SCIENCE LETTERS

eISSN: 2682-8626

Available online at
https://scilett-fsg.uitm.edu.my/

Science Letters 18(2) 2024, 56 – 69.

Science Letters

Understanding Bacterial Persistence under Antibiotic Pressure: A Review

Intan Nurfarzana Mohd Safini, Nur Fatihah Sholehah Zakaria, Muhammad Iqbal Hafiz Saad, Mohd Fakharul Zaman Raja Yahya*, Norashirene Mohamad Jamil*

School of Biology, Faculty of Applied Science, Universiti Teknologi MARA, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 8 March 2024
Revised 15 May 2024
Accepted 1 June 2024
Online first
Published 24 June 2024

Keywords:
Antibiotics
Antibiotic resistance
Multidrug resistance genes
Molecular mechanisms

DOI:
10.24191/sl.v18i2.27017

ABSTRACT

Antibiotic resistance is a significant global health concern, with multidrug-resistant bacteria emerging in hospitals and community settings. This highlights the presence of antibiotic-resistance genes beyond hospital environments. Bacterial adaptations in response to selective pressures drive the growth of antibiotic resistance. To survive these challenges, bacteria develop various defense mechanisms, including chemical modification of antibiotics, enzyme-catalyzed degradation, altered permeability, efflux, mutation of target sites, and biofilm formation. As a result, bacteria become resistant to most currently available antibiotics. This review provides insights into the molecular mechanisms of antibiotic resistance, which can improve strategies for combating resistance and developing new therapeutic approaches to counter multidrug-resistant bacterial infections.

INTRODUCTION

The discovery of antibiotics, their commercialization, and their administration to treat infections has improved therapy and revolutionized modern medicine. Indeed, antibiotic administration has become one of the key medical procedures required for routine clinical interventions such as organ transplantation, surgery, and cancer care. Unfortunately, the significant rise in antibiotic resistance among common bacterial pathogens now threatens this therapeutic achievement, challenging critical patient treatment [1]. Resistance to antibiotics has been described as one of the highest threats to public health in the 21st century [2].

Antibiotic resistance occurs when bacteria have the ability to survive or grow in the presence of an antibiotic concentration that is usually adequate to kill or inhibit their growth [3]. The lateral transfer of antibiotic resistance genes or spontaneous mutations may lead to the resistance trait. The transfer of genes that are resistant to antibiotics can occur via various mechanisms, such as conjugation, transformation, transduction, nanotubes, gene transfer agents (GTA), and membrane vesicles [4]. Conjugation generally transfers genetic material from a donor to a recipient, which requires cell-to-cell contact. The production of nosocomial resistance usually involves the conjugation strategy, which is a highly successful rate of gene transfer method involving mobile genetic elements (MGEs) such as conjugative plasmids, transposon, or integrons. MGEs are vital in disseminating antibiotic resistance genes between clinically relevant species [5]. Transformation is a direct uptake and expression of the extracellular DNA from the environment into a naturally competent recipient cell. Transformation can be the most basic kind of horizontal gene transfer

* Corresponding authors. *E-mail addresses*: fakharulzaman@uitm.edu.my and norashirene@uitm.edu.my

(HGT), but only a few bacterial species may incorporate naked DNA "naturally" for the development of resistance [6]. As for transduction, the mechanism is phage-mediated [7].

In clinical practice, the terms 'resistant' and 'susceptible' are usually used to determine treatment's possible failure or success. Resistance is more likely to occur when the concentration required to kill or inhibit microorganisms fails to be achieved in a patient [3]. In the 2019 AR Threats Report, the Centers for Disease Control and Prevention (CDC) states that over 2.8 million antibiotic-resistant infections are occurring in the US, resulting in over 35,000 deaths each year. It was also highlighted that there were about 223,900 cases of *Clostridioides difficile* infections reported in 2017, which killed at least 12,800 people [8]. An inadequate supply of effective antibiotics exacerbates this condition, making infections nearly incurable and leaving clinicians with no safe options for treating patients who are infected.

Understanding the molecular basis of bacterial resistance mechanisms to combat antibiotic resistance is critically important. Other than becoming intrinsically resistant to antibiotics, microorganisms could also develop resistance (acquired resistance) following exposure to the antibiotic [9]. Intrinsic resistance can be defined as a specific bacteria's innate ability to resist antibiotic actions due to their inherent functional or structural characteristics [10]. This can be achieved by extruding antibiotic molecules through efflux pumps and reducing the permeability of the bacterial outer membrane. In contrast, acquired resistance occurs due to the acquisition of external resistance genetic determinants or mutations in chromosomal genes [11]. Acquired resistance caused the bacteria to resist a particular antibiotic's activity to which it was previously susceptible. Figure 1 summarizes common resistance mechanisms used by pathogenic bacteria.

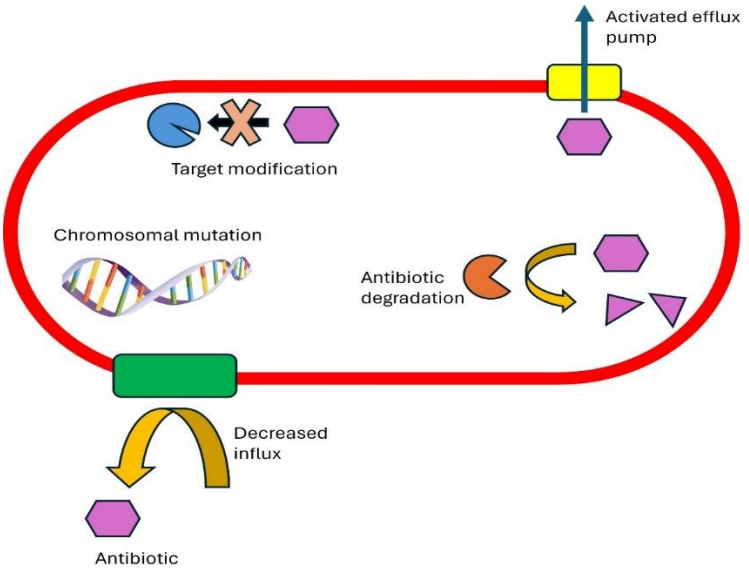

Fig. 1. Antibiotic resistance strategies in bacteria.

CHEMICAL MODIFICATION AND DEGRADATION OF ANTIBIOTICS

Chemical modification and degradation of antibiotics are effective mechanisms for Gram-negative and Gram-positive bacteria to survive in the presence of antibiotics [9, 12]. The chemical modification of the

https://doi.org/10.24191/sl.v18i2.27017

antibiotic is widely seen to occur in the group of antibiotics that inhibits the synthesis of protein at the ribosome level [13].

Antibiotic inactivation

Aminoglycosides are one of the broad-spectrum antibiotics used to treat serious infections such as tuberculosis caused by *Mycobacterium tuberculosis* [14]. Additionally, aminoglycosides are effective against members of the Enterobacteria family, including *Escherichia coli* and *Klebsiella pneumoniae* [15]. Aminoglycoside binds effectively to the A-site located in the 16S rRNA of the 30S ribosomal subunit, inhibiting protein synthesis [16]. From the interaction, aminoglycoside encourages mistranslation by codon misreading, thus resulting in a protein synthesis error that leads to cell damage [17]. Disrupting protein synthesis can kill bacteria [18].

Chemical modification of antibiotics is facilitated by various modifying enzymes, also known as transferases. These enzymes are known as aminoglycoside-modifying enzymes (AMEs), and these enzymes' production is capable of introducing chemical changes to the molecular structure of antibiotics [19]. AMEs covalently modify the amino (-NH_2) or hydroxyl (-OH) group of aminoglycoside molecules and genes encoding AMEs, which are typically carried out by MGEs [19]. These modifying enzymes have different biochemical reactions with various catalytic activities such as phosphorylation (*O*-phosphotransferases), adenylation (*O*-adenyltransferases), and acetylation (*N*-acetyltransferases) [17]. Antibiotics lose their antibacterial potency when AMEs add functional groups to aminoglycosides because of conformational changes that occur, regardless of the specific biochemical reactions involved [20]. Such changes reduce the antibiotics' affinity for binding to their target sites.

Antibiotic inactivation via phosphorylation is a biochemical process that transfers the phosphate group to the hydroxyl group of antibiotics by the action of aminoglycoside phosphotransferases (APHs) enzyme and is widely seen in aminoglycoside and chloramphenicol [21]. An example of this is the phosphorylation of kanamycin and neomycin by APH (3'), which causes the antibiotics to be unable to bind to their ribosomal target site [19]. Adenylation is the process of transferring the AMP molecule to the hydroxyl group of antibiotics, catalyzed by aminoglycoside nucleotidyltransferases (ANTs) and lincosamide nucleotidyltransferases (LNU) [22]. For instance, lincosamide nucleotidyltransferase, encoded by *linB* genes, can inactivate clindamycin, a class of lincosamides, in *E. Coli* and *Staphylococcus aureus* [23]. As for aminoglycosides, ANT (2″) is an example of an enzyme that affects the activity of 4,6-di-substituted aminoglycosides first described in *K. pneumoniae* [16]. On another note, acetylation is a process of transferring the functional group of acetyls to the amino group of antibiotics catalyzed by aminoglycoside acetyltransferases (AACs) enzyme that usually occurs to amikacin, netilmicin, and tobramycin by AAC (6′)-1 enzyme [24, 25].

Another example of resistance through chemical modification of antibiotics can be seen in chloramphenicol. By interacting with the peptidyl-transferase center of the 50S ribosomal subunit, chloramphenicol inhibits bacterial protein synthesis [9]. Producing acetyltranferases enzyme known as chloramphenicol acetyltransferases (CATs) facilitates the modification of chloramphenicol. The transfer of the acetyl group from acetyl-coenzyme A (AcCoA) to the 3-hydroxyl group of chloramphenicol had caused bacterial resistance towards the antibiotic [26]. In Gram-negatives and Gram-positives bacteria, multiple *cat* genes have been found and grouped into two main types: Type A, which results in high-level resistance, and Type B, which results in low-level resistance [27]. These two types of CATs also differ in their structure component, where type A CAT monomers range from 207 to 238 amino acids while type B CAT monomers are smaller, with 209 to 219 amino acids [28].

https://doi.org/10.24191/sl.v18i2.27017

Decreased permeability

Most antibiotics have specific intracellular bacterial targets. The antibiotics must go through the cytoplasmic membrane to be effective; however, some bacteria can prevent the antibiotic from reaching its target sites by lowering the uptake of the antibiotic molecules [9]. The Gram-negative bacteria's outer membrane provides the organism an additional protective layer without compromising the material exchange needed to sustain life [29]. Therefore, Gram-negative bacteria are intrinsically less permeable to many antibiotics than Gram-positive bacteria, as their outer membrane can act as a barrier [30].

The outer membrane of Gram-negative bacteria consists of proteins called porins. Various porin types have been described, and they can be categorized by their selectivity, structure (monomeric vs. trimeric), and regulation of expression. The *Pseudomonas aeruginosa's* OprD, also referred to as protein D2, and the three major proteins in *E. coli* called OmpC, OmpF, and PhoE, are among the best-characterized porins [31]. These two bacteria are also examples of Gram-negative bacteria that employ porin-mediated antibiotic resistance.

Antibiotics with hydrophilic properties, such as tetracyclines, some fluoroquinolones, and β-lactam, are mostly affected by the permeability changes of outer membranes since they use porins to go through the barrier [32]. Permeability changes occurred by three general processes, which are the (i) change in the porin expression level, (ii) shift in the type of porins expressed, and (iii) the impairment of porin functions [33].

One of the general processes of porin-mediated antibiotic resistance is the changes in the expression level of the porin. Data shows that reduction in expression of porin contributes significantly to the development of resistance in Enterobacteriaceae family, *Acinetobacter spp.*, and *Pseudomonas spp.* to newer antibiotics such as cephalosporins and carbapenems to which enzyme degradation usually mediates the resistance [34]. For instance, it has been observed in the Enterobacteriaceae family that in the absence of carbapenemase production, resistance towards carbapenem can still be achieved due to the decrease of porin production (as a result of gene mutation) [34,35].

Another process involved is the shift in the type of porin expressed. When a particular porin is replaced with a different type that forms more selective channels, reduced permeability of the external membrane can be achieved, consequently limiting the entry of antibiotics into the cells of bacteria [30, 36]. An example of the shift in the porin expression can be seen in *K. pneumoniae* strains, multiple drug-resistant bacteria. *K. pneumoniae* strains become less susceptible to β-lactam such as cephalosporins and carbapenems, due to the shift of porin expression from OmpK35 to OmpK36, which possessed a smaller channel size [37]. OmpK36 porin caused a four-to-eight-fold decrease of susceptibility for a wide range of β-lactam antibiotics [38].

Degradation of antibiotic

Enzyme-catalysed antibiotic degradation is another major antibiotic resistance mechanism [39]. There have been thousands of enzymes that could degrade antibiotics of different classes, including macrolides and β-lactams [40]. β-lactamases are degradation enzymes used to hydrolyze the β-lactam antibiotics such as cephalosporins, monobactams, carbapenems, and penicillins [41]. β-lactam antibiotics are bactericidal and act by interfering with the remodeling and synthesis of the peptidoglycan layer of bacteria [42]. β-lactam inactivates the penicillin-binding proteins (PBPs) by forming covalent bonds to it. PBPs are transpeptidases that cross-link the amino acids consisting of β-(1-4)-N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) together in order to form the cell wall of bacteria [43]. Bacteria can develop resistance to β-lactam antibiotics through mutations of the gene that encodes PBPs.

β-lactamases can be acquired via HGT. This can be seen in the penicillin-resistant *S. aureus* that carries plasmid-encoded β-lactamases, which can be readily transmitted between the *S. aureus* strains [44]. β-

https://doi.org/10.24191/sl.v18i2.27017

lactamase genes are generally called *bla*, followed by the specific enzyme name, for example, *bla*KPC is the gene that encodes for *K. pneumoniae* carbapenemase (KPCs) that confer resistant towards carbapenems [45]. It can be found within a chromosome or as part of the accessory genes in MGEs [42]. TEM-1 (class A) is another example of β-lactamase capable of hydrolyzing ampicillin and typically found in Gram-negative bacteria such as *E. coli* and *Klebsiella spp*. It is called TEM-1 after the name Temoneira, the patient in which it was originally found [46, 47].

ANTIBIOTIC EFFLUX

Another common resistant mechanism is antibiotic efflux. This mechanism often occurs in conjunction with other mechanisms, such as modification of a target binding site or the antibiotic. The Efflux pump consists of protein within the channel that works as a pump to extrude the antibiotics out of the cell. It acts on various classes of antibiotics, including β-lactams, aminoglycoside fluoroquinolones, polymyxins, and carbapenems [48]. The genes encoding efflux pumps can be found in bacterial chromosomes or MGEs.

ATP binding cassette (ABC), multidrug and toxin extrusion (MATE), major facilitator superfamily (MFS), small multidrug resistance (SMR), and resistance-nodulation-division (RND) are the five major families of efflux pump [49]. The number of components (multiple or single), sequence, substrate specificity, the number of transmembrane spanning regions, and energy sources have been determined for each of the families [50]. The ABC family utilizes hydrolysis from ATP in the export of substrates, while other families use proton motive force as the energy source. The ABC, MATE, MFS, and SMR families are distributed widely among the Gram-positive and -negative bacteria, while the RND superfamily is specifically found in Gram-negative bacteria.

The tetracycline-specific efflux pump of MFS is one example of efflux-mediated antibiotic resistance that mediates tetracycline resistance [51]. This pump is used to extrude the tetracycline molecules from within the cells at the expense of a proton [52]. Another efflux-mediated antibiotic resistance that can be found is in the resistance of macrolides, where erythromycin can be extruded by the efflux pumps that are encoded by the *mef* genes (*mef* E and *mef*A) [53, 54].

CHANGES IN TARGET SITES

Target protection

Proteins that are physically associated with the antibiotic's target site can defend the bacteria against the antibiotic's inhibitory effects [55]. The genes in this resistance mechanism encode target or ribosomal protection proteins (RPPs) and are usually carried by the mobile genetic elements (MGEs). Generally, this mechanism works by dislodging and preventing the rebinding of the antibiotics [55]. Tetracycline, fusidic acid, and fluoroquinolones are antibiotics affected by this resistance mechanism [56].

Examples of best-characterized RPPs are Tet(M), found in *Streptococcus spp.*, and Tet[O] in *Campylobacter jejuni*, which confer resistance to tetracycline [57]. Tet(M) binds to the ribosome, specifically at domain IV of the 16S rRNA, which is the binding site for the tetracycline. Tet(M) dislodges and releases tetracycline from the ribosome through this interaction. Consequently, the rebinding of the antibiotics is prevented due to conformational changes caused by the interaction of Tet[M] within the drug-binding site [58].

Besides Tet(M), the quinolone resistance protein, Qnr, is another example of a target protection protein that reduces susceptibility to quinolones [59]. Qnr protects DNA gyrase and topoisomerase IV from quinolones inhibition. This plasmid-mediated fluoroquinolone resistance is frequently found in *K.*

https://doi.org/10.24191/sl.v18i2.27017

pneumoniae, making it clinically significant [60]. The target sites for quinolones are the topoisomerase IV and DNA gyrase, bacterial enzymes that are involved in DNA replication [61]. Therefore, by acting as a DNA homolog, Qnr competes for the binding site, preventing the binding of quinolones to topoisomerase IV and DNA gyrase [62].

Alteration of the target sites

Alteration of the target sites is the resistance mechanism affecting almost all families of antibiotics. It consists of mutations of genes encoding target site proteins, enzymatic alterations, and complete replacement or bypass of target sites. However, no matter what type of modification takes place, the final impact of the mechanism is always the same, which is the decrease in the antibiotic's affinity for the target site [63]. This mechanism is a resistance mechanism against a few antibiotic classes, such as aminoglycosides, glycopeptides, β-lactams, macrolides, lincosamide, and streptogramins (MLS) [64]. Examples of those three strategies will be presented below.

Mutations of genes encoding the target site proteins

There are two examples of mutational resistance: the rifampin (RIF) resistance and the fluoroquinolones (FQ) resistance. Rifamycin inhibits the DNA-dependent RNA polymerase and blocks transcription in bacteria. RIF is an effective antibiotic treatment for tuberculosis [65]. RIF acts by inhibiting bacterial RNA polymerase activity and is considered one of the powerful spectrum antibiotics against bacterial pathogens. In *M. tuberculosis*, RIF prevents the elongation of mRNA by binding to the β-subunit of RNA polymerase. The binding of the RIF molecule at this highly conserved pocket will directly block the path of the nascent RNA, subsequently interrupting DNA transcription [65, 66]. The rpoB gene encodes the β subunit within the RNA polymerase, and mutations within this gene have been shown to confer RIF-resistant phenotype. It has been observed among the RIF-resistant isolates that mutations occurred within 81 bp RIF-resistance determining region (RRDR) of *the rpoB* gene [67]. The mutation caused an alteration within the binding pocket (β subunit) that decreased the binding affinity of RIF to the RNA polymerase.

The FQ resistance mechanism is another example of mutational resistance. Fluoroquinolones inhibit the topoisomerase IV and DNA gyrase's supercoiling activity within cells, resulting in cell death (at lethal concentrations) and impaired DNA replication (at lower concentrations) [68]. However, alterations of those crucial enzymes (DNA gyrase and topoisomerase IV) caused resistance toward fluoroquinolone action [61]. This can be achieved by chromosomal mutations in the genes encoding the DNA gyrase subunits (*gyr*A and *gyr*B gene) or enzyme topoisomerase IV (*par*C and *par*E gene) [69]. The region where mutations arise in these genes is known as the quinolone resistance-determining region (QRDR) [70]. The point mutation within the QRDR sequence will result in the substitutions of amino acids that alter the structure of the target protein, followed by the decrease of fluoroquinolone-binding affinity, leading to resistance towards the antibiotic [71].

Enzymatic alteration of target sites

Macrolide resistance through ribosome methylation catalyzed by an enzyme encoded by the *erm* (erythromycin ribosomal methylation) is one of the best examples of this type of resistance [72]. Adenine residue at the A2058 position of domain V of 23S rRNA within the 50S ribosomal subunit can be monomethylated or dimethylated by rRNA methyltransferase. These biochemical changes impair the binding of the macrolide to its target [73].

Other than macrolide resistance, another relevant example is the Cfr-mediated linezolid resistance [74]. This transferable multidrug resistance gene: *cfr* encodes for cfr-methyltransferase, confers resistance towards oxazolidinones, lincosamides, phenicols, streptogramin A and plueromutilins [75]. Adenine residue at the A2503 position of the 23S rRNA can be modified by the cfr-methyltransferase, which results

https://doi.org/10.24191/sl.v18i2.27017

in impaired linezolid binding with their target sites [76]. Subsequently, this will inhibit the protein synthesis by preventing the binding of aminoacyl tRNA to the A site of the ribosome [77]. However, a newer generation of oxazolidinone, namely tedizolid, was generated to overcome the resistance problem by substituting the acetamide group with a smaller hydroxymethyl group. This substitution allows the antibiotic to bind to its target site in the presence of cfr-methyltransferase [78].

Complete replacement or bypass of the target site

Bacteria can prevent the antibiotic molecule's inhibitions by evolving new targets that fulfill the biochemical function of the original target. A bypass of the original target is accomplished by producing an additional low-affinity target. Vancomycin resistant-enterococci (VRE) and methicillin resistant-*S. aureus* (MRSA) has been observed to employ the replacement and bypass strategy to achieve antibiotic resistance [9].

Other than producing *blaZ* encoded-β-lactamase (that hydrolyses the β-lactam ring, rendering it ineffective), MRSA also carries *the mecA* gene that encodes PBP2a (Penicillin-Binding Protein 2a) [79]. Due to its low affinity for β-lactam, PBP2a provides transpeptidase activity that enables the cell wall synthesis by the bacteria to be continued at a concentration that inhibits the β-lactam-sensitive PBPs normally produced by *S. aureus* [80]. PBP2a consists of an active-site serine (S403) at the N-terminus of the α2 helix in the sequence motif SXXK. Through the binding of β-lactam antibiotic with the active-site serine (S403), a rapidly reversible Michaelis complex (EI) was formed. It was converted to a stable covalent adduct by nucleophilic attack by S403 on the β-lactam ring [81]. Even though the binding occurs, the binding of β-lactam to the active site of PBP2a does not inhibit its transpeptidase activity; thus, cross-linking of the peptidoglycan chains to form rigid cell walls is not inhibited.

Another example of a replacement and bypass strategy is observed in VRE. Enterococci, particularly *Enterococcus faecium*, are closely related to the resistance of vancomycin [82]. The vancomycin resistance is conferred by *van* gene clusters involving biochemical machinery that remodels peptidoglycan synthesis. There are two biochemical types of machinery designated in the remodeling of peptidoglycan synthesis: (i) by preventing the binding of vancomycin to the cell wall precursors through destroying the D-Ala-D-Ala ending precursors and (ii) by changing the last D-Ala for either D-serine or D-lactate [83].

BIOFILM FORMATION

Biofilms are complex systems composed of many different types of cells, including bacteria, fungi, and algae. The extracellular matrix surrounding these cells is also heterogeneous, with varying compositions of proteins, lipids, polysaccharides, and other molecules [84-86]. Biofilms are common in natural and human-made environments and are well known to be one of the major contributors to antibiotic resistance [87-89].

Biofilm formation can contribute to antibiotic resistance through several mechanisms: i) physical barrier: biofilms provide a physical barrier between the antibiotics and the bacteria, which makes it difficult for the agent to penetrate and reach the bacterial cells [90]; ii) slow growth rate: biofilms have a slower growth rate and metabolism than planktonic bacteria [91], which reduces their susceptibility to antibiotics, as these antibiotics typically target rapidly growing bacterial cells; iii) quorum sensing: bacteria in biofilms can communicate with each other through quorum sensing by using signaling molecules, allowing them to coordinate their behavior and respond to environmental cues [92]. This can lead to the activation of genes that confer resistance to antibiotics; iv) phenotypic resistance: bacteria in biofilms can exhibit phenotypic resistance, where their gene expression and metabolic activity change in response to their environment [93]. This can make them less susceptible to antibiotics. Overall, biofilm formation can protect bacteria from antibiotics, making them more resistant and difficult to eliminate, leading to persistent infections.

https://doi.org/10.24191/sl.v18i2.27017

Continuous screening for the antibiofilm activity of natural products may shed new light on the control of biofilm formation and antibiotic resistance [94-96]. Resistance mechanisms caused by biofilms are summarized in Figure 2.

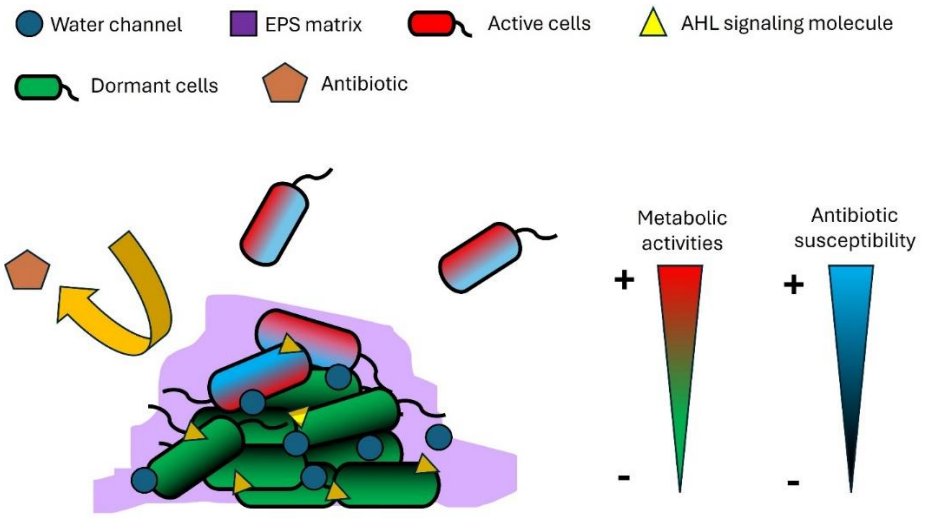

Fig. 2. Role of biofilm formation in antibiotic resistance.

CONCLUSIONS

The emergence of resistance bacteria has increased due to misuse and overuse of antibiotics, patients not finishing the course, and non-laboratory oriented antibiotic therapy. A thorough understanding of the molecular mechanisms of antibiotic resistance is vital to devising new strategies to deal with the threat. Designing better drugs that will not be affected by the bacteria's defence mechanisms or preventing the spread and dissemination of antibiotic-resistance genes needs to be further explored.

ACKNOWLEDGMENTS

The authors would like to thank the Universiti Teknologi Mara (UiTM) for providing the facilities for this research.

AUTHOR'S CONTRIBUTION

Intan Nurfarzana Mohd Safini, Nur Fatihah Sholehah Zakaria, and Muhammad Iqbal Hafiz Saad wrote and revised the article. Mohd Fakharul Zaman Raja Yahya reviewed the article. Norashirene Mohamad Jamil conceptualized the central research idea and approved the article submission.

https://doi.org/10.24191/sl.v18i2.27017

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

REFERENCES

[1] Salam, M.A., Al-Amin, M.Y., Salam, M.T., Pawar, J.S., Akhter, N., Rabaan, A.A., & Alqumber, M.A. (2023). Antimicrobial resistance: a growing serious threat for global public health. In Healthcare (Vol. 11, No. 13, p. 1946). MDPI.

[2] Asghar, A., Khalid, A., Baqar, Z., Hussain, N., Saleem, M.Z., & Rizwan, K. (2024). An insight into emerging trends to control the threats of antimicrobial resistance (AMR): an address to public health risks. *Archives of Microbiology*, *206*(2), 1-18.

[3] Sabtu, N., Enoch, D.A., & Brown, N.M. (2015). Antibiotic resistance: what, why, where, when and how? *British Medical Bulletin*, ldv041.

[4] Blakely, G.W. (2024). Mechanisms of horizontal gene transfer and DNA recombination. In Molecular medical microbiology (pp. 309-324). Academic Press.

[5] Tokuda, M., & Shintani, M. (2024). Microbial evolution through horizontal gene transfer by mobile genetic elements. *Microbial Biotechnology*, e14408.

[6] Thomas, C.M., & Nielsen, K.M. (2005). Mechanisms of, and Barriers to, Horizontal Gene Transfer between Bacteria. *Nature Reviews Microbiology*, *3*(9), 711-721.

[7] Colavecchio, A., Cadieux, B., Lo, A., & Goodridge, L.D. (2017). Bacteriophages Contribute to the Spread of Antibiotic Resistance Genes among Foodborne Pathogens of the Enterobacteriaceae Family – A Review. *Frontiers in Microbiology*, *8*.

[8] Biggest Threats and Data. (2020). Retrieved July 03, 2020, from https://www.cdc.gov/drugresistance/biggest-threats.htm

[9] Munita, J.M., & Arias, C.A. (2016). Mechanisms of Antibiotic Resistance. *Virulence Mechanisms of Bacterial Pathogens*, 481-511.

[10] Cox, G., & Wright, G.D. (2013). Intrinsic antibiotic resistance: Mechanisms, origins, challenges and solutions. *International Journal of Medical Microbiology*, *303*(6-7), 287-292.

[11] Reygaert, W.C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology, 4*(3), 482-501.

[12] Gullberg E. Selection of Resistance at very low Antibiotic Concentrations. PhD thesis. Uppsala University. 2014. ISBN 978-91-554-9101-7.

[13] Wilson, D.N. (2013). Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nature Reviews Microbiology, 12*(1), 35-48.

[14] Labby, K.J., & Garneau-Tsodikova, S. (2013). Strategies to overcome the action of aminoglycoside-modifying enzymes for treating resistant bacterial infections. *Future Medicinal Chemistry, 5*(11), 1285-1309.

[15] Landman, D., Babu, E., Shah, N., Kelly, P., Backer, M., Bratu, S., & Quale, J. (2010). Activity of a novel aminoglycoside, ACHN-490, against clinical isolates of Escherichia coli and Klebsiella pneumoniae from New York City. *Journal of Antimicrobial Chemotherapy, 65*(10), 2123-2127.

[16] Kotra, L.P., Haddad, J., & Mobashery, S. (2000). Aminoglycosides: Perspectives on Mechanisms of Action and Resistance and Strategies to Counter Resistance. *Antimicrobial Agents and Chemotherapy, 44*(12), 3249-3256.

[17] Pradier, L., & Bedhomme, S. (2023). Ecology, more than antibiotics consumption, is the major predictor for the global distribution of aminoglycoside-modifying enzymes. *Elife*, *12*, e77015.

[18] Germovsek, E., Barker, C.I., & Sharland, M. (2016). What do I need to know about aminoglycoside antibiotics? *Archives of Disease in Childhood - Education & Practice Edition, 102*(2), 89-93.

https://doi.org/10.24191/sl.v18i2.27017

[19] Krause, K.M., Serio, A.W., Kane, T.R., & Connolly, L.E. (2016). Aminoglycosides: An Overview. *Cold Spring Harbor Perspectives in Medicine, 6*(6).

[20] Llano-Sotelo, B., Azucena, E.F., Kotra, L.P., Mobashery, S., & Chow, C.S. (2002). Aminoglycosides Modified by Resistance Enzymes Display Diminished Binding to the Bacterial Ribosomal Aminoacyl-tRNA Site. *Chemistry & Biology, 9*(4), 455-463.

[21] Kim, C., & Mobashery, S. (2005). Phosphoryl transfer by aminoglycoside 3′-phosphotransferases and manifestation of antibiotic resistance. *Bioorganic Chemistry, 33*(3), 149-158.

[22] Wright, G.D. (1999). Aminoglycoside-modifying enzymes. *Current Opinion in Microbiology, 2*(5), 499-503.

[23] Morar, M., Bhullar, K., Hughes, D.W., Junop, M., & Wright, G.D. (2009). Structure and Mechanism of the Lincosamide Antibiotic Adenylyltransferase LinB. *Structure, 17*(12), 1649-1659.

[24] Sagar, S., Kaistha, S., Das, A.J., & Kumar, R. (2019). Antibiotic Resistant Bacteria: A Challenge to Modern Medicine.

[25] Shaw, K.J., Rather, P.N., Hare, R.S., & Miller, G.H. (1993). Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiological Reviews, 57*(1), 138-163.

[26] Biswas, T., Houghton, J.L., Garneau-Tsodikova, S., & Tsodikov, O.V. (2012). The structural basis for substrate versatility of chloramphenicol acetyltransferase CATI. *Protein Science, 21*(4), 520-530.

[27] Schwarz, S., Kehrenberg, C., Doublet, B., & Cloeckaert, A. (2004). Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiology Reviews, 28*(5), 519-542.

[28] Roberts, M.C., & Schwarz, S. (2017). Tetracycline and Chloramphenicol Resistance Mechanisms. *Antimicrobial Drug Resistance,* 231-243.

[29] Rajiv, A., Kapse, A., Singh, V.K., Chauhan, M.S., Awasthi, A., & Singh, P. (2024). Chitosan-modified magnesium oxide hybrid nanomaterial: A dual approach for bacterial and cancer cell eradication. Surfaces and Interfaces, 44, 103628.

[30] Dong, X., Liu, Y., Adcock, A.F., Sheriff, K., Liang, W., Yang, L., & Sun, Y.P. (2024). Carbon–TiO2 Hybrid Quantum Dots for Photocatalytic Inactivation of Gram-Positive and Gram-Negative Bacteria. International Journal of Molecular Sciences, 25(4), 2196.

[31] Welte, W., Nestel, U., Wacker, T., & Diederichs, K. (1995). Structure and function of the porin channel. *Kidney International, 48*(4), 930-940.

[32] Pagès, J., James, C.E., & Winterhalter, M. (2008). The porin and the permeating antibiotic: A selective diffusion barrier in Gram-negative bacteria. *Nature Reviews Microbiology, 6*(12), 893-903.

[33] Nikaido, H. (2003). Molecular Basis of Bacterial Outer Membrane Permeability Revisited. *Microbiology and Molecular Biology Reviews, 67*(4), 593-656.

[34] Lavigne, J., Sotto, A., Nicolas-Chanoine, M., Bouziges, N., Pagès, J., & Davin-Regli, A. (2013). An adaptive response of Enterobacter aerogenes to imipenem: Regulation of porin balance in clinical isolates. *International Journal of Antimicrobial Agents, 41*(2), 130-136.

[35] Baroud, M., Dandache, I., Araj, G., Wakim, R., Kanj, S., Kanafani, Z., . . . Matar, G. (2013). Underlying mechanisms of carbapenem resistance in extended-spectrum β-lactamase-producing Klebsiella pneumoniae and Escherichia coli isolates at a tertiary care centre in Lebanon: Role of OXA-48 and NDM-1 carbapenemases. *International Journal of Antimicrobial Agents, 41*(1), 75-79.

[36] Kojima, S., & Nikaido, H. (2013). Permeation rates of penicillins indicate that Escherichia coli porins function principally as nonspecific channels. *Proceedings of the National Academy of Sciences, 110*(28).

https://doi.org/10.24191/sl.v18i2.27017

[37] Tsai, Y., Fung, C., Lin, J., Chen, J., Chang, F., Chen, T., & Siu, L.K. (2011). Klebsiella pneumoniaeOuter Membrane Porins OmpK35 and OmpK36 Play Roles in both Antimicrobial Resistance and Virulence. *Antimicrobial Agents and Chemotherapy, 55*(4), 1485-1493.

[38] Doménech-Sánchez, A., Martínez-Martínez, L., Hernández-Allés, S., Conejo, M.D., Pascual, A., Tomás, J.M., . . . Benedí, V. J. (2003). Role of Klebsiella pneumoniae OmpK35 Porin in Antimicrobial Resistance. *Antimicrobial Agents and Chemotherapy, 47*(10), 3332-3335.

[39] Darby, E.M., Trampari, E., Siasat, P., Gaya, M.S., Alav, I., Webber, M.A., & Blair, J. M. (2023). Molecular mechanisms of antibiotic resistance revisited. *Nature Reviews Microbiology, 21*(5), 280-295.

[40] Egorov, A.M., Ulyashova, M.M., & Rubtsova, M.Y. (2018). Bacterial Enzymes and Antibiotic Resistance. *Acta Naturae, 10*(4), 33-48.

[41] Nordmann, P., Poirel, L., Walsh, T.R., & Livermore, D.M. (2011). The emerging NDM carbapenemases. *Trends in Microbiology, 19*(12), 588-595.

[42] Tang, S.S., Apisarnthanarak, A., & Hsu, L.Y. (2014). Mechanisms of β-lactam antimicrobial resistance and epidemiology of major community- and healthcare-associated multidrug-resistant bacteria. *Advanced Drug Delivery Reviews, 78*, 3-13.

[43] Bourhis, L.L., & Werts, C. (2007). Role of Nods in bacterial infection. *Microbes and Infection, 9*(5), 629-636.

[44] Bush, K. (2013). Proliferation and significance of clinically relevant β-lactamases. *Annals of the New York Academy of Sciences, 1277*(1), 84-90.

[45] Meenakshisundaram, J. (2013). Bla KPC gene Detection in Clinical Isolates of Carbapenem Resistant Enterobacteriaceae in a Tertiary Care Hospital. *Journal Of Clinical And Diagnostic Research.*

[46] Rawat, D., & Nair, D. (2010). Extended-spectrum ß-lactamases in gram negative bacteria. *Journal of Global Infectious Diseases, 2*(3), 263.

[47] Paterson, D.L., & Bonomo, R.A. (2005). Extended-Spectrum β-Lactamases: A Clinical Update. *Clinical Microbiology Reviews, 18*(4), 657-686.

[48] Poole, K. (2005). Efflux-mediated antimicrobial resistance. *Journal of Antimicrobial Chemotherapy, 56*(1), 20-51.

[49] Sun, J., Deng, Z., & Yan, A. (2014). Bacterial multidrug efflux pumps: Mechanisms, physiology and pharmacological exploitations. *Biochemical and Biophysical Research Communications, 453*(2), 254-267.

[50] Nikaido, H. (2011). Structure and Mechanism of RND-Type Multidrug Efflux Pumps. *Advances in Enzymology - and Related Areas of Molecular Biology Advances in Enzymology and Related Areas of Molecular Biology,* 1-60.

[51] Chopra, I., & Roberts, M. (2001). Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiology and Molecular Biology Reviews, 65*(2), 232-260.

[52] Thaker, M., Spanogiannopoulos, P., & Wright, G.D. (2009). The tetracycline resistome. *Cellular and Molecular Life Sciences, 67*(3), 419-431.

[53] Moore, S.D., & Sauer, R.T. (2008). Revisiting the mechanism of macrolide-antibiotic resistance mediated by ribosomal protein L22. *Proceedings of the National Academy of Sciences, 105*(47), 18261-18266.

[54] Li, X., & Nikaido, H. (2009). Efflux-Mediated Drug Resistance in Bacteria. *Drugs, 69*(12), 1555-1623.

[55] Tomlinson, J.H., Thompson, G.S., Kalverda, A.P., Zhuravleva, A., & O'Neill, A.J. (2016). A target-protection mechanism of antibiotic resistance at atomic resolution: Insights into FusB-type fusidic acid resistance. *Scientific Reports, 6*(1).

https://doi.org/10.24191/sl.v18i2.27017

[56] Tran, J.H., Jacoby, G.A., & Hooper, D.C. (2005). Interaction of the Plasmid-Encoded Quinolone Resistance Protein Qnr with Escherichia coli DNA Gyrase. *Antimicrobial Agents and Chemotherapy, 49*(1), 118-125.

[57] Connell, S.R., Tracz, D.M., Nierhaus, K.H., & Taylor, D.E. (2003). Ribosomal Protection Proteins and Their Mechanism ofTetracyclineResistance. *Antimicrobial Agents and Chemotherapy, 47*(12), 3675-3681.

[58] Doenhoefer, A., Franckenberg, S., Wickles, S., Berninghausen, O., Beckmann, R., & Wilson, D. (2012). Structural basis for TetM-mediated tetracycline resistance.

[59] Hooper, D.C., & Jacoby, G.A. (2015). Mechanisms of drug resistance: Quinolone resistance. *Annals of the New York Academy of Sciences, 1354*(1), 12-31.

[60] Martínez-Martínez, L., Pascual, A., & Jacoby, G.A. (1998). Quinolone resistance from a transferable plasmid. *The Lancet, 351*(9105), 797-799.

[61] Jacoby, G.A. (2005). Mechanisms of Resistance to Quinolones. *Clinical Infectious Diseases, 41*(2).

[62] Jacoby, G.A. (2017). Plasmid-Mediated Quinolone Resistance. *Antimicrobial Drug Resistance*, 265-268.

[63] Qu, L., Chai, T., Guo, Z., Zhang, Z., Huang, Z., & Li, N. (2024). Studies on the airborne bacterial communities and antimicrobial resistance genes in duck houses based on metagenome and PCR analysis. *Poultry Science, 103*(2), 103365.

[64] Peterson, E., & Kaur, P. (2018). Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Frontiers in Microbiology, 9*.

[65] Campbell, E.A., Korzheva, N., Mustaev, A., Murakami, K., Nair, S., Goldfarb, A., & Darst, S.A. (2001). Structural Mechanism for Rifampicin Inhibition of Bacterial RNA Polymerase. *Cell, 104*(6), 901-912.

[66] Palomino, J., & Martin, A. (2014). Drug Resistance Mechanisms in Mycobacterium tuberculosis. *Antibiotics, 3*(3), 317-340.

[67] Zaw, M.T., Emran, N.A., & Lin, Z. (2018). Mutations inside rifampicin-resistance determining region of rpoB gene associated with rifampicin-resistance in Mycobacterium tuberculosis. *Journal of Infection and Public Health, 11*(5), 605-610.

[68] Drlica, K., Hiasa, H., Kerns, R., Malik, M., Mustaev, A., & Zhao, X. (2009). Quinolones: Action and Resistance Updated. *Current Topics in Medicinal Chemistry, 9*(11), 981-998.

[69] Piddock, L.J. (1995). Mechanisms of Resistance to Fluoroquinolones. *Drugs, 49*(2), 29-35.

[70] Yoshida, H., Bogaki, M., Nakamura, M., Yamanaka, L.M., & Nakamura, S. (1991). Quinolone resistance-determining region in the DNA gyrase gyrB gene of Escherichia coli. *Antimicrobial Agents and Chemotherapy, 35*(8), 1647-1650.

[71] Hooper, D.C. (2000). Mechanisms of Action and Resistance of Older and Newer Fluoroquinolones. *Clinical Infectious Diseases, 31*(2).

[72] Golkar, T., Zieliński, M., & Berghuis, A.M. (2018). Look and Outlook on Enzyme-Mediated Macrolide Resistance. *Frontiers in Microbiology, 9*.

[73] Fyfe, C., Grossman, T.H., Kerstein, K., & Sutcliffe, J. (2016). Resistance to Macrolide Antibiotics in Public Health Pathogens. *Cold Spring Harbor Perspectives in Medicine, 6*(10).

[74] Schaenzer, A.J., & Wright, G.D. (2020). Antibiotic Resistance by Enzymatic Modification of Antibiotic Targets. *Trends in Molecular Medicine*.

[75] Morales, G., Picazo, J., Baos, E., Candel, F., Arribi, A., Peláez, B., . . . Sánchez-García, M. (2010). Resistance to Linezolid Is Mediated by thecfrGene in the First Report of an Outbreak of Linezolid-ResistantStaphylococcus aureus. *Clinical Infectious Diseases, 50*(6), 821-825.

[76] Locke, J.B., Zurenko, G.E., Shaw, K.J., & Bartizal, K. (2014). Tedizolid for the Management of Human Infections: In Vitro Characteristics. *Clinical Infectious Diseases, 58*(1).

https://doi.org/10.24191/sl.v18i2.27017

[77] Song, Y., Lv, Y., Cui, L., Li, Y., Ke, Q., & Zhao, Y. (2017). Cfr -mediated linezolid-resistant clinical isolates of methicillin-resistant coagulase-negative staphylococci from China. *Journal of Global Antimicrobial Resistance, 8*, 1-5.

[78] Shaw, K.J., Poppe, S., Schaadt, R., Brown-Driver, V., Finn, J., Pillar, C.M., . . . Zurenko, G. (2008). In Vitro Activity of TR-700, the Antibacterial Moiety of the Prodrug TR-701, against Linezolid-Resistant Strains. *Antimicrobial Agents and Chemotherapy, 52*(12), 4442-4447.

[79] Blázquez, B., Llarrull, L.I., Luque-Ortega, J.R., Alfonso, C., Boggess, B., & Mobashery, S. (2014). Regulation of the Expression of the β-Lactam Antibiotic-Resistance Determinants in Methicillin-Resistant Staphylococcus aureus (MRSA). *Biochemistry, 53*(10), 1548-1550.

[80] Fishovitz, J., Hermoso, J. A., Chang, M., & Mobashery, S. (2014). Penicillin-binding protein 2a of methicillin-resistan tStaphylococcus aureus. *IUBMB Life, 66*(8), 572-577.

[81] Fuda, C., Suvorov, M., Vakulenko, S.B., & Mobashery, S. (2004). The Basis for Resistance to β-Lactam Antibiotics by Penicillin-binding Protein 2a of Methicillin-resistantStaphylococcus aureus. *Journal of Biological Chemistry, 279*(39), 40802-40806.

[82] Arias, C.A., & Murray, B.E. (2012). The rise of the Enterococcus: Beyond vancomycin resistance. *Nature Reviews Microbiology, 10*(4), 266-278.

[83] Miller, W.R., Munita, J.M., & Arias, C.A. (2014). Mechanisms of antibiotic resistance in enterococci. *Expert Review of Anti-infective Therapy, 12*(10), 1221-1236.

[84] Yahya, M.F.Z.R., Alias, Z., & Karsani, S.A. (2018). Antibiofilm activity and mode of action of DMSO alone and its combination with afatinib against gram-negative pathogens. *Folia Microbiologica, 63*(1), 23-30.

[85] Yaacob, M.F., Murata, A., Nor, N.H.M., Jesse, F.F.A., & Yahya, M.F.Z.R. (2021). Biochemical composition, morphology and antimicrobial susceptibility pattern of Corynebacterium pseudotuberculosis biofilm. *Journal of King Saud University-Science, 33*(1), 101225.

[86] Kamaruzzaman, A.N.A., Mulok, T.E.T.Z., Nor, N.H.M., & Yahya, M.F.Z.R. (2022). FTIR spectral changes in *Candida albicans* biofilm following exposure to antifungals. *Malaysian Applied Biology, 51*(4), 57-66.

[87] Fan, Q., Zuo, J., Wang, H., Grenier, D., Yi, L., & Wang, Y. (2022). Contribution of quorum sensing to virulence and antibiotic resistance in zoonotic bacteria. *Biotechnology Advances*, 107965.

[88] Michaelis, C., & Grohmann, E. (2023). Horizontal gene transfer of antibiotic resistance genes in biofilms. *Antibiotics, 12*(2), 328.

[89] Hu, X., Zhang, Y., Chen, Z., Gao, Y., Teppen, B., Boyd, S.A., ... & Li, H. (2023). Tetracycline accumulation in biofilms enhances the selection pressure on Escherichia coli for expression of antibiotic resistance. *Science of The Total Environment, 857*, 159441.

[90] Dincer, S., Uslu, F.M., & Delik, A. (2020). Antibiotic resistance in biofilm. In *Bacterial biofilms*. IntechOpen.

[91] Miao, L., Yu, Y., Adyel, T.M., Wang, C., Liu, Z., Liu, S., ... & Hou, J. (2021). Distinct microbial metabolic activities of biofilms colonizing microplastics in three freshwater ecosystems. *Journal of Hazardous Materials, 403*, 123577.

[92] Narla, A.V., Borenstein, D.B., & Wingreen, N.S. (2021). A biophysical limit for quorum sensing in biofilms. *Proceedings of the National Academy of Sciences, 118*(21), e2022818118.

[93] Yinsai, O., Deeudom, M., & Duangsonk, K. (2023). Genotypic Diversity, Antibiotic Resistance, and Virulence Phenotypes of Stenotrophomonas maltophilia Clinical Isolates from a Thai University Hospital Setting. *Antibiotics, 12*(2), 410.

[94] Johari, N.A., Amran, S.S.D., Kamaruzzaman, A.N.A., Man, C.A.I.C. & Yahya, M.F.Z.R. 2020. Anti-biofilm potential and mode of action of Malaysian plant species: a review. *Science Letters, 14*, 34–46.

https://doi.org/10.24191/sl.v18i2.27017

[95] Zawawi, W.M.A.W.M., Ibrahim, M.S.A., Rahmad, N., Hamid, U.M.A. & Yahya, M.F.Z.R. 2020. Proteomic analysis of *Pseudomonas aeruginosa* treated with *Chromolaena odorata* extracts. *Malaysian Journal of Microbiology*, *16*(2), 124-133.

[96] Man, C.A.I.C., Razak, W.R.W.A., & Yahya, M.F.Z.R. (2022). Antibacterial and antibiofilm activities of Swietenia macrophylla King ethanolic extract against foodborne pathogens. *Malaysian Applied Biology*, *51*(4), 45-56.

https://doi.org/10.24191/sl.v18i2.27017