CHARACTERIZATION, PHARMACOKINETICS, IN VITRO CYTOTOXICITY OF OXYTETRACYCLINE-LOADED CaCO3 NANOPARTICLE AND ITS ANTIBACTERIAL AND ANTIBIOFILM EFFECTS AGAINST Corynebacterium pseudotuberculosis ISOLATED FROM GOATS

IDRIS SHERIFAT BANKE

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By

IDRIS SHERIFAT BANKE

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

July 2020

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DEDICATION

This thesis is dedicated to the loving memory of my daughter Khadijah Abubakar Mayaki. You are always alive in my heart…….

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

CHARACTERIZATION, PHARMACOKINETICS, *IN VITRO* CYTOTOXICITY OF OXYTETRACYCLINE-LOADED $CaCO_3$ NANOPARTICLE AND ITS ANTIBACTERIAL AND ANTIBIOFILM EFFECTS AGAINST *Corynebacterium pseudotuberculosis* ISOLATED FROM GOATS

By

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July 2020

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Corynebacterium pseudotuberculosis is the causative agent of caseous lymphadenitis which is one of the most important bacterial diseases of goats causing significant economic losses with high prevalence worldwide. Currently, the treatment of the disease is via the administration of antibiotics combined with surgical excision, flushing and draining of the abscesses. This treatment protocol fails because the antibiotic does not get to the causative agent which is walled away in pus and excision further spreads the organism into the environment. For effective therapy, an improved method of drug delivery is therefore necessary. The main aim of the present study was to determine the effect of cockle shell derived calcium carbonate aragonite nanoparticle encapsulated oxytetracycline against *Corynebacterium pseudotuberculosis* isolated from goat caseous lymphadenitis. Calcium carbonate aragonite nanoparticle (CS-$CaCO_3$NP) was synthesized from cockle shell using top down method and oxytetracycline (OTC) was loaded into it. Characterization to ensure that the CS-$CaCO_3$NP and OTC-CS-$CaCO_3$NP produced had the desired properties was done using Zeta analysis, transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), Fourier transform infra-red spectroscopy (FTIR) and Brunauer-emmett-teller (BET) surface area analysis. Then, the *in vitro* cytotoxicity evaluation of CS-$CaCO_3$NP, OTC-CS-$CaCO_3$NP and OTC was explored using MTT and Trypan blue assay in NIH3T3 cells. The antibacterial and antibiofilm effects of CS-$CaCO_3$NP, OTC-CS-$CaCO_3$NP and OTC against *C. pseudotuberculosis* were also investigated using minimum inhibitory assay (MIC) and minimum biofilm eradication concentration (MBEC) assays. The antibacterial mode of action of OTC-CS-$CaCO_3$NP on planktonic *C. pseudotuberculosis* was assessed using high resolution transmission electron microscopy (HR-TEM) while the antibiofilm effect was investigated using

scanning electron (SEM) and fluorescent microscopy. Furthermore, for the pharmacokinetics study of OTC-CS-CaCO3NP and OTC, a total of 100, 5-6 weeks old female *BALB/c* mice divided into two groups of 50 mice each were used. They were administered 10mg/kg of OTC-CS-$CaCO_3$NP and OTC, respectively. At specific time intervals of 0, 5, 10, 15, 30 minutes and 1, 2, 6, 24 and 48 hrs, five mice from each group were sacrificed. Blood, liver and kidneys were collected.

The results revealed that the synthesized CS-$CaCO_3$NP and OTC-CS-$CaCO_3$NP had a homogeneously spherical appearance on TEM with a mean diameter of 29.90 (nm) and -19.9 (mV) zeta potential which increased to 62.40 nm and -23.5 (mV), respectively after loading with OTC. OTC crystallinity and functionality within CS-$CaCO_3$NP were maintained as showed by XRD and FTIR spectral peaks. The formulation of OTC-CS-$CaCO_3$NP in ratio 1:4 with drug encapsulating efficiency (71%) was used for *in vitro* release study. OTC was sustainably released from OTC-CS-$CaCO_3$NP over a period of 96 hours with pH 4 having the highest drug release percentage (98.2%). Cytotoxicity assay revealed that OTC-CS-$CaCO_3$NP had significantly higher cell viability ($P < 0.05$) compared to OTC in NIH3T3 cells. Loading OTC into CS-$CaCO_3$NP reduced OTC cytotoxicity in NIH3T3 cells. MTT assay overestimated the cytotoxicity of CS-$CaCO_3$NP, OTC-CS-$CaCO_3$NP and OTC when compared to trypan blue assay. The minimum inhibitory concentration (MIC) for OTC-CS-$CaCO_3$NP and OTC was 125 µg/ml and 500 µg/ml while the minimum biofilm eradication concentration (MBEC) was 250 µg/ml and >2000 µg/ml, respectively. However, CS-$CaCO_3$NP alone demonstrated no antibacterial activity. HR-TEM revealed that the antimicrobial mechanism of action of OTC-CS-$CaCO_3$NP was due to damage to the outer envelope of *C. pseudotuberculosis* while SEM and fluorescent microscope showed digestion and death of the bacteria cells within *C. pseudotuberculosis* biofilms. Pharmacokinetic studies demonstrated that OTC-CS-$CaCO_3$NP had a slower elimination rate (0.135 1/hr), longer half-life (5.133 hr), increased area under the curve (AUC) (46.68 µg/ml*h) and increased volume of distribution (1.587 mg/kg/µg/ml) than OTC. Thus, OTC-CS-$CaCO_3$NP is a safe and biocompatible alternative antibiotic delivery system whose antibacterial efficacy is more pronounced compared to OTC.

Keywords: caseous lymphadenitis, oxytetracycline, cockle shell derived nanoparticles, cytotoxicity, biofilms, *BALB/c* mice

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN, FARMAKOKINETIK, SITOTOKSISITI *IN VITRO* NANOPARTIKEL $CaCO_3$ TERMUAT-OKSITETRASIKLINA, DAN KESAN-KESAN ANTIBAKTERIAL DAN ANTIBIOFILEMNYA TERHADAP *Corynebacterium pseudotuberculosis* YANG TERISOLASI DARIPADA KAMBING

Oleh

IDRIS SHERIFAT BANKE

Julai 2020

Pengerusi : Profesor Madya Arifah Abdul Kadir, PhD
Fakulti : Perubatan Veterinar

Corynebacterium pseudotuberculosis adalah agen penyebab penyakit limfadenitis kaseus yang merupakan salah satu daripada penyakit bakterial penting di dalam kambing, dengan kadar prevalens yang tinggi diseluruh dunia, secara langsung menyumbang kepada kerugian yang signifikan di dalam sektor penternakan. Pada ketika ini, kaedah rawatan penyakit ini adalah melalui pemberian kombinasi antibiotik dengan eksisi surgikal, pembersihan dan penyaliran abses tersebut. Protokol rawatan ini sering kali gagal kerana antibiotik tidak mampu sampai ke punca jangkitan, akibat daripada agen diselaputi oleh nanah dan pengeksisian abses akan menyebabkan penyebaran agen ke kawasan setempat. Oleh itu, bagi memberikan terapi yang efektif, satu kaedah penyampaian ubat yang dipertingkatkan adalah sangat diperlukan. Tujuan utama kajian ini adalah untuk menentukan kesan pengkapsulan oksitetrasiklina di dalam nanopartikel kalsium karbonat aragonit terbitan cengkerang kerang, terhadap *Corynebacterium pseudotuberculosis* yang diisolasi daripada limfadenitis kaseus kambing. Nanopartikel kalsium karbonat aragonit (CS-$CaCo_3$NP) telah disintesiskan daripada cengkerang kerang menggunakan kaedah ‘top down’ dan oksitetrasiklina (OTC) telah dimuatkan ke dalamnya. Pencirian CS-$CaCO_3$NP dan OTC-CS-$CaCO_3$NP telah dilakukan melalui analisis Zeta, mikroskopi elektron transmisi (TEM), mikroskopi elektron pengimbasan pancaran medan (FESEM), difraksi sinar-X (XRD), spektroskopi inframerah penjelmaan Fourier (FTIR) dan analisis luas permukaan Brunauer-emmett-teller (BET), bagi memastikan produk tersebut mempunyai sifat-sifat yang diinginkan. Seterusnya, penilaian sitotoksisiti *in-vitro* terhadap CS-$CaCO_3$NP, OTC-CS-$CaCO_3$NP dan OTC telah dilaksanakan melalui ujian MTT dan Trypan biru di dalam sel kultur NIH3T3. Kesan antibakterial dan antibiofilem CS-$CaCO_3$NP, OTC-CS-$CaCO_3$NP dan OTC terhadap *C. pseudotuberculosis* turut dikaji menggunakan ujian kepekatan perencat minimum

(MIC) dan kepekatan eradikasi biofilem minimum (MBEC). Mod tindakan antibakterial OTC-CS-$CaCO_3$NP terhadap planktonik *C. pseudotuberculosis* telah dinilai melalui mikroskopi elektron tansmisi beresolusi tinggi (HR-TEM), sementara kesan antibiofilem dinilai melalui mikroskopi elektromn pengimbasan (SEM) dan mikroskopi floresen. Selain itu, bagi kajian farmakokinetik OTC-CS-$CaCO_3$NP dan OTC, sejumlah 100 ekor tikus BALB/c betina berusia 5-6 minggu telah dibahagikan kepada dua kumpulan yang terdiri daripada 50 ekor tikus setiap satunya. Tikus-tikus tersebut telah diberikan OTC-CS-$CaCO_3$NP dan OTC pada dos 10 mg/kg. Pada selang masa 0, 5, 10, 15, 30 minit, dan 1, 2, 6, 24 dan 48 jam, lima ekor tikus daripada setiap kumpulan telah dimatikan. Darah, hati dan ginjal telah diambil.

Keputusan menunjukan CS-$CaCO_3$NP dan OTC-CS-$CaCO_3$NP yang dihasilkan masing-masing mempunyai permukaan sferikal yang sekata di bawah TEM dengan purata diamter 29.90 (nm) dan keupayaan zeta -19.9 (mV), dan meningkat sehingga 62.40 nm dan -23.5 (mV) setelah dimuatkan OTC. Pengkristalan dan kefungsian OTC di dalam CS-$CaCO_3$NP adalah kekal sama seperti yang ditunjukan oleh puncak spektral XRD dan FTIR. Formulasi OTC-CS-$CaCO_3$NP dalam nisbah 1:4 dengan keberkesanan pengkapsulan ubat (71%) telah digunakan di dalam kajian pelepasan *in-vitro*. OTC telah dibebaskan secara berterusan sepanjang tempoh 96 jam, dengan pH 4 adalah peratusan pembebasan ubat yang tertinggi (92.2%). Ujian sitotoksiti menunjukan OTC-CS-$CaCO_3$NP mempunyai viabiliti sel signifikan ($P<0.05$) yang lebih tinggi berbanding OTC di dalam sel kultur NIH3T3. Pemuatan OTC ke dalam CS-$CaCO_3$NP telah mengurangkan tahap keracunan OTC di dalam sel kultur NIH3T3. Ujian MTT menjangkaui sitotoksiti CS-$CaCO_3$NP, OTC-CS-$CaCO_3$NP dan OTC apabila dibandingkan dengan ujian Trypan biru. Kepekatan perencatan minimum (MIC) bagi OTC-CS-$CaCO_3$NP dan OTC, masing-masing adalah 125 µg/mL dan 500 µg/mL, manakala kepekatan eradikasi biofilem minimum (MBC) adalah 250 µg/mL dan >2000 µg/mL. Selain itu, CS-$CaCO_3$NP sahaja tidak menunjukan sebarang aktiviti antibakterial. HR-TEM menunjukan mekanism tindakan antimikrobial OTC-CS-$CaCO_3$NP adalah berpunca daripada pemusnahan sarung luar *C. pseudotuberculosis*, manakala SEM dan mikroskop floresen menunjukan pencernaan dan kematian sel bakteria di dalam biofilem *C. pseudotuberculosis*. Kajian farmakokinetik menujukan OTC-CS-$CaCO_3$NP mempunyai kadar penyingkiran yang lebih lambat (0.135 L/jam), jangka separuh-hayat yang lebih panjang (5.133 jam), peningkatan kawsan di bawah lekuk (AUC) (46.68 µg/mL/jam) dan peningkatan isipadu penyebaran (1.587 mg/kg/µg/mL) berbanding OTC. Oleh itu, OTC-CS-$CaCO_3$NP adalah satu sistem penyampaian antibiotik alternatif yang selamat dan biokompatibel, di mana keberkesanan antibakterianya adalah lebih baik berbanding OTC sahaja.

Kata Kunci: Limfadeniti kaseus, oksitetrasiklina, nanopartikel terbitan cengkerang kerang, sitotoksiti, biofilem, tikus *BALB/c*

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

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LIST OF ABBREVIATIONS

µg	Microgram
µl	Microlitre
%	Percent
Al	Aluminium
BET	Brunauer–Emmett–Teller
BHI	Brain Heart infusion
BS-12	Dodecyl dimethyl betaine
C	Carbon
Ca	Calcium
$CaCO_3$	Calcium carbonate
Cl	Chlorine
CLA	Caseous Lymphadenitis
cm^3/g	Cubic centimetre/gram
CS-$CaCO_3$	Cockle shell calcium carbonate powder
CS-$CaCO_3$NP	Cockle shell calcium carbonate aragonite nanoparticle
Cu	Copper
DMEM	Dulbecco's modified Eagles Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDX	Energy dispersive X-ray spectroscopy
EDTA	Ethylenediamine tetra-acetic acid
EE	Encapsulation efficiency
EPS	Extracellular polysaccharide matrix
FESEM	Field emission scanning electron microscopy

FBS	Foetal Bovine Serum
FITC	Fluorescein isothiocyanate
FTIR	Fourier transform infrared spectroscopy
HPLC	High performance Liquid Chromatography
HR-TEM	High resolution transmission electron microscopy
ICDD	International Centre for Diffraction Data
IUPAC	International Union of Pure and Applied Chemistry
K	Potassium
KDa	Kilodaltons
LC	Loading capacity
LOD	Limit of detection
LOQ	Limit of quantification
MBEC	Minimum biofilm eradication concentration
m^2/g	Square metre/gram
MFS	Major facilitator superfamily
Mg	Magnesium
MHB	Mueller Hinton broth
MIC	Minimum inhibitory concentration
Ml	Millilitre
MTT	[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]
Na	Sodium
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NIH3T3	Mouse embryonic fibroblast cell line
Nm	Nanometre
O	Oxygen

OTC	Oxytetracycline
OTC-CS-$CaCO_3$NP	Oxytetracycline loaded cockle shell calcium carbonate aragonite nanoparticle
P	Phosphorus
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PDI	Poly dispersity index
pH	Potential of Hydrogen
PLD	Phospholipase D
RNA	Ribonucleic acid
ROS	Reactive oxygen species
Rpm	Revolutions per minute
SEM	Scanning electron microscopy
Si	Silicone
TEM	Transmission electron microscopy
TRITC	Tetramethylrhodamine
tRNA	Transfer RNA
TSB	Tryptic Soy Broth
UV/Vis	Ultraviolet–visible
XRD	X-ray powder diffraction

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of the Study

Corynebacterium pseudotuberculosis (*C. pseudotuberculosis*) is a Gram positive, non spore forming, pleomorphic, intracellular facultative, animal pathogen in the family *Corynebacteriaceae* (Dorella et al., 2006; Oreiby, 2015) *C. pseudotuberculosis* is responsible for major economic losses in small ruminants due to decreased milk yield, reduced weight gain, poor wool and leather quality, high morbidity and mortality with condemnation of meat at slaughter (Soussa et al., 2011; Domenis et al., 2018). This bacterium is the causative agent of caseous lymphadenitis (CLA) or cheesy glands in sheep and goats (Oreiby, 2015; Osman et al., 2015). Caseous lymphadenitis is also known as cheesy gland because of the characteristic chronic suppurating abscess of the lymph node, along lymphatic tracts and or internal organs (Washburn, 2013).

Treatment of CLA with antibiotics have proven unsuccessful due to the ability of the organism to survive in macrophages covered by thick fibrous capsules (Pepin and Paton, 2010). Contributing to its non-response to antibiotics is the formation of biofilms by *C. pseudotuberculosis* which makes the disease chronic in affected animals (Sa et al., 2013). The use of nanoparticles to treat otherwise difficult infectious diseases due to bacterial resistance and/or biofilm formation is a new area in veterinary therapeutics (Aderibigbe, 2017). It is an area of interest because the advantages of the drug can be maximized to reduce its toxicity while increasing its efficacy (Soussa et al., 2011; Nicolosi et al., 2015; Aderibigbe, 2017: Min et al., 2019). This makes the use of nano-antibiotic a better option compared to the conventional antibiotics since the pharmacokinetic parameters/profile of the antibiotic can be altered (Tomuleasa et al., 2014).

Many materials have been used as nanoparticles for drug delivery (Zhang et al., 2010). One of which is the calcium carbonate aragonite nanoparticle derived from bivalve molluscs (*Anadara granosa*) a delicacy in Asian countries (Othman et al., 2013). The high rate of consumption of this seafood results in the deposition of cockle shell into the surrounding environment led to the development of ideas by researchers in applying this biodegradable waste product in the field of engineering and biomedicine (Muthusamy and Sabri, 2012; Islam et al., 2013). Calcium carbonate aragonite nanoparticles derived from cockle shells have been used to successfully to deliver gentamicin, ciprofloxacin and vancomycin, respectively against *Bacillus subtillis*, Salmonella *typhimurium* and methicillin-resistant *Staphylococcus aureus* (Isa et al., 2016; Saidykhan et al., 2016; Pan et al., 2018). Although gold and silver nanoparticles have been used by researchers to enhance *in vitro* intracellular antibacterial activity against *C. pseudotuberculosis* with promising results (Mohamed et al., 2017; Stanisic et al., 2018). The choice of calcium carbonate aragonite nanoparticles in this work was to design a treatment which is readily available and affordable.

One of the antibiotics used in the management of CLA in Malaysia is oxytetracycline (OTC) (Osman et al., 2015). OTC is a broad spectrum antibiotic effective against many Gram negative and Gram positive bacteria. It is produced by *Streptomyces* which belongs to the phylum *Actinobacteria*. OTC is bacteriostatic and acts by binding to the 30S ribosomal subunit to inhibit protein synthesis (Maaland et al., 2013). The therapeutic efficiency of OTC can be improved by designing an alternative method of delivery for it using nanoparticles (Georgescu et al., 2017).

1.2 Statement of the Problem

CLA is a zoonotic and chronic infection of small ruminants with severe economic implications (Mohammed et al., 2017; Santos et al., 2019). Conventional antibiotic therapy in the management of CLA is ineffective because the drug fails to penetrate the thick pus filled fibrous capsule surrounding *C. pseudotuberculosis* (Stanisic et al., 2018). Furthermore, the formation of biofims have been implicated as a contributing factor to the antibiotic treatment failure seen in CLA (Olson et al., 2002; Sá et al., 2013). Therefore, newer alternative approach to treatment of CLA is necessary to combat the menace of this disease (Mohamed et al., 2017; Stanisic et al., 2018; Santos et al., 2019). Loading antibiotics into nanoparticles provides for the development of new alternative drug delivery methods with ability to overcome bacteria resistance mechanisms, increase the antibiotic residence time and sustained drug release profile (Mukherjee et al., 2019; Min et al., 2019; Idris et al., 2020).

1.3 Justification of the Study

The huge financial implications and economic losses caused by CLA in goats yearly makes research in this area necessary (Galvão et al., 2017). The development of newer methods of antibiotic delivery against *C. pseudotuberculosis* with higher volume of distribution, reduced toxicity, efficacy and therapeutic index is now the centre of focus in the search for effective treatment of CLA (Mohamed et al., 2017; Stanisic et al., 2018). With the right delivery method to bypass the protective mechanisms of *C. pseudotuberculosis*, then antibiotics perhaps would be the best method of treating and clearing this microbe completely.

Delivery of antimicrobials using nanoparticles is better compared to the conventional methods of antimicrobial delivery since the bacterial outer membrane plays a role in permeability of these drugs, hence the activity of these drugs would be improved (Torres et al., 2012). Resistance to tetracyclines is caused mostly by induction of cell membrane protein that blocks its entry into the cytosol to prevent its binding to the 30S ribosomal unit (Mukherjee et al., 2019). Developing newer antibiotic agents is capital intensive (Bai et al., 2019), therefore there is the need to rejuvenate older existing antibiotics by loading them into nanoparticles to combat antibiotic resistance and improve antibacterial activity (Mukherjee et al., 2019). Moreover, *C. pseudotuberculosis* a member of the heterogeneous Gram positive-CMNR-group of microorganisms (*Corynebacterium*, *Mycobacterium, Nocardia, and Rhodococcus* genus) which are zoonotic in nature and with it being able to multiply in macrophages,

thus remain undetected by the hosts immune system (Correa et al., 2018).

1.4 Hypothesis

Hypothesis 1

H_o – Oxytetracycline loaded cockle shell calcium carbonate nanoparticle (OTC-CS-$CaCO_3$NP) will not have appropriate physicochemical properties for drug delivery and OTC cannot be sustainably released from it *in vitro*.

H_a - Oxytetracycline loaded into cockle shell calcium carbonate nanoparticle (OTC-CS-$CaCO_3$NP) has appropriate physicochemical properties for drug delivery and OTC can be sustainably released from it *in vitro*.

Hypothesis 2

H_o - OTC-CS-CaCO3NP is toxic to normal mouse fibroblast (NIH3T3) cells

H_a - OTC-CS-$CaCO_3$NP is not toxic to normal mouse fibroblast (NIH3T3) cells

Hypothesis 3

H_o - OTC-CS-CaCO3NP does not possess better antibacterial and antibiofilm activity against *C. pseudotuberculosis in vitro* than free OTC

H_a - OTC-CS-$CaCO_3$NP possess better antibacterial and antibiofilm activity against *C. pseudotuberculosis in vitro* than free OTC

Hypothesis 4

Ho - *In vivo* release of OTC from OTC-CS-$CaCO_3$NP does not provide better plasma pharmacokinetic parameters in *BALB/c* mice than free OTC.

Ha - *In vivo* release of OTC from OTC-CS-CaCO3NP provides better plasma pharmacokinetic parameters in BALB/c mice than free OTC.

1.5 Objectives

The main aim of this study was to evaluate the effect of OTC-CS-$CaCO_3$NP against *C. pseudotuberculosis* isolated from goat CLA cases.

The specific objectives are to:

i. synthesize and characterize OTC loaded CS-$CaCO_3$NP and evaluate the *in vitro* release of OTC.
ii. evaluate the *in vitro* cytotoxicity of OTC-CS-$CaCO_3$NP in normal mouse fibroblast (NIH3T3) cell line using MTT and trypan blue assay.
iii. determine the antibacterial and antibiofilm effect of OTC-CS-$CaCO_3$NP against *C. pseudotuberculosis in vitro.*
iv. investigate the pharmacokinetics of OTC and OTC-CS-$CaCO_3$NP in a *BALB/c* mice model.

REFERENCES

Abd Ghafar, S. L. M., Hussein, M. Z. and Abu Bakar Zakaria, Z. (2017). Synthesis and Characterization of Cockle Shell-Based Calcium Carbonate Aragonite Polymorph Nanoparticles with Surface Functionalization. *Journal of Nanoparticles*, *2017*, 1–12.

Abdal D. A., Hossain, M. K., Lee, S. B., Kim, K., Saha, S. K., Yang, G., Choi, H. Y. and Cho, S. (2017). The role of reactive oxygen species (ros) in the biological activities of metallic nanoparticles. *International Journal of Molecular Sciences*, *18*(1), 1–21.

Abdul Latif, N. A., Abba, Y., Jesse, F. F. A., Chung, E. L. T., Zamri-saad, M., Saharee, A. A., Zakaria, Z., Haron, A. W. and Mohd-Lila, M. A. (2017). Histopathological assessment of chronic *Corynebacterium pseudotuberculosis* infection in the reproductive tract and iliac lymph node of Katjang does. *Comparative Clinical Pathology*, *26*(1) 147–154.

Achenie, L. E. K. and Pavurala, N. (2017). Modelling of drug release from a polymer matrix system, *Novel Approaches in Drug Designing and Development*, *2*(3),1-10.

Aderibigbe, B. A. (2017). Metal-based nanoparticles for the treatment of infectious diseases. *Molecules*, 22, 1-27.

Adhikari, M. D., Goswami, S., Panda, B. R., Chattopadhyay, A. and Ramesh, A. (2013). Membrane-directed high bactericidal activity of (gold nanoparticle)–polythiophene composite for niche applications against pathogenic bacteria. *Advanced Healthcare Materials*, *2*, 599–606.

Aktas I. and Yarsan E. (2017). Pharmacokinetics of conventional and long-acting oxytetracycline preparations in kilis goat. *Frontiers in Veterinary Medicine*, *4*, 1-5.

Albanese, A., Tang, P. S. and Chan, W. C. W. (2012). The effect of nanoparticle size , shape , and surface chemistry on biological systems. *Annual Review of Biomedical Engineering*, *14*, 1-17

Armbruster, C. and Parsek, M. (2018). New insight into the early stages of biofilm formation. *Proceedings of the National Academy of Sciences of the United States of America*, *115* (17), 4317-4319.

Arvizo, R. R., Miranda, O. R., Moyano, D. F., Walden, C. A., Giri, K., Bhattacharya, R., Robertson, J. D., Rotello, V. M., Reid, J. M. and Mukherjee, P. (2011). Modulating pharmacokinetics, tumour uptake and biodistribution by engineered nanoparticles. *Plos One*, *6*, 3-8.

Ata, S., Hamid, F., Ahmed, M., Hamid, M., Wattoo, S., Ahmed, S. and Wadood, A. (2015). A method optimization study for atomic absorption spectrophotometric

determination of total zinc in insulin using direct aspiration technique. *Alexandria Journal of Medicine*, *51*(1), 19–23.

Auad, J., Cerutti, J., Cooper, L. G., Camussone, C. M., Lozano, N. A., Crespo, F. M. and Lozano, A. (2018). Humoral immune response of pregnant goats to two *Corynebacterium pseudotuberculosis* bacterin formulations. *Australian Journal of Veterinary Science*, *105*, 101–105.

Awang-Hazmi ,A. J., Zuki A. Z., Noordin, M. M., Jalila, A. and Norimah, Y. (2007). Mineral and physicochemical characterization of cockle (*Anadara granosa*) shells as an alternative biomaterial for bone tissue engineering. *The Medical Journal of Malaysia*, *63*, 93–94.

Bahadar, H., Maqbool, F. Niaz, K. and Abdollahi, M. (2016). Toxicity of nanoparticles and an overview of current experimental models. *Iranian Biomedical Journal*, *20*(1), 1–11.

Bai, D., Lin, X., Huang, Y. & Zhang , X. (2018). Theranostics Aspects of Various Nanoparticles in Veterinary Medicine. *International Journal of Molecular Sciences*, *19*, 3299-3331.

Bailer, A. J. (1988). Testing for the equality of area under the curves when using destructive measurement techniques. *Journal of Pharmacokinetics and Biopharmaceutics*, *16*, 303–9.

Barzegar-jalali, M., Adibkia, K., Valizadeh, H., Reza, M. and Shadbad, S. (2008). Kinetic analysis of drug release from nanoparticles, *Journal of Pharmacy and Pharmaceutical Sciences*, *11*(1), 167–177.

Basavaraju, M., Sisnity, V. S. and Palaparthy, R. (2016). ScienceDirect quorum quenching : signal jamming in dental plaque biofilms. *Journal of Dental Sciences*, *11*(4), 349–352.

Bastos, B. L., Portela, R. W. D., Dorella, F. A., Ribeiro, D., Seyffert, N., Castro, T. L., Miyoshi, A., Oliveira, S. C., Meyer, R. and Azevedo, V. (2012). *Corynebacterium pseudotuberculosis*: immunological responses in animal models and zoonotic potential. *Journal of Clinical and Cellular Immunology*, *01*(4), 1–15.

Beaudoin, T., Zhang, L., Hinz, A. J., Parr, C. J. and Mah, T. (2012). The biofilm-specific antibiotic resistance gene ndvb is important for expression of ethanol oxidation genes in *Pseudomonas aeruginosa*. *Journal of Bacteriology*, *194*(12), 3128–3136.

Bernard, K. (2012). The genus *Corynebacterium* and other medically relevant Coryneform-Like bacteria. *Journal of Clinical Microbiology*, *50*(10), 3152–3158.

Bernardes, E.T., Charron-Mazindol, L., Reading, D. J., Reckseidler-Zenteno, S. L. and Lewenza, S. (2017). Exopolysaccharides representing small molecules with

antibiofilm and antivirulence activity against *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*,*61*(5), 1–15.

Bhatia S. (2016) Nanoparticles types, classification, characterization, fabrication methods and drug delivery applications. In: natural polymer drug delivery systems. Springer, Cham pp 33-93.

Billington, S. J., Esmay, P. A., Songer, J. G. and Jost, B. H. (2002). Identification and role in virulence of putative iron acquisition genes from *Corynebacterium pseudotuberculosis*. *FEMS Microbiology Letters*, *208*, 41–45.

Bjarnsholt, T. (2013). The role of bacterial biofilms in chronic infections. *Acta Pathologica, Microbiologica et Immunologica Scandinavica, 121*,1-51.

Boulos, R. A., Zhang, F., Tjandra, E. S., Martin, A. D., Spagnoli, D. and Raston, C. L. (2014). Spinning up the polymorphs of calcium carbonate. *Scientific Reports*, *4*, 1–6.

Brandenburg, K. S., Calderon, D. F., Kierski, P. R., Brown, L., Shah, N. M., Abbott, N. L., Schurr, M. J., Murphy, C. J., McAnulty, J. F. and Czuprynsky, C. J. (2016). Inhibition of *Pseudomonas aeruginosa* biofilm formation on wound dressings. *Wound Repair and Regeneration*, *23*(6), 842–854.

Braun, K., Martina, C., Biskupek, J., Kaiser, U., Kirchho, F. and Lindén, M. (2018). Comparison of different cytotoxicity assays for in vitro evaluation of mesoporous silica nanoparticles. *Toxicology in Vitro*, *52*, 214–221.

Burkovski, A. (2013). Cell envelope of *Corynebacteria*: structure and influence on pathogenicity. *ISRN Microbiology*, *2013*, 1-11

Carne, H. R. and Onon, E. O. (1978). Action of *Corynebacterium ovis* exotoxin on endothelial cells of blood vessels. *Nature*, *271*(5642):246-248.

Castaneda, P. Mclaren, A. and Tavaziva, G. (2016). Biofilm antimicrobial susceptibility increases with antimicrobial exposure time. *Clinical Orthopaedics and Related Research*, *474*(7), 1659–1664

Castillo-martínez, J. C., Martínez-castañón, G. A., Martínez-gutierrez, F., Zavala-alonso, N. V., Patiño-marín, N. and Niño-martinez, N. (2015). Antibacterial and antibiofilm activities of the photothermal therapy using gold nanorods against seven different bacterial strains, *Journal of Nanomaterials*, *2015*, 1-7.

Cavaliere, R., Ball, J. L., Turnbull, L. and Whitchurch, C. B. (2014). The biofilm matrix destabilizers, EDTA and DNase I enhance the susceptibility of nontypeable *Hemophilus influenzae* biofilms to treatment with ampicillin and ciprofloxacin. *Microbiology Open*, 3(4), 557–567.

Chakraborty, S. P., Sahu, S. K. and Pramanik, P. (2012). Biocompatibility of folate-modified chitosan nanoparticles. Asian *Pacific Journal of Tropical Biomedicine, 2*, 215–219.

Chapman, C. K. and Kennedy, M. J. (2017). Caseous lymphadenitis management in Goats. *Agriculture extension, Utah State University*, *1*, 1-4.

Chen, J. and Xiang, L. (2009). Controllable synthesis of calcium carbonate polymorphs at different temperatures. *Powder Technology*, *189*(1), 64–69.

Chen, J., Hessler, J. A., Putchakayala, K., Khan, D. P., Hong, S., Mullen, D. G., DiMaggio, S. C., Som, A., Tew, G. N., Lopatin, A. N., Baker, Jr. J. R., Holl, M.M. B. and Orr, B. G. (2009). Cationic nanoparticles induce nanoscale disruption in living cell plasma membranes. *The Journal of Physical Chemistry B*, *113*, 11179–11185.

Chi, Z., Liu, R., You, H., Ma, S., Cui, H. and Zhang, Q. (2014). Probing the *in vitro* cytotoxicity of the veterinary drug oxytetracycline, *Plos One*, *9*(7), 3–10.

Chopra, I. and Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews*, *65*(2), 232–60.

Chung, P. Y. and Toh, Y. S. (2014). Anti-biofilm agents : recent breakthrough against multi-drug resistant *Staphylococcus aureus. Pathogens and disease*, *70*, 231–239.

Corrêa, J. I., Stocker, A., Trindade, S. C., Vale, V., Brito, T., Bastos, B., Raynal, J. T., Miranda, P. M., Alcantara, A. C., Freire, S. M., Costa, L. M. and Meyer, R. (2018). *In vivo* and *in vitro* expression of five genes involved in *Corynebacterium pseudotuberculosis* virulence. *AMB Express*, *8*, 88-98.

Dakal, T. C., Kumar, A., Majumdar, R. S. and Yadav, V. (2016). Mechanistic basis of antimicrobial actions of silver nanoparticles. *Frontiers in Microbiology*, *7*.1–17.

Danmaigoro, A., Selvarajah, G. T., Hezmee, M. and Noor, M. (2017). Development of cockleshell (Anadara granosa) derived $CaCO_3$ nanoparticle for doxorubicin delivery. *Journal of Computational and Theoretical Nanoscience*, *14*, 5074-5086.

Das, S. and Chaudhury, A. (2011). Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. *AAPS PharmSciTech*, *12*, 62–76.

Davies, D. G. and Marques, N. H. (2009). A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. *Journal of Bacteriology*, *191*(5), 1393–1403.

Dawson, C. C., Intapa, C. and Jabra-rizk, M. A. (2011). " Persisters " : Survival at the cellular level. *Plos Pathogens*, *7*(7), 2–4.

Deng, H., Shen, X. C., Wang, X.M. and Du, C. (2013). Calcium carbonate crystallization controlled by functional groups: A mini-review. *Frontiers of Material Science*, *7*(1),62-68.

Dhand, C., Dwivedi, N., Loh, J. and Jie, N. (2015). Methods and strategies for the synthesis of diverse nanoparticles and their applications : a comprehensive overview, *RSC Advances*, *5*, 105003–105037.

Dijaz, S. M., Jalali, M. B., Zarrintan, M.H., Adibkhia, K. M. and Lotfipour, F. (2015). Calcium carbonate nanoparticles as cancer drug delivery system. *Expert opinion in Drug Delivery*, *12*, 1649-1660.

Domenis, L., Spedicato, R., Pepe, E. Orusa R. and S. Robetto. (2018). Caseous lymphadenitis caused by *Corynebacterium pseudotuberculosis* in alpine chamois (*Rupicapra r. rupicapra*): a Review of 98 Cases. *Journal of Comparative Pathology*, *161*, 11-19.

Dorella, F., Pacheco, G. L. C., Oliveira, S., Miyoshi, S. and Azevedo, V. (2006). *Corynebacterium pseudotuberculosis*: microbiology , biochemical properties , pathogenesis and molecular studies of virulence. *Veterinary Research*, *37*(2), 201-218.

Dorey, L., Pelligand, L., Cheng, Z. and Lees, P. (2017). Pharmacokinetic / pharmacodynamic integration and modelling of oxytetracycline for the porcine pneumonia pathogens *Actinobacillus pleuropneumoniae* and *Pasteurella multocida*. *Journal of Veterinary Pharmacology and Therapeutics*, *40*(5), 505–516.

El-Ghannam, A., Ahmed, K. and Omran, M. (2005). Nanoporous delivery system to treat osteomyelitis and regenerate bone: gentamicin release kinetics and bactericidal effect. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *73B*(2), 277–284.

Eroshenko, D., Polyudova, T. and Korobov, V. (2017). N-acetylcysteine inhibits growth, adhesion and biofilm formation of Gram-positive skin pathogens. *Microbial Pathogenesis*, *105*, 145–152.

Fard, J. K., Jafari, S. and Eghbal, M. A. (2015). A review of molecular mechanisms involved in toxicity of nanoparticles. *Advanced Pharmaceutical Bulletin*, 5(4), 447–54.

Faustino-Vega, A., Alvarez-Polo, M. A., Gasca, B. and Bernad-Bernad, M. (2009). Influence of three different colloidal systems on the oxytetracycline-lecithin behaviour. *Pharmazie*, *64*, 05–509.

Ferraz, M. P., Mateus, A. Y., Sousa, J. C. and Monteiro, F. J. (2007). Nanohydroxyapatite microspheres as delivery system for antibiotics: Release kinetics, antimicrobial activity, and interaction with osteoblasts. *Journal of Biomedical Materials Research Part A*, *81A* (4), 994–1004.

Fu, W., Forster, T., Mayer, O., Curtin, J. J., Lehman, S. M. and Donlan, R. M. (2010). Bacteriophage cocktail for the prevention of biofilm formation by pseudomonas aeruginosa on catheters in an *in vitro* model system. *Antimicrobial Agents and Chemotherapy*, *54*(1), 397–404.

Fu, W., Hezmee, M., Noor, M., Yusof, L. M., Ibrahim, A. T., Keong, Y. S., Jaji, A. Z. and Zakaria, M. Z. A. B. (2017). *In vitro* evaluation of a novel pH sensitive drug delivery system based cockle shell-derived aragonite nanoparticles against osteosarcoma, *Journal of Experimental Nanoscience, 12*(1), 161-187.

Fuente-Nunez, C., Reffuveille, F., Fernandez, L. and Hancock, R. E. (2013). Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. *Current Opinion in Microbiology*, *16*,580-589.

Galvão, C. E., Fragoso, S. P., de Oliveira, C. E., Forner, O., Pereira, R. R. B., Soares, C. O. and Rosinha, G. M. S. (2017). Identification of new Corynebacterium pseudotuberculosis antigens by immunoscreening of gene expression library. *BMC Microbiology*, *17*(1), 202-210.

Gao, W., Thamphiwatana, S., Angsantikul, P. and Zhang, L. (2015). Nanoparticle approaches against bacterial infections. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *6*(6), 532–547.

Garrigós, C., Murillo, O., Lora-Tamayo, J., Verdaguer, R., Tubau, F., Cabellos, C. and Ariza, J. (2012). Efficacy of daptomycin-cloxacillin combination in experimental foreign-body infection due to methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, *56*(7), 3806–3811.

Gbanbari, M., Klose, V., Crispie, F. and Cotter, P. D. (2019) The dynamics of the antibioticresistome in the feces of freshly weaned pigs following therapeutic administration of oxytetracycline. *Scientific Reports*, 9, 1-11.

Georgescu, D., Brezoiu, A. and Mitran, R. (2017). Mesostructured silica titania composites for improved oxytetracycline delivery systems. *Comptes Rendus - Chimie*, *20*(11–12), 1017–1025.

Ghaji, M. S., Abu, Z., Zakaria, B., Shameha, A. R. I., Noor, M., Hezmee, M. and Hazilawati, H. (2017). Novelty to synthesize nanoparticles from cockle shell via mechanical method to delivery and controlled Release of Cytarabine. *Journal of Computational and Theoretical Nanoscience*, *14*, 1–9.

Goel, S. and Mishra, P. (2018). Thymoquinone inhibits biofilm formation and has selective antibacterial activity due to ROS generation. *Applied Microbiology and Biotechnology*, *102*, 1955–1967.

Gökçen, A., Vilcinskas, A. and Wiesner, J. (2013). Methods to identify enzymes that degrade the main extracellular polysaccharide component of *Staphylococcus epidermidis* biofilms. Virulence, *4*(3), 260-270.

Guerra, W., Silva-caldeira, P. P., Terenzi, H. and Pereira-maia, E. C. (2016). Impact of metal coordination on the antibiotic and non-antibiotic activities of tetracycline-based drugs. *Coordination Chemistry Reviews*, *328*, 188–199.

Gupta, A. (2015). Biofilm quantification and comparative analysis of MIC (minimum inhibitory concentration) and MBIC (minimum biofilm inhibitory concentration

) value for different antibiotics against *E . coli*. *International .Journal of Current Microbiological and Applied Science*, *4*(2), 198–224.

Hammadi, N. I., Abba, Y., Hezmee, M. N. M., Razak, I. S. A., Jaji, A. Z., Isa, T. and Zakaria, M. Z. A. B. (2017). Formulation of a sustained release docetaxel loaded cockle shell-derived calcium carbonate nanoparticles against breast cancer. *Pharmaceutical Research*, *34*, 1193–1203.

Hariharan, M., Varghese, N., Cherian, A. B., Sreenivasan, P. V and Paul, J. (2014). Synthesis and characterisation of $CaCO_3$ (calcite) nano particles from cockle shells using chitosan as precursor. *International Journal of Scientific and Research Publications*, *4*(10), 1–5.

Harja, M. and Ciobanu, G. (2018). Science of the total environment studies on adsorption of oxytetracycline from aqueous solutions onto hydroxyapatite. *Science of the Total Environment*, *629*, 36–43.

Harms, A. C. A., Papich, M. G., Stamper, A., Patricia, M. R., Rodriguez, M. X. and Hohn, A. A. (2004). Pharmacokinetics of oxytetracycline in loggerhead sea turtles (Caretta caretta) after single intravenous and intramuscular injections. *Journal of Zoo and Wildlife Medicine*, *35*(4), 477-488.

Harper, D. R., Parracho, H. M. R. T., Walker, J., Sharp, R., Hughes, G. and Werthé, M. (2014). Bacteriophages and Biofilms. *Antibiotic*s, *3*, 270–284.

Harrison, J. J., Ceri, H., Yerly, J., Stremick, C. A., Hu, Y., Martinuzzi, R. and Turner, R. J. (2006). The use of microscopy and three-dimensional visualization to evaluate the structure of microbial biofilms cultivated in the Calgary Biofilm Device, *Biological Procedures Online*, *8*(1), 194–215.

He, Y., Ingudam, S., Reed, S., Gehring, A., Jr, T. P. S. and Irwin, P. (2016). Study on the mechanism of antibacterial action of magnesium oxide nanoparticles against foodborne pathogens. *Journal of Nanobiotechnology*, *14*, 1–9.

Hochbaum, A. I., Kolodkin-gal, I., Foulston, L., Kolter, R., Aizenberg, J. and Losick, R. (2011). Inhibitory effects of D -amino acids on *Staphylococcus aureus* biofilm development. *Journal of Bacteriology*, *193*(20), 5616–5622.

Hoiby, N., Bjarnsholt, T., Givskov, M., Molin, S. and Ciofu, O. (2010). Antibiotic resistance of bacterial biofilms. *International Journal of Antimicrobial Agents*, *35*, 322–332.

Horie, M., Nishio, K., Kato, H., Endoh, S., Fujita, K., Nakamura, A., Kinugasa, S., Hagihara, Y., Yoshida, Y. and Iwahashi, H. (2014). Evaluation of cellular influences caused by calcium carbonate nanoparticles. *Chemico-Biological Interactions*, *210*, 64–76.

Hu, D., Li, H., Wang, B., Ye, Z., Lei, W., Jia, F., Jin, Q., Ren, K. and Ji, J. (2017). Surface adaptive gold nanoparticles with effective adherence and enhanced

photothermal ablation of methicillin resistant *Staphylococcus aureus* biofilms. *ACS Nano*, *11*, 9330-9339.

ICH (2019) Harmonized guideline, bioanalytical method validation M10: Text and Methodology Q2(R1). ICH Secretariat, Geneva 13, Switzerland, Pp 1-51.

Idris, S. B, Arifah, A. K., Jesse, F. F.A., Ramanoon, S. Z Basit, M. A. and Zakaria, Z. A. B (2020). Pharmacokinetics of free oxytetracycline and oxytetracycline loaded cockle shell calcium carbonate-based nanoparticle in BALB/c mice. *Frontiers in Veterinary Science*, *7*, 1-5.

Isa, T., Zakaria, Z. A. B., Rukayadi, Y., Mohd Hezmee, M. N., Jaji, A. Z., Imam, M. U., Hammadi, N. I. and Mahmood, S. K. (2016). Antibacterial activity of ciprofloxacin-encapsulated cockle shells calcium carbonate (aragonite) nanoparticles and its biocompatibility in macrophage J774A.1. *International Journal of Molecular Sciences*, *17*(5), 1-17.

Izgür, M. İ., Akan, M., Lhan, Z. İ. and Lu, N. Y. Ğ. (2010). Studies on vaccine development for ovine caseous lymphadenitis. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *57*, 161-165.

Jaji, A. Z., Abu, Z., Zakaria, B., Mahmud, R., Loqman, M. Y. and Abba, Y. (2017). Safety assessments of subcutaneous doses of aragonite calcium carbonate nanocrystals in rats. *Journal of Nanoparticle Research*, *19*, 1-18

Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M. A., Hussain, T., Ali, M., Rafiq, M. and Kamil, A. M. (2018). ScienceDirect Bacterial biofilm and associated infections. *Journal of the Chinese Medical Association*, *81*(1), 7–11.

Jamal, M., Tasneem, U., Hussain, T. and Andleeb, S. (2015). Bacterial biofilm: its composition, formation and role in human infections. *Research and Reviews: Journal of Microbiology and Biotechnology*, *4*(3), 1–14.

Jakstys, B., Ruzgys, P., Tamosiunas, M., Satkauskas, S. (2015). Different cell viability assays reveal inconsistent results after bleomycin electro transfer *in vitro*. *The Journal of Membrane Biology*, 248, 857-853

Jermy, A. (2012). Biofilms: disassembly instructions included. *Nature Reviews Microbiology*, *10*(6), 376-376.

Jesse F. F. A., Abba, Y., Nurul, S. Y., Adamu, L., Bitrus, A. A., Chung, E. L. T., Sadiq, M. A., Idris, U. H., Haron, W. and Mohhamed L. M. A. (2017). Clinical case of caseous lymphadenitis in a goat : case management. *Malaysian Journal of Veterinary Research*, *8*(1), 31–35.

Jones, D. E., Ghandehari, H. and Facelli, J. C. (2015). Predicting cytotoxicity of PAMAM dendrimers using molecular descriptors. *Beilstein Journal of Nanotechnology*, *6*(1), 1886–1896.

Jijie, R., Barras, A., Teodorescu, F., Boukherroub, R. and Szunerits, S. (2016). Advancements on the molecular design of nanoantibiotics: current level of development and future challenges. *Molecular Systems Design and Engineering*, 2, 349-369.

Kalghatgi, S., Spina, C. S., Costello, J. C., Liesa, M., Ruben, J., Slomovic, S., Molina, A., Shirihai, O. S and Collins, J. J. (2013). Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in mammalian cells. *Science Translational Medicine*, *5*(192), 1-22.

Kamba, A. S., Ismail, M., Azmi T. I. T. and Zakaria, Z. A. B. (2014). Biocompatibility of bio based calcium carbonate nanocrystals aragonite polymorph on NIH3T3 fibroblast cell line. *African Journal of Traditional, Complementary and Alternative Medicines*, *11*(4), 31–38.

Kamba, S., Ismail, M., Hussein-Al-Ali, S., Ibrahim, T. and Zakaria, Z. (2013). *In Vitro* delivery and controlled release of doxorubicin for targeting osteosarcoma bone cancer. *Molecules*, *18*(9), 10580–10598.

Kaya, S., Yarsan, E., Baydan, E., Akkaya, R., Aksoy, A. (2001). Comparison of the pharmacokinetics of conventional and long acting formulations of oxytetracycline in sheep. *Turkish Journal of Veterinary and Animal Sciences*, *25*, 173-177.

Khan, N. T. (2017). Nanoparticles Mediated Drug Delivery. *Journal of Pharmacogenomics and Pharmacoproteomics*, *8*(3): 8–10.

Khanna, P., Ong, C. Bay, B. H. and Baeg, G. H. (2015) Nanotoxicity: an interplay of oxidative stress, inflammation and cell death. *Nanomaterials*, *5*(3),1163–80.

Kim, J., Pitts, B., Stewart, P. S., Camper, A. and Yoon, J. (2008). Comparison of the antimicrobial effects of chlorine, silver ion , and tobramycin on biofilm. *Antimicrobial Agents and Chemotherapy*, *52*(4), 1446–1453.

Kostakioti, M., Hadjifrangiskou, M. and Hultgren, S. J. (2013). Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the post antibiotic era. *Cold Spring Harbour Perspectives*, *3*,1–24.

Kot, B., Wierzchowska, K., Grużewska, A. and Lohinau, D. (2018). The effects of selected phytochemicals on biofilm formed by five methicillin-resistant *Staphylococcus aureus*, *Natural Product Research*, *32*(11), 1299-1302.

Kou, L., Sun, J., Zhai, Y. and He, Z. (2013). The endocytosis and intracellular fate of nanomedicines : Implication for rational design. *Asian Journal of Pharmaceutical Sciences*, *8*(1), 1–10.

Kumar, A., Alam, A., Rani, M., Ehtesham, N. Z. and Hasnain, S. E. (2017). Biofilms : Survival and defence strategy for pathogens, *International Journal of Medical Microbiology*, *307*, 481–489.

Kuria, J. K. N., Mbuthia, P. G., Kang, E. K. and Wahome, R. G. (2001). Caseous Lymphadenitis in goats: the pathogenesis, incubation period and serological response after experimental infection. *Veterinary Research Communications*, *25*, 89–97.

Lagast, N., Carlier, C. and Ceelen, W. P. (2018). Pharmacokinetics and tissue transport of intraperitoneal chemotherapy. *Surgical Oncology Clinics of North America*, *27*(3), 477–494

Lambert, G., Bergman, A., Zhang, Q., Bortz, D. and Austin, R. (2014). Physics of biofilms : the initial stages of biofilm formation and dynamics. *New Journal of Physics*, 16, 1-23.

Larbi-Bouamrane, O., Bal, Y., Aliouche, D., Cote, G. and Chagnes, A. (2016). Preparation and characterization of cross-linked chitosan microcapsules for controlled delivery of oxytetracycline. *Indian Journal of Pharmaceutical Sciences*, *78*(6), 715–724.

Lee, J., Kim, M., Kim, H., Lee, J. k., Jeong, J., Kim, Y., Oh, J. and Choi, S. (2015). The fate of calcium carbonate nanoparticles administered by oral route: absorption and their interaction with biological matrices. *International Journal of Nanomedicine*, *10*, 2273-2293.

Leroueil, P. R., Hong, S., Mecke, A., Jr, J. R. B., Orr, G. and Holl, M. M. B. (2007). Nanoparticle Interaction with Biological Membranes: Does nanotechnology present a Janus face? *Accounts of Chemical Research*, *40*(5), 335–342.

Li, H., Yang, H., Zhou, Z., Li, X., Yi, W., Xu, Y., Wang, Z. and Hu, S. (2018). Isolation, antibiotic resistance, virulence traits and phylogenetic analysis of *Corynebacterium pseudotuberculosis* from goats in southwestern China. *Small Ruminant Research*, *168*(160), 69–75.

Li, Y., Zhang, Y., Yang, T., Li, H., Guo, J., Zhao, Q. and Xie, J. (2015). Pharmacokinetics and tissue distribution study of Isovitexin in rats by HPLC-MS/MS Panel. *Journal of Chromatography*, B. *991*, 13-20

Limoli, D. H., Jones, C. J., Wozniak, D. J. and Cruz, S. (2015). Bacterial extracellular polysaccharides in biofilm formation and function. *Microbiology Spectrum*, *3*(3), 1–30.

Love, S. A., Maurer-jones, M. A., Thompson, J. W., Lin, Y. and Haynes, C. L. (2012). Assessing nanoparticle toxicity. *Annual Review of Analytical Chemistry*, 25, 181–205.

Lucero-Acuna and Guzmán, R. (2015). Nanoparticle encapsulation and controlled release of a hydrophobic kinase inhibitor : Three stage mathematical modelling and parametric analysis, *International Journal of Pharmaceutics*, *494*, 249–257.

Lye, G., Jacob, A., Pomroy, W., Stafford, K. and Singh, P. (2019). Pharmacokinetics of subcutaneously administered doramectin in alpacas. *Journal of Veterinary Pharmacology and Therapeutics*, *43*,123–1288.

Ma, S., Li, H. U. I., Yan, C., Wang, D. A. N., Li, H., Xia, X., Dong, X., Zhao, Y., Sun, T., Hu, P. and Guan, W. (2014). Antagonistic effect of protein extracts from Streptococcus sanguinis on pathogenic bacteria and fungi of the oral cavity. *Experimental and Therapeutic Medicine*, 1486–1494.

Maaland, M. G., Papich, M. G., Turnidge, J. and Guardabassi, L. (2013). Pharmacodynamics of doxycycline and tetracycline against *Staphylococcus pseudintermedius*: proposal of canine-specific breakpoints for doxycycline. *Journal of Clinical Microbiology*, *51*(11), 3547–3554.

Mah, T. (2012). Regulating Antibiotic Tolerance within Biofilm Microcolonies. *Journal of Bacteriology*, *194*(18), 4791–4792.

Mah, T. C. and O'Toole, G. A. O. (2001). Mechanisms of biofilm resistance to antimicrobial agents. *Trends in Microbiology*, *9*(1), 34–39.

Masarudin, M. J., Cutts, S. M., Evison, B. J. and Pigram, P. J. (2015). Factors determining the stability , size distribution , and cellular accumulation of small , monodisperse chitosan nanoparticles as candidate vectors for anticancer drug delivery : application to the passive encapsulation of [14 C] -doxorubicin, *Nanotechnology Science and Applications*, *8*, 67–80.

Maynard, A. D., Warheit, D. B. and Philbert. M. A. (2011). The new toxicology of sophisticated materials: nanotoxicology and beyond. *Toxicological Sciences*, *120*(Suppl 1): S109–S129.

McKean, S. C., Davies, J. K., Moore, R. J. and Moore, R. J. (2018). Expression of phospholipase D, the major virulence factor of *Corynebacterium pseudotuberculosis*, is regulated by multiple environmental factors and plays a role in macrophage death. *Microbiology*, *153*, 2203–2211.

Min, H. K., Jang, E., Jae, H., Hwang, Y., Ryu, J., Moon, J. and Cheon, S. (2019). Journal of Industrial and Engineering Chemistry pH-Responsive mineralized nanoparticles for bacteria-triggered topical release of antibiotics. *Journal of Industrial and Engineering Chemistry*, *71*, 210–219.

Minozzi, G., Mattiello, S., Grosso, L., Crepaldi, P., Chessa, S. and Pagnacco, G. (2017). First insights in the genetics of caseous lymphadenitis in goats. *Italian Journal of Animal Science*, *16*(1), 31–38.

Mishra, D., Khare, P., Shanker, K., Singh, D. K. and Luqman, S. (2016). Controlled delivery systems of cellulose matrix for oxytetracycline : *In vitro* dissolution. *New Horizons in Translational Medicine*, *3*(2), 66–72.

Mohamad, A. T., Kaur, J., Azwadi, N., Sidik, C. and Rahman, S. (2018). Nanoparticles : a review on their synthesis, characterization and

physicochemical properties for energy technology industry. *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, *46*(1), 1–10.

Mohamed, M. M., Fouad, S. A., Elshoky, H. A., Mohammed, G. M. and Salaheldin, T. A. (2017). Antibacterial effect of gold nanoparticles against *Corynebacterium pseudotuberculosis*. *International Journal of Veterinary Science and Medicine*, *5*(1), 23–29.

Molobela, I. P., Cloete, T. E. and Beukes, M. (2010). Protease and amylase enzymes for biofilm removal and degradation of extracellular polymeric substances (EPS) produced by *Pseudomonas fluorescens* bacteria. *African Journal of Microbiology Research*, *4*(14), 1515-1524.

Motwani, S. K., Chopra, S., Talegaonkar, S., Kohli, K., Ahmad, F. J. and Khar, R. K. (2008). Chitosan-sodium alginate nanoparticles as sub microscopic reservoirs for ocular delivery: Formulation, optimisation and in vitro characterisation. *European Journal of Pharmaceutics and Biopharmaceutics*, *68*(3), 513–525.

Mu, H., Tang, J., Liu, Q., Sun, C., Wang, T. and Duan, J. (2016). Potent antibacterial nanoparticles against biofilm and intracellular bacteria. *Scientific Reports*, *6*, 1–9.

Mudshinge, S. R., Deore, A. B., Patil, S. and Bhalgat, C. M. (2011). Nanoparticles: emerging carriers for drug delivery. *Saudi Pharmaceutical Journal*, *19*(3), 129–141.

Muhamad, I. I. and Selvakumaran, S. (2014). Designing polymeric nanoparticles for targeted drug delivery system outline: *Nanomedicine, 11*, 287–313.

Mukherjee, R., Dutta, D., Patra, M., Chatterjee, B., and Basu, T. (2019). Nanonized tetracycline cures deadly diarrheal disease 'shigellosis' in mice, caused by multidrug-resistant *Shigella flexneri 2a* bacterial infection. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *18*, 402–413.

Mukherjee, R., Patra, M., Dutta, D., Banik, M. and Basu, T. (2016). Tetracycline-loaded calcium phosphate nanoparticle (Tet-CPNP): Rejuvenation of an obsolete antibiotic to further action. *Biochimica et Biophysica Acta*, *1860*(9), 1929–1941.

Mulla, S., Kumar, A. and Rajdev, S. (2016). Comparison of MIC with MBEC Assay for *in vitro* antimicrobial susceptibility testing in biofilm forming clinical bacterial isolates, *Advances in Microbiology*, *6*, 73–78.

Murdock, R. C., Braydich-Stolle, L., Schrand, A. M., Schlager, J. J. and Hussain, S. M. (2008). Characterization of nanomaterial dispersion in solution prior to *in vitro* exposure using dynamic light scattering technique. *Toxicological Sciences*, *101*(2), 239–253.

Muthusamy, K. and Sabri, N. A. (2012). Cockle shell: a potential partial coarse aggregate replacement in concrete. *International Journal of Science, Environment and Technology*, *1*(4),260-267.

Mydin, R. B. S. M. N., Zahidi, I. N. M., Ishak, N. N. and Shaida, N. (2018). Potential of calcium carbonate nanoparticles for therapeutic applications. *Malaysian Journal of Medicine and Health Sciences*, *14*(12), 201–206.

Narayanan, D., Anitha, A., Jayakumar, R., Nair, S.V. and Chennazhi, K. P. (2012). Synthesis, characterization and preliminary *in vitro* evaluation of PTH 1-34 loaded chitosan nanoparticles for osteoporosis. *Journal of Biomedical Nanotechnology*, *8*, 98–106.

Neto, O. and Silva, C. (2017). Nitric oxide and immune response in infection control of Caseous Lymphadenitis. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *69*(6), 1565–1572.

Ng, F. S. W., Wright, D. M. and Seah, S. Y. K. (2011). Characterization of a phosphotriesterase-like lactonase from *Sulfolobus solfataricus* and its immobilization for disruption of quorum sensing. *Applied and Environmental Microbiology*, *77*(4), 1181–1186.

Nguyen, F., Starosta, A. L., Arenz, S., Sohmen, D., Dönhöfer, A. and Wilson, D. N. (2014). Tetracycline antibiotics and resistance mechanisms. *Biological Chemistry*, 395(5), 559-575.

Ni, M. and Ratner, B. D. (2008). Differentiation of calcium carbonate polymorphs by surface analysis technique - an XPS and TOF-SIMS study. *Surface and Interface Analysis*, *40*(10), 1356–1361.

Nicolosi, D., Cupri, S., Genovese, C., Tempera, G., Mattina, R. and Pignatello, R. (2015). Nanotechnology approaches for antibacterial drug delivery: preparation and microbiological evaluation of fusogenic liposomes carrying fusidic acid. *International Journal of Antimicrobial Agents*, *45*, 622–626.

Norambuena-subiabre, L., Gonz, M. P. and Contreras-lynch, S. (2018). Oxytetracycline depletion and withdrawal time estimation following intraperitoneal administration in three species from Chilean salmon farming. *Aquaculture Research*, *49*, 593–602.

Nouws, J. F., Vree, T. B., Termond, E., Lohuis, J., Lith, P., Binkhorst, G. J. and Breukink, H. J. (1985). Pharmacokinetics and renal clearance of oxytetracycline after intravenous and intramuscular administration to dairy cows. *The Veterinary Quarterly*, *7*(4), 296–305.

Nurul Islam, K., Eaqub Ali, M., Zuki Bin Abu Bakar, M., Loqman, M., Islam, A., Saiful Islam, M., Mahfujur Rahman, M., and Ullah, M. (2013). A novel catalytic method for the synthesis of spherical aragonite nanoparticles from cockle shells. *Powder Technology*, *246*, 434-440.

Nurul Islam, K., Zuki, A. Z., Ali, M. E., Hussein, M. Z. B., Noordin, M. M., Loqman, M. Y., Wahid, H., Hakim, M. A. and Abd Hamid, S. B. (2012). Facile synthesis of calcium carbonate nanoparticles from cockle shells. *Journal of Nanomaterials*, *1*, 1-5.

O'Toole, G. A. O. (2011). Microtiter dish biofilm formation assay. *Journal of Visualized Experiments*, *47*, 10–11

Oliveira Neto, M. G., Santos, H. A., Fraga, R. E., Pacheco, A. S., Sampaio, G. P., Moura-Costa, L. F., Meyer, R., Costa Silva, M., Trindade, S. C. and Vale, V. L. C. (2017). Nitric oxide and immune response in infection control of Caseous Lymphadenitis. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 69(6), 1565–1572.

Olsen, I. (2015). Biofilm-specific antibiotic tolerance and resistance, *European Journal of Clinical Microbiology and Infectious Diseases*, *34*,877–886.

Olson, M. E., Ceri, H., Morck, D. W., Buret, A. G. and Read, R. R. (2002). Biofilm bacteria : Formation and comparative susceptibility to antibiotics. *The Canadian Journal of Veterinary Research, 66,* 86–92.

Opperman, T.J., Kwansny, S. M., Williams, J. D., Khan, A.R., Peet, N.P., Moir, D.T. and Bowlin, T.L. (2009). Arylrhodanines specifically inhibit staphylococcal and enterococcal biofilm formation. *Antimicrobial Agents and Chemotherapy,* 53: 4357–4367.

Oreiby, A. F. (2015). Diagnosis of caseous lymphadenitis in sheep and goat. *Small Ruminant Research*, *123*(1), 160–166.

Oreiby, A. F. and Hegazy, Y. M. (2016). Diagnosis of ovine caseous lymphadenitis by blood and milk gamma interferon assays. *Small Ruminant Research*, *144*, 109–112.

Osman, A. Y., Lim, E., Chung, T., Abba, Y. Sadiq, M. A., Mohammed, K., Lila, M. A. M., Haron, A. W. and Saharee, A.A. (2015). Caseous lymphadenitis in a Goat: A case report. *International Journal of Livestock Research*, *5*(3), 128-132.

Osman, A. Y., Nordin, M. L., Kadir, A. A. and Saharee, A. A. (2018). The Epidemiology and Pathophysiology of Caseous lymphadenitis: a review. *Journal of Veterinary Medicine and Research, 5*(3), 1128-1135.

Othman, H., Hisham, B., Bakar, A., Don, M. M., Azmi, M. and Johari, M. (2013). Cockle shell ash replacement for cement and filler in concrete. *Malaysian Journal of Civil Engineering*, *25*(2), 201–211.

Palza, H. (2015). Antimicrobial polymers with metal nanoparticles, *International Journal of Molecular Sciences, 16,* 2099–2116.

Pan, X., Chen, S., Li, D., Rao, W., Zheng, Y. and Yang, Z. (2018). The Synergistic Antibacterial Mechanism of Gentamicin-Loaded CaCO3 Nanoparticles. *Frontiers in Chemistry*, *5*, 1–9.

Park, D. J., Min, K. H., Lee, H. J., Kim, K., Kwon, I. C., Jeong, S. Y., and Lee, S. C. (2016). Photosensitizer-loaded bubble-generating mineralized nanoparticles for ultrasound imaging and photodynamic therapy. *Journal of Materials Chemistry B*, *4*, 1219–1227. .

Pépin, M. and Paton, M. W. (2010). Caseous lymphadenitis in sheep and goats. *Infectious and parasitic diseases of livestock*, *86*:1153-1165.

Perlman, D., Heuser, L. J., Dutcher, J. D., Barrett, J. M. and Boska, J. A. (1960). Biosynthesis of tetracycline by 5-hydroxy-tetracycline-producing cultures of *Streptomyces rimosus*. *Journal of Bacteriology*, *80*, 419–420.

Porter, W. P., Bitar, Y. M., Charache, P. C. and Strandberg, J. D. (1985) A comparison of subcutaneous and intraperitoneal oxytetracycline injection methods for control of infectious disease in the rat. *Laboratory Animals*, *19*, 3-6.

Posimo, J. M., Unnithan, A. S., Gleixner, A. M., Choi, H. J., Jiang, Y., Pulugulla, S. H. and Leak, R. K. (2014). Viability assays for cells in culture. *Journal of Visualized experiments*, *2*, 1–14.

Pozo, J. D. and Patel, R. (2015). The challenge of treating biofilm-associated bacterial infections. *Translational Medicine*, *82*,(2)1-7.

Puech, V., Chami, M., Lemassu, A., Laneelle, M. B., Schiffler, B., Gounon, P., Bayan, N., Benz, R. and Daffe, M. (2001). Structure of the cell envelope of *Corynebacteria* : importance of the non-covalently bound lipids in the formation of the cell wall permeability barrier and fracture plane. *Microbiology*, *147*, 1365–1382.

Pujalté, I., Passagne, I., Brouillaud, B., Tréguer, M., Durand, E., Ohayon-Courtès, C. and L'Azou. B. (2014). Cytotoxicity and oxidative stress induced by different metallic nanoparticles on human kidney cells. *Beilstein Journal of Nanotechnology 5*(1), 1590–1602.

Qin, H., Cao, H., Zhao, Y., Zhu, C., Cheng, T., Wang, Q., Peng, X., Cheng, M., Wang, J., Jin, G., Jiang, Y., Zhang, X., Liu, X. and Chu, P. K. (2014). *In vitro* and *in vivo* anti-biofilm effects of silver nanoparticles immobilized on titanium. *Biomaterials*, *35*, 9114–9125.

Qiu, L., Lai, W. S., Stumpo, D. J. and Blackshea, P. J. (2016). Mouse embryonic fibroblast cell culture and stimulation. *Bio-Protocol*, 6(13), 1-8.

Quan, X., Sun, W., Gu, J., Wang, X., Sun, J., Yin, Y. and Duan M. (2016). Variable effects of oxytetracycline on antibiotic resistance gene abundance and the bacterial community during aerobic composting of cow manure. *Journal of Hazardous Materials*, *315*,61-69

Raad, I. I., Fang, X., Keutgen, X. M., Xiang, Y., Sherertz, R. and Hachem, R. (2008). The role of chelators in preventing biofilm formation and catheter related blood stream infections. *Current opinion in infectious diseases*, *21(*4), 385-392.

Rajput A and Kumar M. (2018). Anti-biofilm peptides: a new class of quorum quenchers and their prospective therapeutic applications. In: Kalia V. (eds) Biotechnological applications of quorum sensing inhibitors. Springer, Singapore, pp 87-110.

Rao, C. R. M., Kumar, L. C. A. and Sekharan, C. B. (2015). Quantitative analysis of oxytetracycline residues in honey by high performance liquid chromatography. *International Research Journal of Biological Sciences*, *4*(5), 59-65.

Rawat, P. and Rajput, Y. S. (2016). A method for synthesis of gold nanoparticles using 1-Amino-2-Naphthol-4- sulphonic acid as reducing agent. *Research Communications*, *110*(12), 1–5.

Razalia, N.I.M., Pramanika, S., Abu Osmana, N., Radzib, Z. and Pingguan-Murphya, B.(2016). Conversion of calcite from cockle shells to bioactive nanorod hydroxyapatite for biomedical applications. *JCPR*, *17*: 699-706.

Ribble, D., Goldstein, N. B., Norris, D. A. and Shellman, Y. G. (2005). A simple technique for quantifying apoptosis in 96-well plates, *BMC Biotechnology*, *7*, 1–7.

Ribeiro, M., Malheiro, J., Grenho. L., Fernandes, M. H. and Simões, M. (2018). Cytotoxicity and antimicrobial action of selected phytochemicals against planktonic and sessile *Streptococcus mutans*. *Peer J, 6*, 1-13.

Rizvi, S. A. A. and Saleh, A. M. (2018). Applications of nanoparticle systems in drug delivery technology. *Saudi Pharmaceutical Journal*, *26*(1), 64–70.

Robaj, A., Hamidi, A., Bytyqi, H. and Sylejmani, D. (2017). Frequency and antimicrobial susceptibility of bacterial isolates from caseous lymphadenitis in sheep in Kosovo. *Bulgarian Journal of Agricultural Science*, *23*(6), 1033–1036.

Rocha, V., Marques C., Figueiredo, J. L., Galo, A. R., Costa, P. C., Sousa Lobo, J. M., and Almeida, I. F. (2017). *In vitro* cytotoxicity evaluation of resveratrol-loaded nanoparticles: Focus on the challenges of *in vitro* methodologies. *Food and Chemical Toxicology*, *103*: 213-222.

Rudramurthy, G. R., Swamy, M. K., Sinniah, U. R. and Ghasemzadeh, A. (2016). Nanoparticles: alternatives against drug-resistant pathogenic microbes. *Molecules*, *21*, 1–30.

Rycroft, T., Trump, B., Poinsatte-Jones, K. and Linkov, I. (2018). Nanotoxicology and nanomedicine : making development decisions in an evolving governance environment. *Journal of Nanoparticle Research, 20,* 1-9.

Sá, M. C. A., Veschi, J. L. A., Santos, G. B., Amanso, E. S., Oliveira, S. A. S., Mota, R. A., Veneroni-gouveia, G. and Costa, M. M. (2013). Activity of disinfectants and biofilm production of *Corynebacterium pseudotuberculosis*. *Pesquisa Veterinária Brasileira*, *33*(11), 1319–1324.

Sadat, S. M. A, Tasnim Jahan, S. and Haddadi, A. (2016). Effects of size and surface charge of polymeric nanoparticles on *in vitro and in vivo* applications. *Journal of Biomaterials and Nanobiotechnology*, *7*(7), 91–108.

Saidykhan, L., Abu Bakar, M. Z. B., Rukayadi, Y., Kura, A. U. and Saiful Yazan, L. (2016). Development of nanoantibiotic delivery system using cockle shell. *International Journal of Nanomedicine*, *11*, 661–673.

Sambanthamoorthy, K., Gokhale, A. A., Lao, W., Parashar, V., Neiditch, M. B., Semmelhack, M. F., Lee, I. and Waters, C. M. (2011). Identification of a novel benzimidazole that inhibits bacterial biofilm formation in a broad-spectrum manner. *Antimicrobial Agents and Chemotherapy*, *55*(9), 4369–4378.

Santana-Jorge, K.T., Santos, T.M., Tartaglia, N.R., Aguiar, E.L., Souza, R.F., Mariutti, R.B., Eberle, R.J., Arni, R.K., Portela, R.W., Meyer, R. and Azevedo, V. (2016). Putative virulence factors of *Corynebacterium pseudotuberculosis* FRC41: vaccine potential and protein expression. *Microbial Cell Factories*, *15*, 1-13.

Santos, L. M., Stanisic, D., Menezes, U. J., Mendonça, M. A., Barral, T. D., Seyffert, N., Azevedo, V. Durian, N., Meyer, R., Tasic, L. and Portela, R. W. (2019). Biogenic silver nanoparticles as a post-surgical treatment for *Corynebacterium pseudotuberculosis* infection in small ruminants, *Frontiers in Microbiology*, *10*, 1–11.

Scarascia, G., Wang, T. and Hong, P. (2016). Quorum sensing and the use of quorum quenchers as natural biocides to inhibit sulfate-reducing bacteria. *Antibiotics*, *5*, 1-20.

Secinti, K. D., Ozalp, H., Attar, A. and Sargon, M. F. (2011). Nanoparticle silver ion coatings inhibit biofilm formation on titanium implants. *Journal of Clinical Neuroscience*, *18*, 391–395.

Sellyei, B., Bányai, K., Bartha, D., Hajtós, I., Fodor, L. and Makrai, L. (2017). Multilocus sequencing of *Corynebacterium pseudotuberculosis* biotype *Ovis* strains, *BioMed Research International*, *2017*, 1-7.

Senturk, S and Temizel, M. (2006). Clinical efficacy of rifamycin SV combined with oxytetracycline in the treatment of caseous lymphadenitis in sheep. *Veterinary Record*, 159, 216-217.

Sepehr, S., Rahmani-badi, A., Babaie-naiej, H. and Soudi, M. R. (2014). Unsaturated fatty acid, cis-2-decenoic acid , in combination with disinfectants or antibiotics removes pre-established biofilms formed by food-related bacteria. *Plos One*, *9*(7), 1-9.

Shabir G. A (2004). Practical approach to validation of HPLC methods under current good manufacturing practices. *Journal of Validation Technology*, *1*, 29–37.

Shahridon, S. A., Zamri-saad, M., Zakaria, Z. and Rozaihan, M. (2016). Development of recombinant cells encoding surface proteins of *Corynebacterium pseudotuberculosis* against caseous lymphadenitis in goats. *International Journal of Biosciences*, *9*(2), 16–26.

Shang, L., Nienhaus, K. and Nienhaus, G. U. (2014). Engineered nanoparticles interacting with cells: Size matters. *Journal of Nanobiotechnology*, *12*(1), 1–11.

Silva, T.M. D. O., Silvestre, F., Bezerra, B., Barros, R., Pinho, D., Begnini, R. H., Seixas, F. K., Collares, T., Portella, R. D., Azevedo, V., Dellagostin, O. and Borsuk, S. (2018). Association of *Corynebacterium pseudotuberculosis* recombinant proteins rCP09720 or rCP01850 with rPLD as immunogens in caseous lymphadenitis immunoprophylaxis. *Vaccine*, *36*(1), 74–83.

Sim, S., Hong, E., Kim, Y. and Lee, H. (2014). Analysis of cepA encoding an efflux pump-like protein in *Corynebacterium glutamicum*. *Journal of Microbiology*, *52*(4), 278–283.

Skogman, M. E., Vuorela, P. M. and Fallarero, A. (2016). A platform of anti-biofilm assays suited to the exploration of natural compound libraries. *Journal of Visualized Experiments*, *118*, 1-10.

Soares, S. C., Silva, A., Trost, E., Blom, J., Ramos, R., Carneiro, A., Ali, A., Santos, R. A., Pinto, A. C., Diniz, C., Barbosa, E. G. V., Dorella, F. A., Aburjaile, F., Rocha, F.S., Nascimento, K. K. F., Guimaraes L. C., Almeida, S., Hassan, S. S., Bakktiar, S.M. Pereira, U. P., Abreu, V. A. C., Schneider, M. P. C., Miyoshi, A., Tauch, A. and Azevedo, V. (2013). The Pan-Genome of the Animal Pathogen Corynebacterium pseudotuberculosis Reveals Differences in Genome Plasticity between the Biovar *ovis* and *equi* Strains. *Plos One*, *8*(1), 1-14.

Solano, C., Echeverz, M. and Lasa, I. (2013). Biofilm dispersion and quorum sensing. *Current Opinion in Microbiology*, *18*, 96–104.

Song, M., Kim, S. and Kim, E. (2014). Cytotoxicity of newly developed pozzolan cement and other root-end filling materials on human periodontal ligament cell. *Restorative Dentistry and Endodontics*, *39*, 39–44.

Sousa, C., Botelho, C. and Oliveira, R. (2011). Nanotechnology applied to medical biofilms control. *Science against microbial pathogens: communicating current research and technological advances*, *1*, 878–888.

Souza, C. De, Faria, Y. V., Oliveira, L. De, Anna, S., Viana, V. G., Seabra, S. H., Souza, M. C., Vieira, Junior, R. H., Moreira, L. O. and Mattos-guaraldi, A. L. (2015). Biofilm production by multi resistant *Corynebacterium striatum* associated with nosocomial outbreak. *Memorias do Instituto Oswaldo Cruz*, *110*(2), 242–248.

Stanisic, D., Fregonesi, N. L., Barros, C. H. N., Pontes, G. M., Fulaz, S., Menezes, U. J., Nicoleti, J. L., Castro, T. L. P., Azevedo, V., Duran, N., Portela, R. W. and Tasic, L. (2018). Treatment of superficial caseous lymphadenitis in small ruminants. *RSC Advances*, *8*, 40778–40786.

Stefanska, I., Gierynska, M., Rzewuska, M. and Binek, M. (2010). Survival of *Corynebacterium pseudotuberculosis* within macrophages and induction of phagocytes death. *Polish Journal of Veterinary Sciences*, *13*(1), 143-149.

Stefanska, I., Rzewuska, M. and Binek, M. (2008). Evaluation of three methods for dna fingerprinting of *Corynebacterium pseudotuberculosis* strains isolated from goats in Poland. *Polish Journal of Microbiology*, *57*(2), 105–112.

Stephens, C. R., Conover, L. H., Hochstein, F. A., Regna, P. P., Pilgrim, F. J., Brunings, K. J. and Woodward, R. B. (1952). Terramycin viii structure of aureomycin and terramycin. *Journal of the American Chemical Society*, *74*(19), 4976–4977.

Stewart, P. S. and Costerton, J. W. (2001). Antibiotic resistance of bacteria in biofilms. *The lancet*, *358*, 135–138.

Stockert, J. C., Blázquez-castro, A., Ca, M. and Horobin, R. W. (2012). MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. *Acta Histochemica*, *114*, 785–796.

Stockert, J. C., Horobin, R. W., Colombo, L. L. and Blázquez-castro, A. (2018). Tetrazolium salts and formazan products in cell biology : viability assessment, fluorescence imaging, and labelling perspectives. *Acta Histochemica*, *120*(3), 159–167.

Tankhiwale, S. (2016). Beta-lactamases in *P. aeruginosa*: A threat to clinical therapeutics. *Current Pediatric Research*, *20*(1), 253–257.

Thaker, M., Spanogiannopoulos, P. and Wright, G. D. (2010). The tetracycline resistome. *Cellular and Molecular Life Sciences*, *67*, 419–431.

Tomuleasa, C., Braicu, C., Irimie, A., Craciun, L. and Berindan-Neagoe, I. (2014). Nanopharmacology in translational haematology and oncology. *International Journal of Nanomedicine*, *9*(1), 3465–3479.

Torres, L. F. C., Ribeiro, D., Jr, R. H., Gustavo, L., Pacheco, L. G. C., Souza, M. C., Santos, L. S., Santos, C. S., Salah, M., Costa, M. M., Ribeiro, M. G., Selim, S. A., Azevedo, V. A. C. and Mattos-guaraldi, A. L. (2013). Multiplex polymerase chain reaction to identify and determine the toxigenicity of *Corynebacterium spp* with zoonotic potential and an overview of human and animal infections. *Memorias do Instituto Oswaldo Cruz*, *108*(3), 272–279.

Torres, S. K., Campos, V. l., Leon, C. G., Rodrıguez-Llamazares, S. M., Rojas, S. M., Gonzalez, M., Smith, C. and Mondaca, M. A. (2012). Biosynthesis of

selenium nanoparticles by*Pantoea agglomerans* and their antioxidant activity. *Journal of Nanoparticle Research*, *14*, 1236-1245.

Tote, K., Horemans, T., Vanden Berghe, D., Maes, L. and Cos, P. (2010). Inhibitory effect of biocides on the viable masses and matrices of *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. *Applied and environmental Microbiology*, *76*(10), 3135–3142.

Tsuzuki, T. and Cormick, P. G. (2004). Mechanochemical synthesis of nanoparticles. *Journal of Material Science*, *9*, 5143–5146.

Ueno, R., kinoshita, A. and Wakabayashi, J. (2004). Comparative pharmacokinetics of oxytetracycline in eel and its fate in a closed aquatic environment. *Aquaculture*, *235*(1), 53-63.

Vorbacha, B. S., Chandasanac, H., Derendorfc, H. and Yanong, R.P.E. (2019). Pharmacokinetics of Oxytetracycline in the Giant Danio (*Devario aequipinnatus*) following bath immersion. *Aquaculture*, *498*, 12-16.

Wallace, D. R. (2015).Nanotoxicology and metalloestrogens: possible involvement in breast cancer. *Toxics, 3* (4), 390–413.

Wang, L. and Hu, C. (2017). The antimicrobial activity of nanoparticles : present situation and prospects for the future. *International Journal of Nanomedicine*, *12*, 1227–1249.

Washburn, K. E., Fajt, V. R., Lawhon, S. D., Adams, L. G., Tell, L. A. and Bissett, W. T. (2013). Caprine abscess model of tulathromycin concentrations in interstitial fluid from tissue chambers inoculated with corynebacterium pseudotuberculosis following subcutaneous or intra chamber administration. *Antimicrobial Agents and Chemotherapy*, *57*(12), 6295–6304.

Williamson, L.H. (2001). Caseous lymphadenitis in small ruminants. *Veterinary Clinics of North America: Food Animal Practice, 17*: 359–371.

Windsor, P. A. and Bush, R. D. (2016). Caseous lymphadenitis : Present and near forgotten from persistent vaccination ? *Small Ruminant Research, 142*, 6–10.

Xiong, M. H., Li, Y. J, Bao, Y., Yang, X. Z., Hu, B. and Wang, J. (2012). Bacteria-responsive multifunctional nanogel for targeted antibiotic delivery. *Advanced Materials*, *24*, 6175–6180.

Xu, W., Pengjin, G., Zhang, N., Liu, X. and Xie, J. (2018). Macroporous silica nanoparticles for delivering Bcl2-function converting peptide to treat multidrug resistant-cancer cells. *Journal of Colloid and Interface Science, 527*, 141-150.

Xu, X., Wang, Y., Chen, R., Feng, C., Yao, F., Tong, S., Wang, L., Yamashita, F. and Yu, J. (2011). Formulation and pharmacokinetic evaluation of tetracycline-loaded solid lipid nanoparticles for subcutaneous injection in mice. *Chemical and Pharmaceutical Bulletin, 59*, 260-265.

Xu, Z., Xu, X., Qi, D., Yang, L., Li, B., Li, L., Li, X. and Chen, D. (2017). Effect of aminoglycosides on the pathogenic characteristics of microbiology. *Microbial Pathogenesis*, *113*, 357-354.

Yalçın Enis, İ., Küçükali Öztürk, M., Sezgin, H. and Ismar, E. (2017). An overview of Nanotoxicology. *Engineering Sciences (NWSAENS)*, *12*(1), 57–65.

Yuan, J. (1993). Estimation of variance for AUC in animal studies. *Journal of Pharmaceutical Sciences*, *82*,761–3.

Yuyama, K. T. and Abraham, W. R. (2016). Cis-2-alkenoic acids as promising drugs for the control of biofilm infections. *Medicinal Chemistry*, *13*:3-12.

Zarei, M., Jamnejad, A. and Khajehali, E. (2014). Antibacterial effect of silver nanoparticles against four foodborne pathogens. *Jundishapur Journal of Microbiology*, *7*(1), 1–4.

Zazo, H., Colino, C. I. and Lanao, J. M. (2016). Current applications of nanoparticles in infectious diseases. *Journal of Controlled Release*, *224*, 86–102.

Zhang, L., Hinz, A. J., Nadeau, J. and Mah, T. (2011). *Pseudomonas aeruginosa* tssC1 links type VI secretion and biofilm-specific antibiotic resistance. *Journal of Bacteriology*, *193*(19), 5510–5513.

Zhang, Y., Huo, M., Zhou, J. and Xie, S. (2010) PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Computer Methods and Programs in Biomedicine*, 99(3): 306-314

Zhang, L., Pornpattananangkul, D., Hu, C. J. and Huang, C. (2010). Development of nanoparticles for antimicrobial drug delivery. *Current Medicinal Chemistry*, *17*, 585–594.

Zhao, Q., Han, B., Wang, Z., Gao, C., Peng, C. and Shen, J. (2007). Hollow chitosan-alginate multilayer microcapsules as drug delivery vehicle : doxorubicin loading and *in vitro and in vivo* studies. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *33*, 63–74.

Zhao, T. and Liu, Y. (2010). N-acetylcysteine inhibit biofilms produced by *Pseudomonas aeruginosa. BMC Microbiology*, *10*, 1-8.

Ziołkowski, H., Grabowski, T., Jasiecka, A., Zuska-Prot, M., Barski, D. and Jaroszewski, J. J. (2019). Pharmacokinetics of oxytetracycline in broiler chickens following different routes of administration. *The Veterinary Journal*, *208*, 96-98.