POTENTIAL OF *ELATERIOSPERMUM TAPOS* BLUME EXTRACT AS CONJUGATION INHIBITOR FOR PREVENTING DISSEMINATION OF TETRACYCYLINE RESISTANCE GENE TRANSFER

By

NUR AINAA NABIHAH BINTI MOHD GHAZALI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

December 2021

FPSK (m) 2021 47

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

POTENTIAL OF *ELATERIOSPERMUM TAPOS* BLUME EXTRACT AS CONJUGATION INHIBITOR FOR PREVENTING DISSEMINATION OF TETRACYCLINE RESISTANCE GENE TRANSFER

By

NUR AINAA NABIHAH BINTI MOHD GHAZALI

December 2021

Chair : Azmiza Syawani Jasni, PhD
Faculty : Medicine and Health Sciences

Dissemination of antibiotic resistance has caused major threat to human health especially in causing hospital-acquired infections. Horizontal gene transfer, mainly conjugation is the responsible transfer mechanism of antibiotic resistance genes among antibiotic resistant bacteria. The search for specific compounds or molecules that are able to inhibit conjugation is therefore significant for preventing antibiotic resistance gene transfer. Unsaturated fatty acids specifically oleic, linoleic and alpha-linolenic acids are known as conjugation inhibitors due to their conjugation inhibition ability, although most of the previous studies used synthetic unsaturated fatty acids. In this study, *Elateriospermum tapos* seed oil (ETSO) with high unsaturated fatty acids composition was obtained using Soxhlet extraction method and used to determine its potential in preventing conjugal transfer of tetracycline resistant-gene (*tet*M). Antimicrobial susceptibility testing was carried out to determine resistance profiles of bacterial strains prior to conjugation assay. Filter mating experiment between *Lactococcus garvieae* KHS-97051, *Enterococcus faecalis* JH2-2, *Clostridium difficile* ribotype 014 and *Enterococcus faecalis* ATCC 51299 were conducted to determine suitable donor and recipient cells for conjugation inhibition study. Successful transfer of *tet*(M) gene in transconjugants was confirmed by colony re-streaking, Gram-staining and polymerase chain reaction (PCR). *E. faecalis* JH2-2 transconjugants conferring *tet*(M) resistance were produced from mating between *L. garvieae* KHS-97051 donor and *E. faecalis* JH2-2 recipient. Transfer frequency decreased from $(6.11 \pm 1.80) \times 10^{-6}$ to $(5.99 \pm 3.44) \times 10^{-10}$ transconjugant per donor and $(5.85 \pm 1.58) \times 10^{-8}$ to $(5.89 \pm 2.31) \times 10^{-10}$ transconjugant per recipient after exposed to ETSO. Transconjugant colonies following ETSO exposure was reduced from 163 to 9 colonies with 94.48% of

conjugation inhibition. Although, no transconjugant was observed from two conjugation sets of *C. difficile* donor and *E. faecalis* JH2-2 and *E. faecalis* ATCC 51299 recipients proven that mating is a donor-recipient specific event. Scanning (SEM) and transmission electron microscopy (TEM) were used to identify the effects of ETSO exposure towards donor and recipient cells during conjugation. The average size of pili produced from control and ETSO exposed mating cells were 0.213 ± 0.029 and 0.093 ± 0.042 μm, respectively hence demonstrated that there is significant difference between the two groups. Aside from conjugative pili, there was no appearance of short, numerous attachment pili known as fimbriae on the cell surface of both cells. However, less cell-to-cell contact and pili formations were captured in the exposed donor and recipient cells. Smooth continuous cell wall and membrane of non-exposed recipient cell was observed but the cell thickness increased in the exposed recipient cell. There was no difference in cell diameter before and after ETSO exposure. No damaging effects were observed towards the exposed cells. The COIN potential of ETSO highlighted in this study will extend the current knowledge on the antimicrobial resistance in bacteria and enable the future development of a promising alternative bottom-up strategy to control the emergence and spread of antimicrobial-resistant bacteria.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

POTENSI EKSTRAK *ELATERIOSPERMUM TAPOS* BLUME SEBAGAI PENGHALANG KONJUGASI BAKTERIA UNTUK MENCEGAH PEMINDAHAN GEN RINTANGAN TETRACYCLINE

Oleh

NUR AINAA NABIHAH BINTI MOHD GHAZALI

Disember 2021

Pengerusi : Azmiza Syawani Jasni, PhD
Fakulti : Perubatan dan Sains Kesihatan

Penyebaran rintangan antibiotik telah menyumbang kepada ancaman besar terhadap kesihatan manusia terutamanya dalam menyebabkan jangkitan di hospital. Pemindahan gen mendatar, terutamanya konjugasi merupakan mekanisma utama yang terlibat dalam pemindahan gen rintangan antibiotik. Asid lemak tak tepu khususnya asid oleik, linoleik dan alpha-linolenik dikenali sebagai penghalang konjugasi berikutan keupayaannya dalam menghalang konjugasi bakteria. Namun begitu, kebanyakan kajian yang lepas hanya menggunakan asid lemak tak tepu tiruan. Menerusi kajian ini, minyak biji *Elateriospermum tapos* atau juga dikenali sebagai buah Perah yang tinggi dengan kandungan asid lemak tak tepu telah diekstrak menggunakan kaedah Soxhlet dan digunakan dalam menentukan keupayaannya dalam mencegah pemindahan konjugasi gen rintangan tetracycline (*tet*M). Ujian kerentanan antibiotik telah dilakukan untuk menentukan profil ketahanan strain bakteria sebelum ujian konjugasi. Saringan pengawanan melibatkan sekumpulan bakteria jenis *Lactococcus garvieae* KHS-97051, *Enterococcus faecalis* JH2-2, *Clostridium difficile* ribotype (RT) 014 dan *Enterococcus faecalis* ATCC 51299 telah dijalankan untuk menentukan sel penderma dan penerima yang sesuai untuk kajian perencatan konjugasi. Kejayaan pemindahan gen *tet*(M) telah disahkan melalui pencoretan semula, pewarnaan Gram dan tindak balas berantai polimerase (PCR). Transkonjugan *E. faecalis* JH2-2 mengandungi gen *tet*(M) berjaya dihasilkan melalui pengawanan antara sel penderma *L. garvieae* KHS-97051 dan sel penerima *E. faecalis* JH2-2. Kekerapan pemindahan menurun daripada $(6.11 \pm 1.80) \times 10^{-6}$ kepada $(5.99 \pm 3.44) \times 10^{-10}$ transkonjugan per penderma dan daripada $(5.85 \pm 1.58) \times 10^{-8}$ kepada $(5.89 \pm 2.31) \times 10^{-10}$ transkonjugan per penerima selepas pendedahan terhadap minyak biji buah

Perah. Jumlah koloni sel transkonjugan yang dihasilkan berikutan pendedah minyak biji buah Perah berkurang daripada 163 ke 9 koloni dengan peratusan perencatan konjugasi sebanyak 94.48%. Walau bagaimanapun, pengawanan antara dua kumpulan bakteria penderma *C. difficile* dengan sel penerima *E. faecalis* JH2-2 serta *E. faecalis* ATCC 51299 tidak menghasilkan sebarang transkonjugan. Hal ini menunjukkan bahawa proses pengawanan bakteria mememerlukan sel penderma dan sel penerima yang khusus. Mikroskopi elektron penskanan dan trasmisi telah digunakan untuk mengenal pasti kesan pendedahan minyak biji buah Perah terhadap sel penderma dan penerima semasa konjugasi. Purata saiz pili yang terhasil dari proses konjugasi control dan yang terdedah kepada minyak biji buah Perah adalah 0.213 ± 0.029 dan 0.093 ± 0.042 μm. Ini jelas menunjukkan bahawa terdapat perbezaan dalam saiz pili antara dua kumpulan tersebut. Selain pili konjugasi, tiada cerapan kemunculan pili pendek dan bercantum yang dikenali sebagai fimbriae dihasilkan. Namun demikian, terdapat pengurangan dalam pembentukan pili konjugasi dan jarak perhubungan antara sel yang terdedah kepada minyak biji buah Perah. Kajian ini mendapati bahawa pendedahan terhadap minyak biji buah Perah tidak menyebabkan perbezaan dalam diameter sel bakteria dan kerosakan kepada sel yang terdedah. Potensi perencatan konjugasi oleh minyak biji buah Perah yang diketengahkan dalam kajian ini dapat memperluas pengetahuan terkini mengenai ketahanan antimikrob dalam bakteria dan membolehkan pengembangan strategi alternatif dalam mengawal kemunculan dan penyebaran bakteria tahan antimikrob.

ACKNOWLEDGEMENTS

My sincere gratitude to Dr. Azmiza Syawani Jasni, for her unwavering support and patience throughout this long research journey. Special thanks to my co-supervisors, Assoc. Prof. Dr. Hasnah Bahari and Dr. Rosni Ibrahim, who assisted me in completing this study with their ideas, suggestions, and critical remarks.

I would like to thank all of my colleagues at the Faculty of Medicine and Health Sciences, particularly those in the departments of Medical Microbiology, Human Anatomy and Nutrition, as well as those at the Microscopy Unit, Institute of Bioscience, for their kindness and assistance with my research.

Also, thank you to my family, mostly to my devoted parents, whose love and encouragement made me the person I am today.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Azmiza Syawani Jasni, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Hasnah Bahari, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Rosni Ibrahim, MD, MPath
Medical Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

_____
ZALILAH BINTI MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 13 April 2023

Declaration by the Graduate Student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and the copyright of the thesis are fully-owned by Universiti Putra Malaysia, as stipulated in the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from the supervisor and the office of the Deputy Vice-Chancellor (Research and innovation) before the thesis is published in any written, printed or electronic form (including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials) as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld in accordance with the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2015-2016) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No.: Nur Ainaa Nabihah Binti Mohd Ghazali

Declaration by Members of the Supervisory Committee

This is to confirm that:

- the research and the writing of this thesis were done under our supervision;
- supervisory responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2015-2016) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xiii
LIST OF FIGURES		xiv
LIST OF APPENDICES		xv
LIST OF ABBREVIATIONS		xvi
CHAPTER		
1	**INTRODUCTION**	1
	1.1 Background	1
	1.2 Problem Statement	2
	1.3 Objectives of the Study	2
	1.3.1 General Objectives	2
	1.3.2 Specific Objectives	2
2	**LITERATURE REVIEW**	3
	2.1 Antibiotic Resistance	3
	2.2 Antibiotic resistance Threats to Humans	4
	2.3 Tetracycline Resistance	5
	2.4 Antibiotic Resistant Bacteria	6
	2.4.1 *Enterococcus faecalis*	6
	2.4.2 *Lactococcus garvieae*	7
	2.4.3 *Clostridium difficile*	8
	2.5 Mechanisms of Antibiotic Resistance	9
	2.5.1 Mutation	9
	2.5.2 Horizontal Gene Transfer	9
	2.5.2.1 Conjugation	10
	2.5.2.2 Transduction	10
	2.5.2.3 Transformation	11
	2.6 Control of Antibiotic Resistance	11
	2.6.1 Appropriate Antibiotic Use and Awareness	11
	2.6.2 Conjugation Inhibitors	12
	2.7 Unsaturated Fatty Acids	13
	2.8 *Elateriospermum tapos*	13
	2.8.1 Physical Characteristics	14
	2.8.2 Uses	14

3	**SCREENING FOR SUITABLE CONJUGATION MODEL**		16
	3.1	Introduction	16
	3.2	Materials and Methods	16
		3.2.1 Bacterial Strains and Growth Condition	16
		3.2.2 Storage of Bacterial Strains	17
		3.2.3 Media and Reagents	17
		3.2.4 Antibiotic Susceptibility Testing (Disc Diffusion)	17
		3.2.5 Filter Mating Experiment	17
		3.2.6 Transfer Experiment and Transconjugant Selection	18
		3.2.7 Transfer Frequency	19
		3.2.8 DNA Extraction	19
		3.2.9 Polymerase Chain Reaction	19
	3.3	Results	19
		3.3.1 Antibiotic Susceptibility Profiles	19
		3.3.2 Conjugal Transfer Experiment	20
		3.3.3 Detection of *tet*(M) in *E. faecalis* JH2-2 Transconjugants	21
	3.4	Discussion	22
	3.5	Conclusion	23
4	***ELATERIOSPERMUM TAPOS* AS CONJUGATION INHIBITOR**		24
	4.1	Introduction	24
	4.2	Materials and Methods	24
		4.2.1 Bacterial Strains	24
		4.2.2 Extraction of *Elateriospermum tapos* Seed Oil	24
		4.2.3 Fatty Acid Analysis of *Elateriospermum tapos* Seed Oil	25
		4.2.4 Confirmation on Antibiotic Resistance Profiles	25
		4.2.5 Conjugation Assay and Transconjugant Selection	25
		4.2.6 Percentage of Conjugation Inhibition	25
	4.3	Results	26
		4.3.1 Fatty Acids Composition of *Elateriospermum tapos* Seed Oil	26
		4.3.2 Confirmation on Antibiotic Resistance Profiles	26
		4.3.3 Detection of *tet*(M) Gene in *E. faecalis* Transconjugants	27
		4.3.4 Frequency of Conjugal Transfer	27
		4.3.5 DNA Sequence Analysis	28
	4.4	Discussion	28

4.5 Conclusion 29

5 PHENOTYPIC EFFECTS OF DONOR AND RECIPIENT CELLS FOLLOWING ETSO EXPOSURE 30

5.1 Introduction 30
5.2 Materials and Methods 30
5.2.1 Filter Mating Experiment 30
5.2.2 Sample Processing 31
5.2.3 Scanning Electron Microscopy 31
5.3.4 Transmission Electron Microscopy 31
5.3 Results 32
5.3.1 Cell Morphology in Exposed and Non-Exposed Cells 32
5.3.2 Conjugative Pili Formation 34
5.3.3 Ultrastructural Changes in Exposed and Non-Exposed Cells 36
5.4 Discussion 39
5.5 Conclusion 39

6 SUMMARIZING DISCUSSION 40

7 FINAL CONCLUSION AND RECOMMENDATIONS 43

7.1 Conclusion 43
7.2 Recommendations 43

REFERENCES 44
APPENDICES 57
BIODATA OF STUDENT 69
LIST OF PUBLICATIONS 70

LIST OF TABLES

Table		Page
3.2.1	List of bacterial strains used in this study	17
3.2.9	List of primers used for detection of *tet*(M) gene	19
3.3.1	Antibiotic susceptibility patterns in selected bacterial strains	20
3.3.2	List of conjugation models and their transfer frequency	21
4.3.1	Fatty acids content in ETSO	26
4.3.2	Confirmation of antibiotic resistance using antibiotics supplemented media	26
4.3.4	Number of transconjugant colony from the average of 3 independent experiments run in triplicates	28
5.3.2	Length of conjugative pili	34

LIST OF FIGURES

Figure		Page
2.5.2	Types of horizontal gene transfer in bacteria	9
2.8.1	Fresh *E. tapos* seed	14
3.2.5	Nitrocellulose membrane filter placed on freshly-prepared BHI agar to allow mating	18
3.3.3	Gel electrophoresis image of *tet*(M) gene detection	22
4.3.3	Detection of *tet*(M) gene in *E. faecalis* JH2-2 transconjugants	27
5.3.1	SEM images of cells morphology before and after ETSO exposure	33
5.3.2	SEM images of mixed cultures of donor (*L. garvieae* KHS-97051) and recipient (*E. faecalis* JH2-2) during conjugation	35
5.3.3a	TEM images of exposed and non-exposed mixed donor and recipient cells during conjugation	37
5.3.3b	TEM images of *E. faecalis* JH2-2 before and after ETSO exposure	38

LIST OF APPENDICES

Appendix		Page
A	List of materials	57
B	Antibiotic stocks preparation	59
C	Preparation of media and buffer	60
D	Data from control and ETSO exposed conjugation	61
E	Resistance profiles of bacterial strains prior to filter mating	62
F	Sequencing analysis	66

LIST OF ABBREVIATIONS

%	Universiti Putra Malaysia
°C	degree Celsius
µg	Microgram
µg/ml	Microgram per millilitre
µL	Microliter
µm	Micrometre
Amp	Ampicillin
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BHI	Brain Heart Infusion
BLASTn	Basic Local Alignment Search Tool nucleotide
bp	Base pair
C. difficile	*Clostridium difficile*
CDC	Centers for Disease Control
CFU	Colony Forming Unit
Cn	Gentamicin
DMSO	Di-methyl sulfonium oxide
DNA	Deoxyribonucleic acid
E. faecalis	*Enterococcus faecalis*
E. tapos	*Elateriospermum tapos*
ETSO	*Elateriospermum tapos* seed oil
Fa	Fusidic acid

g	Gram
Kb	Kilobase
L	Litre
L. garvieae	*Lactococcus garvieae*
MRSA	Methicillin-resistant *Staphylococcus aureus*
NCBI	National Center of Biotechnology Information
NIH	National Institute of Health
nm	nanometer
OD_{600}	Optical density 600
PCR	Polymerase Chain Reaction
Rif	Rifampicin
rpm	rotation per minute
RT	PCR ribotyping
SEM	Scanning Electron Microscopy
Tc	Tetracycline
TEM	Transmission Electron Microscopy
tet(M)	Tetracycline M
V	Volts

CHAPTER 1

INTRODUCTION

1.1 Background

Antibiotics discovery is one of the most significant medical breakthroughs of the 20th century. Antibiotics have helped many physicians in saving patients' lives and successfully played a key role in achieving major advances in medicine and surgery (Gould and Bal, 2013). Aside from being used for preventing or treating infections in patients who are receiving chemotherapy treatments and those with chronic diseases, most modern medical procedures today are made possible with antibiotics. This includes organ transplants, joint replacements and open-heart surgery (Golkar et al., 2014). However, the overuse and misuse of antibiotics have unfortunately caused the evolution of antibiotic resistance which now has become a major threat to human health (Rossolini et al., 2014).

The rapid rise of antibiotic resistance has made many previously efficient antimicrobial drugs turn out to be no longer effective for some infections. Lack of access to quality antibiotics remains a global issue and antibiotic shortages are affecting countries of all levels of development (Carlet et al., 2012). Bacteria acquired antibiotic resistant genes through horizontal gene transfer and spontaneous mutation. Previous studies have confirmed that antibiotic resistance is mainly transferred among bacteria of different species via bacterial conjugation (van Hoek et al., 2011). These antibiotic-resistant bacteria may then infect humans and animals thus, causing severe illnesses, increasing mortality rates and risk of complications and admission to hospital.

The World Health Organisation (WHO, 2016) has introduced a global action plan on antimicrobial resistance with five strategic objectives mainly focusing on improving awareness and understanding, strengthening surveillance and research, reducing the incidence of infection, optimizing the use of antimicrobial drugs and ensuring sustainable investment in countering antimicrobial resistance. In the mid of losing the battle against infectious diseases, scientists are continuously searching for a specific molecule or compound that may be able to inhibit antibiotic resistant genes transfer during bacterial conjugation, henceforward preventing the widespread of antibiotic resistance (Cabezón et al., 2017). Studies have proven that unsaturated fatty acids such as oleic-, linoleic- and alpha-linolenic acids share similar characteristics allowing these compounds to inhibit bacterial conjugation and are known as specific conjugation inhibitors. However, previous researches have mostly used synthetic oleic and linolenic acids to investigate their conjugation inhibitory

potential (Ripoll-Rozada et al., 2016), yet the status of unsaturated fatty acids derived from natural sources are still unknown.

In Malaysia, the seed of *Elateriospermum tapos* or also known as *buah perah* is discovered as a new source of seed oil containing a high percentage of unsaturated fatty acids such as oleic-, linoleic- and alpha-linolenic acids (Tan et al., 2014). Due to the availability of this underutilized species of plant in Peninsular Malaysia, this potential local plant to be further explored as the next conjugation inhibitor to tackle the worldwide antibiotic resistance problem.

1.2 Problem Statement

The fast spread of antibiotic resistance demands effective means to stop, or at least slow down their dissemination. Since antibiotic resistance genes disseminate mostly by conjugation, strategies to control conjugation could provide effective means to curb antibiotic resistance dissemination. Hence, the search for compounds that can specifically inhibit bacterial conjugation is crucial in the fight against antibiotic resistant bacteria.

1.3 Objectives of the Study

1.3.1 General Objectives

The main objective of this study is to investigate the potential of *E. tapos* extract as a conjugation inhibitor in preventing the spread of tetracycline resistance gene transfer.

1.3.2 Specific Objectives

i. To screen for suitable donor and recipient strains as conjugation model.

ii. To determine the potential of *E. tapos* seed oil as a conjugation inhibitor for antibiotic resistant gene transfer.

iii. To observe the phenotypic effects of the donor and recipient cells in response to *E. tapos* seed oil exposure during conjugation.

REFERENCES

Ab Rahman N, Teng CL, Sivasampu, S. Antibiotic prescribing in public and private practice: a cross-sectional study in primary care clinics in Malaysia. *BMC Infect Dis.* 2016;16(208).

Abubakar AR, Haque M. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. *J Pharm Bioall Sci*. 2020;12:1-10.

Ahmadi, M.H. Global status of tetracycline resistance among clinical isolates of *Vibrio cholerae*: a systematic review and meta-analysis. *Antimicrob Resist Infect Control* 2021;10(115):1-12.

Alderliesten JB, Duxbury SJ, Zwart, M.P, de Visser GM, Stegeman A, Fischer EA. Effect of donor-recipient relatedness on the plasmid conjugation frequency: a meta-analysis. *BMC Microbiol.* 2020;20:135.

Aljayyousi GF, Abdel-Rahman ME, ElHeneidy A, Kurdi R, Faisal E. Public practices on antibiotic use: a cross-sectional study among Qatar University students and their family members. PLoS ONE. 2019;14(11):e0225499.

Archambaud C, Derré-Bobillot A, Lapaque N, Rigottier-Gois, Serror P. Intestinal translocation of enterococci requires a threshold level of enterococcal overgrowth in the lumen. *Sci Rep*. 2019;9:8926.

Arutyunov D, Frost LS. F conjugation: back to beginning. *Plasmid*. 2013;70:18-32.

Baquero F, Coque TM, de la Cruz, F. Ecology and evolution as targets: the need for novel eco-evo drugs and strategies to fight antibiotic resistance. *Antimicrob Agents Chemother.* 2011;55(8):3649-3660.

Baym M, Stone LK, Kishony R. Multidrug evolutionary strategies to reverse antibiotic resistance. *Science.* 2016;351(6268):aad3292.

Bazinet R, Layé S. Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nat Rev Neurosci.* 2014;15(12)**:**771–785.

Blokesch M. (2015) Protocols for Visualizing Horizontal Gene Transfer in Gram-Negative Bacteria Through Natural Competence. In: McGenity T.J., Timmis K.N., Nogales B. (eds) Hydrocarbon and Lipid Microbiology Protocols. Springer Protocols Handbooks. Springer, Berlin, Heidelberg.

Borghi AA, Palma MS. Tetracycline: production, waste treatment and environmental impact assessment. *Braz J Pharm Sci.* 2014;50(1):25-40.

Brown-Jaque M, Calero-Cáceres W, Muniesa M. Transfer of antibiotic-resistance genes via phage-related mobile elements. *Plasmid*. 2015;79:1-7.

Cabezón E, de la Cruz F, Arechaga I. Conjugation inhibitors and their potential use to prevent dissemination of antibiotic resistance genes in bacteria. *Front Microbiol*. 2017;8:2329.

Capita R, Riesco-Peláez F, Alonso-Hernando A, Alonso-Calleja C. Exposure of *Escherichia coli* ATCC 12806 to sublethal concentrations of food-grade biocides influences its ability to form biofilm, resistance to antimicrobials, and ultrastructure. *Appl Environ Microbiol.* 2014;80(4):1268-1280.

Carlet J, Jarlier V, Harbarth S, et al. Ready for a world without antibiotics? The Pensières Antibiotic Resistance Call to Action. *Antimicrob Resist Infect Control*. 2012;1(1):11.

Cavanagh D, Fitzgerald GF, McAuliffe O. From field to fermentation: the origins of *Lactococcus lactis* and its domestication to the dairy environment. *Food Microbiol*. 2015;47:45–61.

Cavanagh D, Fitzgerald GF, McAuliffe O. From field to fermentation: the origins of *Lactococcus lactis* and its domestication to the dairy environment. *Food Microbiol.* 2015;47:45–61.

Ceci M, Delpech G, Sparo M, Mezzina V, Bruni SS, Baldaccini B. Clinical and microbiological features of bacteremia caused by *Enterococcus faecalis*. *J Infect Dev Ctries*. 2015; 9(11):1195-1203.

Centers for Disease Prevention and Control (CDC). *Antibiotic Resistance Threats in the United States, Atlanta, Georgia*. 2013.

Charpentier X, Polard P, Claverys JP. Induction of competence for genetic transformation by antibiotics: convergent evolution of stress responses in distant bacterial species lacking SOS?. *Curr Opin Microbiol*. 2012;15(5):570-576.

Chen A, Chen Y, Ding C, Liang H, Yang B. Effects of tetracycline on simultaneous biological wastewater nitrogen and phosphorus removal. *RSC Adv.* 2015;5:59326–59334.

Chen G, Zhao L, Dong YH. Oxidative degradation kinetics and products of chlortetracycline by manganese dioxide. *J Hazard Mater*. 2011;193:128–138.

Choksi TT, Dadani F. Reviewing the emergence of *Lactococcus garvieae*: a case of catheter associated urinary tract infection caused by *Lactococcus garvieae* and *Escherichia coli* coinfection. *Case Rep Infect Dis*. 2017:5921865.

Choo SJ, Chang CT, Lee J, Munisamy V, Tan CK, Raj JD et al. A cross-sectional study on public belief, knowledge and practice towards antibiotic use in the state of Perak, Malaysia. J Infect Dev Ctries. 2018;12(11), 960–969.

Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev*. 2001;65:232–260.

Collins DA, Riley TV. Routine detection of *Clostridium difficile* in Western Australia. *Anaerobe*. 2016;37:34–37.

Collins MD, Farrow JA, Phillips BA, Kandler O. *Streptococcus garvieae* sp. nov. and *Streptococcus plantarum* sp. nov. *J Gen Microbiol*. 1983;129(11):3427-3431.

Connell SR, Tracz, DM, Nierhaus KH, Taylor DE. Ribosomal protection proteins and their mechanism of tetracycline resistance. *Antimicrob Agents Chemother.* 2003;47:3675–3681.

Corner, EJH. Wayside Trees of Malaya, third ed. United Selangor Press, Kuala Lumpur. 1989.

Curry A, Appleton H, Dowsett B. Application of transmission electron microscopy to the clinical study of viral and bacterial infections: present and future. *Micron*. 2006;37(2):91-106.

Daghrir R, Drogui P. Tetracycline antibiotics in the environment: a review. *Environ Chem Lett*. 2013;11(3):209–227.

de Lorgeril M, Salen P, Laporte F, de Leiris J. Alpha-linolenic acid in the prevention and treatment of coronary heart disease. *Eur Heart J Suppl.* 2001;3 Suppl D:D26–D32.

Dunny GM, Leonard BA. Cell-cell communication in Gram-positive bacteria. *Annu Rev Microbiol*. 1997;51:527-564.

Evans CT, Safdar N. Current trends in the epidemiology and outcomes of *Clostridium difficile* infection. *Clin Infect Dis*. 2015;60 Suppl 2:S66-S71.

Falagas ME, Vardakas KZ, Kapaskelis A, Triarides NA, Roussos NS. Tetracyclines for multidrug-resistant *Acinetobacter baumannii* infections. *Int J Antimicrob Agents*. 2015;45:455-460.

Faruque AS, Alam K, Malek MA, Khan MG, Ahmed S, Saha D, et al. Emergence of multidrug-resistant strain of Vibrio cholerae O1 in Bangladesh and reversal of their susceptibility to tetracycline after two years. *J Health Popul Nutr.* 2007;25(2):241-243.

Fernandez-Lopez R, Machón C, Longshaw CM, et al. Unsaturated fatty acids are inhibitors of bacterial conjugation. *Microbiology (Reading)*. 2005;151(Pt 11):3517-3526.

Fleming H, Fowler SV, Nguyen L, Hofinger DM. *Lactococcus garvieae* multi-valve infective endocarditis in a traveller returning from South Korea. *Travel Med Infect Dis.* 2012;10(2):101–104.

Garcillán-Barcia MP, Jurado P, González-Pérez B, Moncalián G, Fernández LA, de la Cruz F. Conjugative transfer can be inhibited by blocking relaxase activity within recipient cells with intrabodies. *Mol Microbiol*. 2007;63(2):404-416.

Gargano V, Sciortino S, Gambino D, Costa A, Agozzino V, Reale S, Alduina R, Vicari, D. Antibiotic susceptibility profile and tetracycline resistance genes detection in Salmonella spp. strains isolated from animals and food. *Antibiotics* 2021;10(7):809.

Garrido AM, Gálvez A, Pulido RP. Antimicrobial Resistance in Enterococci. *J Infect Dis Ther*. 2014;2(4):150.

Getino M, de la Cruz F. Natural and artificial strategies to control the conjugative transmission of plasmids. *Microbiol Spectr*. 2018;6(1):10.

Getino M, Fernández-López R, Palencia-Gándara C, et al. Tanzawaic acids, a chemically novel set of bacterial conjugation inhibitors. *PLoS One*. 2016;11(1):e0148098.

Getino M, Sanabria-Ríos DJ, Fernández-López R, et al. Synthetic fatty acids prevent plasmid-mediated horizontal gene transfer. *mBio*. 2015;6(5):e01032-15.

Gibello A, Galán-Sánchez F, Blanco MM, Rodríguez-Iglesias M, Domínguez L, Fernández-Garayzábal JF. The zoonotic potential of *Lactococcus garvieae:* an overview on microbiology, epidemiology, virulence factors and relationship with its presence in foods. *Res Vet Sci.* 2016;109:59–70.

Golkar Z, Bagasra O, Pace DG. Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. *J Infect Dev Ctries*. 2014;8(2):129-136.

Gould IM, Bal AM. New antibiotic agents in the pipeline and how they can help overcome microbial resistance. *Virulence*. 2013;4(2):185-191.

Griffiths AJF, Miller JH, Suzuki DT, et al. An Introduction to Genetic Analysis. 7th edition. New York: W. H. Freeman; 2000. Bacterial conjugation.Grohmann E, Muth G, Espinosa M. Conjugative plasmid transfer in Gram-positive bacteria. *Microbiol Mol Biol Rev*. 2003;67(2):277-301.

Grossman TH. Tetracycline Antibiotics and Resistance. *Cold Spring Harb Perspect Med* 2016;6:a025387. Published by Cold Spring Harbor Laboratory Press.

Guillaume G, Verbrugge D, Chasseur-Libotte M, Moens W, Collard J. PCR typing of tetracycline resistance determinants (Tet A-E) in *Salmonella enterica* serotype Hadar and in the microbial community of activated sludges from hospital and urban wastewater treatment facilities in Belgium. *FEMS Microbiol Ecol*. 2000;32(1):77–85.

Hamidah S, Yian LN, Mohd A. Comparison of physico-chemical properties and fatty acid compostion of *Elateriospermum tapos* (buah perah), palm oil and soybean oil. *World Acad Sci Eng Technol*. 2011;81:855-858.

Handa SS, Khanuja SPS, Longo G, Rakesh DD. (2008). Extraction Technologies for Medicinal and Aromatic Plants (1st edition, no. 66), United Nations Industrial Development Organization and the International Centre for Science and High Technology, Italy.

Harvey N, Dennison E, Cooper, C. Osteoporosis: impact on health and economics. *Nature reviews Rheumatology*. 2010;6:99–105.

Hashem YA, Abdelrahman KA, Aziz RK. Phenotype–genotype correlations and distribution of key virulence factors in *enterococcus faecalis* isolated from patients with urinary tract infections. *Infect Drug Resist*. 2021;14:1713-1723.

Hawkings NJ, Butler CC, Wood F. Antibiotics in the community: a typology of user behaviours. *Patient Educ Couns*. 2008; 73(1):146–152.

Hazebroek HP, Kashim A, eds. *National Parks of Sarawak*, Natural History Publication, Borneo; 2000.

Hirakawa TF, da Costa FAA, Vilela MC, Rigon M, Abensur H, de Araujo MRE. *Lactococcus garvieae* endocarditis: first case report in Latin America. A*rq Bras Cardiol*. 2011;97(5):e108–e110.

Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in enterococcus. *Virulence*. 2012;3(5):421-433.

Hormaeche I, Alkorta I, Moro F, Valpuesta JM, Gon FM, de la Cruz F. Purification and properties of trwb, a hexameric, atp-binding integral

membrane protein essential for r388 plasmid conjugation. *J Biol Chem*. 2002;277(48):46456–46462.

Hubeny J, Buta M, Zieliński W, Harnisz M, Korzeniewska E, Nowrotek M et al. The prevalence of *tet*(A) and *tet*(M) tetracycline resistance genes in municipal wastewater. *J Ecol Eng*. 2019;20(10):1-6.

Husin N, Tan NAH, Muhamad II, Mohd N. Physicochemical and biochemical characteristics of the underutilized. *J Teknol*. 2013;64:57-61.

Huys G, D'Haene K, Collard JM, Swings J. Prevalence and molecular characterization of tetracycline resistance in Enterococcus isolates from food. *Appl Environ Microbiol*. 2004;70(3):1555-1562.

Hyder SL, Streitfeld MM. Transfer of erythromycin resistance from clinically isolated lysogenic strains of *Streptococcus pyogenes* via their endogenous phage. *J Infect Dis*. 1978;138(3):281-286.

Isidro J, Mendes AL, Serrano M, Henriques AO, Oleastroet M. Overview of *Clostridium difficile* infection: life cycle, epidemiology, antimicrobial resistance and treatment. Enany, Shymaa, editor. *Clostridium difficile* - a comprehensive overview. *IntechOpen*, 2017.

Jahantigh IM, Samadi K, Dizaji RE and Salari S. Antimicrobial resistance and prevalence of tetracycline resistance genes in *Escherichia coli* isolated from lesions of colibacillosis in broiler chickens in Sistan, Iran. *BMC Vet Res*. 2020;16(1):267.

Jasni AS, Mullany P, Hussain H, Roberts, AP. Demonstration of conjugative transposon (Tn*5397*)-mediated horizontal gene transfer between *Clostridium difficile* and *Enterococcus faecalis. Antimicrob Agents Chemother*. 2010;54(11): 4924-4926.

Jenkins R, Maddocks S. 2019. Academic Press. Chapter 3 – Microscopy and Staining Pages. In Bacteriology Methods for the Study of Infectious Diseases 53-72.

Jeong J, Song W, Cooper WJ, Jung J, Greaves J. Degradation of tetracycline antibiotics: mechanisms and kinetic studies for advanced oxidation/reduction processes. *Chemosphere*. 2010;78(5):533–540.

Johnston C, Martin B, Fichant G, Polard P, Claverys JP. Bacterial transformation: distribution, shared mechanisms and divergent control. *Nat Rev Microbiol*. 2014;12(3):181-196.

Kar SK, Pal BB, Khuntia HK, Achary KG, Khuntia CP. Emergence and spread of tetracycline resistant *Vibrio cholerae* O1 El Tor variant during 2010

cholera epidemic in the tribal areas of Odisha, India. *Int J Infect Dis*. 2015;33:45–49.

Kardas, P., Devine, S., Golembesky, A. & Roberts, C. A systematic review and meta-analysis of misuse of antibiotic therapies in the community. *Int J Antimicrob Agents*. 2005;26:106–113.

Kenzaka T, Tani K. (2012). Scanning Electron Microscopy Imaging of Bacteria Based on Nucleic Acid Sequences, Scanning Electron Microscopy, Dr. Viacheslav Kazmiruk (Ed.), ISBN: 978-953-51-0092-8, InTech.

Kochan TJ, Somers MJ, Kaiser AM, Shoshiev MS, Hagan AK., Hastie JL et al. Intestinal calcium and bile salts facilitate germination of *Clostridium difficile* spores. *PLoS Pathog*. 2017;13:e1006443.

Kollef MH. Broad-spectrum antimicrobials and the treatment of serious bacterial infections: getting it right up front. *Clin Infect Dis*. 2008;47 Suppl 1:S3-S13.

Lawrence JG, Retchless AC. The interplay of homologous recombination and horizontal gene transfer in bacterial speciation. *Methods Mol Biol*. 2009;532:29-53.

Levitus M, Rewane A, Perera TB. Vancomycin-Resistant Enterococci. In: StatPearls. Treasure Island: StatPearls Publishing.

Li R, Zhang Y, Lee CC, Liu L, Huang Y. Hydrophilic interaction chromatography separation mechanisms of tetracyclines on amino-bonded silica column. J Sep Sci. 2011;34(13), 1508–1516.

Li W, Atkinson GC, Thakor NS, lar Allas U, Lu C, Chan KY. Mechanism of tetracycline resistance by ribosomal protection protein Tet(O). *Nat Commun*. 2013;4:1477.

Lim KK, Teh CC. Cross sectional study of public knowledge and attitude towards antibiotics in Putrajaya, Malaysia. *South Med Rev*. 2012;5(2):26-33.

Ling OA, Hassali MA, Al-Haddad MS, Syed Sulaiman SA, Shafie AA, Awaisu A. Public knowledge and attitudes towards antibiotic usage: a cross-sectional study among the general public in the state of Penang, Malaysia. *J Infect Dev Ctrie*s. 2010;5: 338-347.

Ling SK, Fukumori S , Tomii K , Tanaka T, Kouno I. Isolation, purification and identification of chemical constituents from *Elateriospermum tapos*. *J Trop For Sci*. 2006;18(1):81-85.

Little P, Gould C, Williamson I, Warner G, Gantley M, Kinmonth AL. Reattendance and complications in a randomised trial of prescribing strategies for sore throat: the medicalising effect of prescribing antibiotics. *BMJ*. 1997;315(7104):350-352.

Livermore D. Current epidemiology and growing resistance of Gram-negative pathogens. *Korean J Intern Med*. 2012;27:128–142.

Llor C, Bjerrum L. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. *Ther Adv Drug Saf*. 2014;5(6):229-241.

Lotfi MN, Husseinsyah, S, Osman H, Ismail H. Surface modification of *Elateriospermum tapos* seed shell recycled polypropylene composites. *Bioresources*, 2015;10(2):3409–3425.

Lushniak BD. Antibiotic resistance: a public health crisis. *Public Health Rep*. 2014;129(4):314-316.

Magnet S, Blanchard JS. Molecular insights into aminoglycoside action and resistance. *Chem Rev*. 2005;105(2):477-498.

Mahamallik P, Saha S, Pal A. Tetracycline degradation in aquatic environment by highly porous MnO2 nanosheet assembly. *Chem Eng J.* 2015;276:155–165.

Malek A, de la Hoz A, Gomez-Villegas SI, Nowbakht C, Arias CA. *Lactococcus garvieae*, an unusual pathogen in infective endocarditis: case report and review of the literature. *BMC Infect Dis.* 2019;19:301:1-10.

Martinez JL, Baquero F. Mutation frequencies and antibiotic resistance. *Antimicrob Agents Chemother.* 2000;44(7):1771-1777.

Mazaheri Nezhad Fard R, Barton MD, Heuzenroeder MW. Bacteriophage-mediated transduction of antibiotic resistance in enterococci. *Lett Appl Microbiol*. 2011;52(6):559-564.

Meltzer E, Smollen G, Huppert A, Fluss R, Tal I, Gilboa M, et al. Universal screening for *Clostridioides difficile* in a tertiary hospital: risk factors for carriage and clinical disease. *Clin Microb Infect*. 2019; 25:1127–32.

Miranda JM, Rodriguez JA, Galan-Vidal CA. Simultaneous determination of tetracyclines in poultry muscle by capillary zone electrophoresis. *J Chromatogr A*. 2009;1216(15):3366–3371.

Modi SR, Lee HH, Spina CS, Collins JJ. Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. *Nature*. 2013;499(7457):219-222.

Mofredj A, Baraka D, Cadranel JF, LeMaitre P, Kloeti G, Dumont JL. *Lactococcus garvieae* septicemia with liver abscess in an immunosuppressed patient. *Am J Med*. 2000;109(6):513–514.

Monaghan TM, Robins A, Knox A, Sewell HF, Mahida YR. Circulating antibody and memory B-Cell responses to *C. difficile* toxins A and B in patients with *C. difficile*-associated diarrhoea, inflammatory bowel disease and cystic fibrosis. *PLoS One*. 2013;8(9):e74452.

Morgan DJ, Okeke IN, Laxminarayan R, Perencevich EN, Weisenberg S. Non-prescription antimicrobial use worldwide: a systematic review. *Lancet Infect Dis*. 2011;11(9):692-701.

Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. *Microbiol Spectr*. 2016;4(2):1-37.

National Institute for Health and Clinical Excellence: Centre for Clinical Practice at NICE (UK). *Respiratory Tract Infections - Antibiotic Prescribing: Prescribing of Antibiotics for Self-Limiting Respiratory Tract Infections in Adults and Children in Primary Care*. London. July 2008.

Navas ME, Hall G, El Bejjani D. A case of endocarditis caused by *Lactococcus garvieae* and suggested methods for identification. *J. Clin Microbiol*. 2013;51(6):1990-1992.

Neela FA, Nagahama N, Suzuki S. Cell-to-cell contact is required for transfer of tetracycline resistance gene *tet*(M) in marine bacteria. *Interdisciplinary Studies on Environmental Chemistry – Biological Responses to Contaminants*. 2008;349-353.

Neela FA, Nonaka L, Rahman MH, Suzuki S. Transfer of the chromosomally encoded tetracycline resistance gene *tet*(M) from marine bacteria to *Escherichia coli* and *Enterococcus faecalis*. *World J Microbiol Biotechnol*. 2009;25:1095–1101.

Neidell MJ, Cohen B, Furuya Y, et al. Cost of healthcare and community associated infections with antimicrobial resistant versus antimicrobial susceptible organisms. *Clin Infect Dis*. 2012;55(6):807-815.

Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: mechanisms, inhibition and biological effects. *Biochem Biophys Res Commun*. 2005;338(1):668-676.

Norman A, Hansen LH, Sørensen SJ. Conjugative plasmids: vessels of the communal gene pool. *Philos Trans R Soc Lond B Biol Sci*. 2009;364(1527):2275-2289.

Oppegaard H, Steinum TM, Wasteson Y. Horizontal transfer of a multi-drug resistance plasmid between coliform bacteria of human and bovine origin in a farm environment. *Appl Environ Microbiol*. 2001;67(8):3732–3734.

Osada N, Takeda H, Kawaguchi H, Furukawa A, Awang M. Estimation of crown characters and leaf biomass from leaf litter in a Malaysian canopy species, *Elateriospermum tapos* (Euphorbiaceae). *Forest Ecology and Management*. 2003;177:379–386.

Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L. Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. *Antimicrob Agents Chemother*. 2010;54(11):4851-4863.

Plachouras D, Kavatha D, Antoniadou A, et al. Dispensing of antibiotics without prescription in Greece, 2008: another link in the antibiotic resistance chain. *Euro Surveill*. 2010;15(7):19488.

Prudhomme M, Attaiech L, Sanchez G, Martin B, Claverys JP. Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*. *Science*. 2006;313(5783):89-92.

Raissy M, Ansari M. Antibiotic susceptibility of *Lactococcus garvieae* isolated from rainbow trout (*Oncorhynchus mykiss*) in Iran fish farms. *Afr J Biotechnol*. 2011;10(8):1473-1476.

Rawling RA, Granato PA. *Lactococcus garvieae* native valve endocarditis. *Clin Microbiol Newsl*. 2014;36(22):182-183.

Rayamajhi N, Cha SB, Yoo HS. Antibiotic resistances: past, present and future. *J Biomed Res*. 2010;11(2): 65-80.

Ripoll-Rozada J, García-Cazorla Y, Getino M, et al. Type IV traffic ATPase TrwD as molecular target to inhibit bacterial conjugation. *Mol Microbiol*. 2016;100(5):912-921.

Roberts AP, Braun V, Eichel-Streiber CV, and Mullany P. Demonstration that the group ii intron from the Clostridial conjugative transposon tn5397 undergoes splicing in vivo. *J Bacteriol*. 2001;18(4):1296-1299.

Romaniuk JA, Cegelski L. Bacterial cell wall composition and the influence of antibiotics by cell-wall and whole-cell NMR. *Philos Trans R Soc Lond B Biol Sci*. 2015;370(1679):20150024.

Rossolini GM, Arena F, Pecile P, Pollini S. Update on the antibiotic resistance crisis. *Curr Opin Pharmacol*. 2014;18:56-60.

Rupnik M, Wilcox MH, Gerding DN. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol*. 2009;7(7):526-536.

Salton MRJ, Kim KS. Structure. In: Baron S, eds. Medical Microbiology. 4th ed. Galveston, TX: University of Texas Medical Branch at Galveston; 1996. Chapter 2.

Schleifer KH, Kraus J, Dvorak C, Kilpper-Bälz R, Collins MD, Fischer W. Transfer of *streptococcus lactis* and related streptococci to the genus lactococcus gen. nov. Syst Appl Microbiol. 1985;6(2):183–195.

Schnappinger D, Hillen W. Tetracyclines: antibiotic action, uptake, and resistance mechanisms. *Arch Microbiol*. 1996;165(6):359–369.

Sellect EM, Van Tyne D, Gilmore MS. Pathogenicity of Enterococci. *Microbiol Spectr*. 2019;7(4):1-38.

Smillie C, Garcillán-Barcia MP, Francia MV, Rocha EP, de la Cruz, F. Mobility of plasmids. *Microbiol Mol Biol Rev*. 2010;74(3):434-452.

Sowa BA, Moore D, Ippen-Ihler K. Physiology of F-pilin synthesis and utilization. *J Bacteriol*. 1983;153(2):962-968.

Sun D. Pull in and push out: Mechanisms of horizontal gene transfer in bacteria. *Front Microbiol*. 2018;9:2154.

Sun X, Hirota SA. The roles of host and pathogen factors and the innate immune response in the pathogenesis of *Clostridium difficile* infection. *Mol Immunol*. 2015;63(2):193-202.

Tan NAH, Siddique BM, Muhamad II, Kok FS. The effect of solvents on the soxhlet extraction of omega 3 from perah oil. *Jurnal Teknol*. 2014;67:51-54.

Tang C, Cui L, Xu Y, *et al*. The incidence and drug resistance of *Clostridium difficile* infection in Mainland China: a systematic review and meta-analysis. *Sci Rep*. 2016;6:37865.

Thomas CM, Nielsen KM. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nat Rev Microbiol*. 2005;3(9):711-721.

Tiong TW, Chua SS. Knowledge and attitude of university students on antibiotics: a cross-sectional study in Malaysia. *Drug Health Patient Saf.* 2020;12:135-144.

Ubukata K, Konno M, Fujii R. Transduction of drug resistance to tetracycline, chloramphenicol, macrolides, lincomycin and clindamycin with phages induced from *Streptococcus pyogenes*. *J Antibiot (Tokyo)*. 1975;28(9):681-688.

van Hoek AH, Mevius D, Guerra B, Mullany P, Roberts AP, Aarts HJ. Acquired antibiotic resistance genes: an overview. *Front Microbiol*. 2011;2:203.

Varga M, Kuntová L, Pantůček R, Mašlaňová I, Růžičková V, Doškař J. Efficient transfer of antibiotic resistance plasmids by transduction within methicillin-resistant *Staphylococcus aureus* USA300 clone. *FEMS Microbiol Lett*. 2012;332(2):146-152.

Vendrell D, Balcázar JL, Ruiz-Zarzuela I, de Blas I, Gironés O, Múzquiz JL. *Lactococcus garvieae* in fish: a review. *Comp Immunol Microbiol Infect Dis*. 2006;29(4):177-198.

Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *PT*. 2015;40(4):277-283.

von Wintersdorff CJ, Penders J, van Niekerk JM, et al. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front Microbiol*. 2016;7:173.

Wang CY, Shie HS, Chen SC, Huang JP, Hsieh IC, Wen MS et al. *Lactococcus garvieae* infections in humans: possible association with aquaculture outbreaks. *Int J Clin Pract.* 2007;61(1):68–73.

Werner G, Coque TM, Franz CM, Grohmann E, Hegstad K. Antibiotic resistant enterococci-tales of a drug resistance gene trafficker. *Int J Med Microbiol.* 2013;303(6-7):360-379.

World Health Organization. Global action plan on antimicrobial resistance. Available at: https://www.who.int/publications/i/item/9789241509763. Published January 1, 2016. Accessed on: July 8, 2020.

Wozniak RA, Waldor MK. Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. *Nat Rev Microbiol*. 2010;8(8):552-563.

Wright GD. Bacterial resistance to antibiotics: enzymatic degradation and modification. *Adv Drug Deliv Rev*. 2005;57(10):1451-1470.

Yong OY, Salimon J. Characteristics of *Elateriospermum tapos* seed oil as a new source of oilseed. *Ind Crops Prod.* 2006;24:146-151.

Zhanel GG, Homenuik K, Nichol K, Noreddin A, Vercaigne L, Embil J, et al. The glycylcyclines: a comparative review with the tetracyclines. *Drugs*. 2004;64:63-88.

Zhou X, Li Y, eds. Basic Biology of Oral Microbes. In: *Atlas of Oral Microbiology: From Healthy Microflora to Disease*. Academic Press; 2015:1-14.

Zlotkin A, Eldar A, Ghittino C, Bercovier H. Identification of *Lactococcus garvieae* by PCR. *J Clin Microbiol*. 1998;36(4):983–985.