick, C. G., Keri, J., Tanaka, S. K., Furey, N., Damiani, G., Johnson, J. L., & Grada, A. (2021). Antibacterial mechanisms and efficacy of sarecycline in animal models of infection and inflammation. Antibiotics, 10(4), 439. **https://doi.org/10.3390/antibiotics10040439**

Burdett, V. (1991). Purification and characterization of Tet (M), a protein that renders ribosomes resistant to tetracycline. Journal of Biological Chemistry, 266(5), 2872-2877. **https://doi.org/10.1016/S0021-9258(18)49928-0**

Burdett, V. I. C. K. E. R. S. (1986). Streptococcal tetracycline resistance mediated at the level of protein synthesis. Journal of bacteriology, 165(2), 564-569. **https://doi.org/10.1128/jb.165.2.564-569.1986**

Cholera, E. P. E. C., & Neutropenia, P. (2022). Clinical syndromes. Schlossberg's Clinical Infectious Disease, 335.

Dalhoff, A. (2021). Selective toxicity of antibacterial agents—still a valid concept or do we miss chances and ignore risks?. Infection, 49, 29-56. **https://doi.org/10.1007/s15010-020-01536-y**

Dmitriev, S. E., Vladimirov, D. O., & Lashkevich, K. A. (2020). A quick guide to small-molecule inhibitors of eukaryotic protein synthesis. Biochemistry (Moscow), 85, 1389-1421. **https://doi.org/10.1134/S0006297920110097**

Du, D., Wang-Kan, X., Neuberger, A., Van Veen, H. W., Pos, K. M., Piddock, L. J., & Luisi, B. F. (2018). Multidrug efflux pumps: structure, function and regulation. Nature Reviews Microbiology, 16(9), 523-539. **https://doi.org/10.1038/s41579-018-0048-6**

Fitzgerald, J. (2012). Engineering of Biosynthetic Pathways to the Type II Polyketides Frenolicin B and A-74528. Stanford University.

Hayes, K., O'Halloran, F., & Cotter, L. (2020). A review of antibiotic resistance in Group B Streptococcus: the story so far. Critical reviews in microbiology, 46(3), 253-269. **https://doi.org/10.1080/1040841X.2020.1758626**

Henderson, P. J., Maher, C., Elbourne, L. D., Eijkelkamp, B. A., Paulsen, I. T., & Hassan, K. A. (2021). Physiological functions of bacterial "multidrug" efflux pumps. Chemical reviews, 121(9), 5417-5478. **https://doi.org/10.1021/acs.chemrev.0c01226**

Hendriksen, R. S., Lukjancenko, O., Munk, P., Hjelmsø, M. H., Verani, J. R., Ng'eno, E., ... & Aarestrup, F. M. (2019). Pathogen surveillance in the informal settlement, Kibera, Kenya, using a metagenomics approach. PLoS One, 14(10), e0222531. **https://doi.org/10.1371/journal.pone.0222531**

Hong, X. (2018). Regulation of the enzymes that recycle the universal glycan carrier dictates bacterial outer membrane composition (Doctoral dissertation, Yale University).

Hörömpöli, D., Ciglia, C., Glüsenkamp, K. H., Haustedt, L. O., Falkenstein-Paul, H., Bendas, G., ... & Brötz-Oesterhelt, H. (2021). The antibiotic negamycin crosses the bacterial cytoplasmic membrane by multiple routes. Antimicrobial Agents and Chemotherapy, 65(4), e00986-20. **https://doi.org/10.1128/AAC.00986-20**

Ives, C. L., & Bott, K. F. (1990). Characterization of chromosomal DNA amplifications with associated tetracycline resistance in Bacillus subtilis. Journal of bacteriology, 172(9), 4936-4944. **https://doi.org/10.1128/jb.172.9.4936-4944.1990**

Kartalidis, P., Skoulakis, A., Tsilipounidaki, K., Florou, Z., Petinaki, E., & Fthenakis, G. C. (2021). Clostridioides difficile as a dynamic vehicle for the dissemination of antimicrobial-resistance determinants: review and in silico analysis. Microorganisms, 9(7), 1383. **https://doi.org/10.3390/microorganisms9071383**

Keet, R., & Rip, D. (2021). Listeria monocytogenes isolates from Western Cape, South Africa exhibit resistance to multiple antibiotics and contradicts certain global resistance patterns. AIMS microbiology, 7(1), 40. **https://doi.org/10.3934%2Fmicrobiol.2021004**

Koonin, E. V., Dolja, V. V., Krupovic, M., Varsani, A., Wolf, Y. I., Yutin, N., ... & Kuhn, J. H. (2020). Global organization and proposed megataxonomy of the virus world. Microbiology and molecular biology reviews, 84(2), e00061-19. **https://doi.org/10.1128/MMBR.00061-19**

Korry, B. J., Cabral, D. J., & Belenky, P. (2020). Metatranscriptomics reveals antibiotic-induced resistance gene expression in the murine gut microbiota. Frontiers in Microbiology, 11, 322. **https://doi.org/10.3389/fmicb.2020.00322**

Kyriakidis, I., Vasileiou, E., Pana, Z. D., & Tragiannidis, A. (2021). Acinetobacter baumannii antibiotic resistance mechanisms. Pathogens, 10(3), 373. **https://doi.org/10.3390/pathogens10030373**

Lakemeyer, M., Zhao, W., Mandl, F. A., Hammann, P., & Sieber, S. A. (2018). Thinking outside the box—Novel antibacterials to tackle the resistance crisis. Angewandte Chemie International Edition, 57(44), 14440-14475. **https://doi.org/10.1002/anie.201804971**

Li, G., Zhang, J., Guo, Q., Jiang, Y., Wei, J., Zhao, L. L., ... & Wan, K. (2015). Efflux pump gene expression in multidrug-resistant Mycobacterium tuberculosis clinical isolates. PloS one, 10(2), e0119013. **https://doi.org/10.1371/journal.pone.0119013**

Lima, T., Domingues, S., & Da Silva, G. J. (2020). Manure as a potential hotspot for antibiotic resistance dissemination by horizontal gene transfer events. Veterinary sciences, 7(3), 110. **https://doi.org/10.3390/vetsci7030110**

Low, C. X., Tan, L. T. H., Ab Mutalib, N. S., Pusparajah, P., Goh, B. H., Chan, K. G., ... & Lee, L. H. (2021). Unveiling the impact of antibiotics and alternative methods for animal husbandry: A review. Antibiotics, 10(5), 578. **https://doi.org/10.3390/antibiotics10050578**

Michael-Kordatou, I., Karaolia, P., & Fatta-Kassinos, D. (2018). The role of operating parameters and oxidative damage mechanisms of advanced chemical oxidation processes in the combat against antibiotic-resistant bacteria and resistance genes present in urban wastewater. Water research, 129, 208-230. **https://doi.org/10.1016/j.watres.2017.10.007**

Niu, P., Chen, Z., Ren, X., Han, W., Dong, H., Shen, R., ... & Yu, S. (2021). A Riemerella anatipestifer metallophosphoesterase that displays phosphatase activity and is associated with virulence. Applied and Environmental Microbiology, 87(11), e00086-21. **https://doi.org/10.1128/AEM.00086-21**

Ogawara, H. (2019). Comparison of antibiotic resistance mechanisms in antibiotic-producing and pathogenic bacteria. Molecules, 24(19), 3430. **https://doi.org/10.3390/molecules24193430**

Osman, G. E., Abulreesh, H. H., Elbanna, K., & Shaaban, M. R. (2019). Samreen, iqbal ahmad, recent progress in metal-microbe interactions: Prospects in bioremediation. J Pure Appl Microbiol, 13(1), 13-26. **https://dx.doi.org/10.22207/JPAM.13.1.02**

Prajapati, J. D., Kleinekathöfer, U., & Winterhalter, M. (2021). How to enter a bacterium: bacterial porins and the permeation of antibiotics. Chemical reviews, 121(9), 5158-5192. **https://doi.org/10.1021/acs.chemrev.0c01213**

Price, T. K., Hilt, E. E., Dune, T. J., Mueller, E. R., Wolfe, A. J., & Brubaker, L. (2018). Urine trouble: should we think differently about UTI?. International urogynecology journal, 29, 205-210. **https://doi.org/10.1007/s00192-017-3528-8**

Ramachanderan, R., & Schaefer, B. (2021). Tetracycline antibiotics. ChemTexts, 7(3), 18. **https://doi.org/10.1007/s40828-021-00138-x**

Roberts, M. C. (2019). Tetracyclines: Mode of action and their bacterial mechanisms of resistance. Bacterial Resistance to Antibiotics–from Molecules to Man, 101-124. **https://doi.org/10.1002/9781119593522.ch5**

Ropponen, H. K., Richter, R., Hirsch, A. K., & Lehr, C. M. (2021). Mastering the Gram-negative bacterial barrier–Chemical approaches to increase bacterial bioavailability of antibiotics. Advanced Drug Delivery Reviews, 172, 339-360. **https://doi.org/10.1016/j.addr.2021.02.014**

Rusu, A., & Buta, E. L. (2021). The development of third-generation tetracycline antibiotics and new perspectives. Pharmaceutics, 13(12), 2085. **https://doi.org/10.3390/pharmaceutics13122085**

Salyers, A. A., Speer, B. S., & Shoemaker, N. B. (1990). New perspectives in tetracycline resistance. Molecular microbiology, 4(1), 151-156. **https://doi.org/10.1111/j.1365-2958.1990.tb02025.x**

Sánchez-López, E., Gomes, D., Esteruelas, G., Bonilla, L., Lopez-Machado, A. L., Galindo, R., ... & Souto, E. B. (2020). Metal-based nanoparticles as antimicrobial agents: an overview. Nanomaterials, 10(2), 292. **https://doi.org/10.3390/nano10020292**

Sanganyado, E., & Gwenzi, W. (2019). Antibiotic resistance in drinking water systems: Occurrence, removal, and human health risks. Science of the Total Environment, 669, 785-797. **https://doi.org/10.1016/j.scitotenv.2019.03.162**

Sawa, T., Kooguchi, K., & Moriyama, K. (2020). Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. Journal of intensive care, 8, 1-13. **https://doi.org/10.1186/s40560-020-0429-6**

Sharkey, L. K., & O'Neill, A. J. (2018). Antibiotic resistance ABC-F proteins: bringing target protection into the limelight. ACS Infectious Diseases, 4(3), 239-246. **https://doi.org/10.1021/acsinfecdis.7b00251**

Sharma, P., Dutta, J., & Thakur, D. (2018). Future prospects of actinobacteria in health and industry. In New and Future Developments in Microbial Biotechnology and Bioengineering (pp. 305-324). Elsevier. **https://doi.org/10.1016/B978-0-444-63994-3.00021-7**

Shen, J., Liu, Z., Yu, H., Ye, J., Long, Y., Zhou, P., & He, B. (2020). Systematic stress adaptation of Bacillus subtilis to tetracycline exposure. Ecotoxicology and Environmental Safety, 188, 109910. **https://doi.org/10.1016/j.ecoenv.2019.109910**

Sony, M., Sumithra, T. G., Anusree, V. N., Amala, P. V., Reshma, K. J., Alex, S., & Sanil, N. K. (2021). Antimicrobial resistance and virulence characteristics of Vibrio vulnificus, Vibrio parahaemolyticus and Vibrio harveyi from natural disease outbreaks of marine/estuarine fishes. Aquaculture, 539, 736608. **https://doi.org/10.1016/j.aquaculture.2021.736608**

Strätker, K., Haidar, S., Dubiel, M., Estévez-Braun, A., & Jose, J. (2021). Autodisplay of human PIP5K1α lipid kinase on Escherichia coli and inhibitor testing. Enzyme and Microbial Technology, 143, 109717. **https://doi.org/10.1016/j.enzmictec.2020.109717**

Sun, J., Chen, C., Cui, C. Y., Zhang, Y., Liu, X., Cui, Z. H., ... & Liu, Y. H. (2019). Plasmid-encoded tet (X) genes that confer high-level tigecycline resistance in Escherichia coli. Nature microbiology, 4(9), 1457-1464. **https://doi.org/10.1038/s41564-019-0496-4**

Taiariol, L., Chaix, C., Farre, C., & Moreau, E. (2021). Click and bioorthogonal chemistry: the future of active targeting of nanoparticles for nanomedicines?. Chemical Reviews, 122(1), 340-384. **https://doi.org/10.1021/acs.chemrev.1c00484**

Terreni, M., Taccani, M., & Pregnolato, M. (2021). New antibiotics for multidrug-resistant bacterial strains: latest research developments and future perspectives. Molecules, 26(9), 2671. **https://doi.org/10.3390/molecules26092671**

Tripathi, K. D. (2020). Essentials of pharmacology for dentistry. Jaypee Brothers Medical Publishers.

Tshibangu-Kabamba, E., & Yamaoka, Y. (2021). Helicobacter pylori infection and antibiotic resistance—from biology to clinical implications. Nature Reviews Gastroenterology & Hepatology, 18(9), 613-629. **https://doi.org/10.1038/s41575-021-00449-x**

Vasudevan, U. M., & Lee, E. Y. (2020). Flavonoids, terpenoids, and polyketide antibiotics: Role of glycosylation and biocatalytic tactics in engineering glycosylation. Biotechnology advances, 41, 107550. **https://doi.org/10.1016/j.biotechadv.2020.107550**

Wang, X., Yin, S., Bai, J., Liu, Y., Fan, K., Wang, H., ... & Wang, W. (2019). Heterologous production of chlortetracycline in an industrial grade Streptomyces rimosus host. Applied microbiology and biotechnology, 103, 6645-6655. **https://doi.org/10.1007/s00253-019-09970-1**

Weiner-Lastinger, L. M., Abner, S., Edwards, J. R., Kallen, A. J., Karlsson, M., Magill, S. S., ... & Dudeck, M. A. (2020). Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: summary of data reported to the National Healthcare Safety Network, 2015–2017. Infection Control & Hospital Epidemiology, 41(1), 1-18. **https://doi.org/10.1017/ice.2019.296**

Wu, W., Feng, Y., Tang, G., Qiao, F., McNally, A., & Zong, Z. (2019). NDM metallo-β-lactamases and their bacterial producers in health care settings. Clinical microbiology reviews, 32(2), e00115-18. **https://doi.org/10.1128/CMR.00115-18**