

# **UNIVERSITI PUTRA MALAYSIA**

# ANTIMICROBIAL SUSCEPTIBILITY TESTING OF CORYNEBACTERIUM PSEUDOTUBERCULOSIS ORIGINATED FROM CAPRINE CASEOUS LYMPHADENITIS (CLA) CASES

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A project paper submitted to the Facultly of Veterinary Medicine, Universiti Putra Malaysia In prtial fulfillment of the requirement for the DEGREE OF DOCTOR OF VETERINARY MEDICINE Universiti Putra Malaysia Serdang, Selangor Darul Ehsan.

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It is hereby certified that we have read this project paper entitled "Antimicrobial Susceptibility Testing of *Corynebacterium pseudotuberculosis* Originated from Caprine Caseous Lymphadenitis (CLA) Cases", by Chew Ying Yi and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 Final Year Project.

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DEDICATION



This project and this project paper dedicated to my family and everyone that supports me throughout my life.

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CONTENT
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TITLE	Ι
CERTIFICATION	II
DEDICATION	III
ACKNOWLEDGEMENTS	IV
CONTENTS	V
LIST OF TABLES AND FIGURE	VII
LIST OF ABBREVIATIONS	VIII
ABSTRACT	IX
ABSTRAK	XI
1.0 INTRODUCTION	1
2.0 LITERATURE REVIEW	3
2.1 Corynebacterium pseudotuberculosis	3
2.1.1 Aetiological agent	3
2.1.2 Distribution and Economic Impact	4
2.1.3 Prevalence in Malaysia	4
2.1.4 Sources of Infection and Form of Transmission	5
2.1.5 Virulence Factors and Pathogenesis	5
2.1.6 Incubation Period and Clinical Manifestation	6
2.1.7 Diagnosis	7
2.1.8 Treatment, Prevention and Control	8
2.1.9 Antimicrobial Susceptibility Testing	9
2.1.10 Public Health Concerns	10
3.0 MATERIALS AND METHODS	11

3.1 Bacterial isolation and identification	11
3.2 Antimicrobial susceptibility testing	11
3.1.1 Disc Diffusion Methods	11
3.1.2 Broth Microdilution Methods	12
4.0 RESULT AND DISCUSSION	13
5.0 CONCLUSIONS	18
6.0 RECOMMENDATIONS	19
REFERENCES	20

LIST OF TABLES AND FIGURE

Table 1: Antibiotic concentration of neomycin, gentamycin, penicillin G and erythromycin in 96-well plates ( $\mu g/mL$ )

Table 2: Zone of inhibition (mm) of selected antibiotics againstCorynebacterium pseudotuberculosis

 Table 3: Minimal bactericidal concentration and minimal inhibitory

 concentration of selected antibiotics towards *C.pseudotuberculosis*

Figure 1: Zone of inhibition (mm) of selected antibiotics against Corynebacterium pseudotuberculosis

#### LIST OF ABBREVIATIONS

- CLA Caseous Lymphadenitis
- BAT Bacterial Agglutination Test
- ELISA Enzyme Linked Immunosorbent Assay
- MIC Minimal Inhibitory Concentration
- MBC Minimal Bactericidal Concentration
- PLD Phospholipase D
- AML Amoxicillin
- AMC Amoxicillin with Clavulanic acid
- AMP Ampicillin
- CL Cephalexin
- EN Enrofloxacin
- E Erythromycin
- CN Gentamicin
- N Neomycin
- OT Oxytetracycline
- P Penicillin G
- PB Polymixin B
- S Streptomyxin
  - SXT Sulfamethoxazole-Trimethoprim
  - TE Tetracycline

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project

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#### ABSTRACT

Caseous lymphadenitis (CLA) is a chronic bacterial infectious disease caused by a gram-positive bacteria named *Corynebacterium pseudotuberculosis* infecting animals especially small ruminant resulting in economic losses to a farm. Despite of contagious infection, the use of antibiotics in most cases do not eliminate the disease totally. The possibility of antibiotic resistance towards available antibiotics should be considered. Using antimicrobial susceptibility testing, this study was aimed to identify the susceptibility of the microorganism

selected antibiotics. Laboratory of*Corynebacterium* towards strain pseudotuberculosis was used to test against 14 antibiotics available using disc diffusion method and zone of inhibition was measured to determine the susceptibility. The microorganism was also tested against 4 selected antibiotics available using broth microdilution method and minimal inhibitory concentration(MIC) and minimum bactericidal concentration (MBC) were determined. The microorganism showed susceptibility to amoxicillin with or without clavulanic acid, ampicillin, cephalexin, enrofloxacin, erythromycin, gentamicin, neomycin, oxytetracycline, penicillin G, sulfamethoxazoletrimethoprim and tetracycline but showed resistance to streptomycin and polymixinB. Minimal bactericidal concentration for neomycin, gentamycin, penicillin g and erythromycin are 1.875 µg,0.25 µg, 20 µg, and 20 µg respectively. Minimum inhibitory concentration for neomycin, gentamycin, penicillin g and erythromycin are 0.9375 µg, 0.125 µg, 10 µg and 10 µg, respectively. From the study, the microorganism is susceptible to most of the antibiotic groups including penicillin, cephalosporin, macrolides, tetracycline and aminoglycosides except streptomycin. The microorganism is resistant to cyclic peptide group of antibiotic (polymixin B).

Keywords: Caseous Lymphadenitis, CLA, Corynebacterium pseudotuberculosis, AST, antibiotic

#### ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk

memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Ilmiah Tahun Akhir

### KEPEKAAN CORYNEBACTERIUM PSEUDOTUBERCULOSIS TERHADAP

#### ANTIBIOTIK BERASAL DARIPADA KES KAMBING YANG MENYEBABKAN

## LIMFADENITIS KASEUS

Oleh

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Limfadenitiskaseus (CLA) merupakan penyakit jangkitan yang kronik oleh bakteria grampositif yang dikenali sebagai *Corynebacterium pseudotuberculosis* yang menjangkiti haiwan, terutamanya ruminant kecil serta mengakibatkan kerugian. Antibiotik tidak dapat merawat penyakit ini sepenuhnya walaupun disebabkan oleh bakteria. Oleh itu, kemungkinan rintangan antibiotic terhadap antibiotik yang sedia ada harus dipertimbangkan. Kajian ini bertujuan untuk mengenalpasti kepekaan bakteria tersebut terhadap antibiotic terpilih dengan

menggunakan ujian kepekaan antimicrobial. Strain makmal Corynebacterium pseudotuberculosis telah digunakan untuk menguji terhadap 14 antibiotik dengan menggunakan kaedah cakera penyebaran dan zon perencatan akan diukur untuk menentukan kepekaannya. Mikroorganisma tersebut juga diuji terhadap 4 jenis antibiotic terpilih yang sedia ada, dengan kaedah mikro pencairan, konsentrasi minima perencatan (MIC) dan konsentrasi minima bakteriasida (MBC). Mikroorganisma berkenaan memaparkan kepekaan terhadap amoxicillin dengan dan tanpa asid Klavutanik, ampicillin, cephalexin, enrofloxacin, erythromycin, gentamicin, neomycin, oxytetracycline, penicilin G, sulfamethoxazoletrimethoprimdan tetracycline. Tetapi ia merintang terhadap streptomycin danpolymixin B. Konsentrasi perencatan minima bagi neomycin, gentamycin, penicilin G dan erythromycin masing-masing adalah 0.9375 µg, 0.125 µg, 10 µg dan 10 µg. Berdasarkan kajian ini, mikroorganisma ini peka terhadap kebanyakan kumpulan antibiotik, termasuklah penicilin, cephalosporin, macrolides, tetracycline dan aminoglycosides selain streptomycin secara in vitro. Bakteria ini juga bertahan terhadap kumpulan antibiotik yang mempunyai peptide kitaran (polymixin B).

Kata kunci: Limfadenitiskaseus, CLA, Corynebacterium pseudotuberculosis, AST, antibiotic

#### 1.0 INTRODUCTION

Caseous lymphadenitis (CLA) is a chronic bacterial infectious disease in small ruminant caused by *Corynebacterium pseudotuberculosis*, a gram- positive, facultative intracellular pleomorphic form bacteria (Williamson, 2001). The incubation periods range from 7 to 140 days (Kuria *et al*, 2001; Jeber*et al.*, 2016) which would result in abscess formation in the body.In Malaysia, this disease is known to be common in semi-intensive sheep-goat rearing practices (Osman, *et al.* 2012). This disease is distributed worldwide and affecting most of the continents while prevalence of about 7% was determined in a study done in goats in Peninsular Malaysia (Bahaman *et al.*, 1989). At the east coast of Malaysia, the prevalence was estimated at 11.1% (Osman, *et al.* 2012). In another state in Peninsular Malaysia, 75% of the farms were affected by this disease (Ismail *et al.*, 2012).

This disease causes significant consequences to the farmers in both production and productivity (Jeber*et al.*, 2016).Carcasses and skin are condemned at meat inspection in abattoirs (Williamson, 2001). Affected animal would have their meat and milk yield production decreased as well as affecting animal reproductive efficacy (Stanford *et al.*, 1997). Besides meat condemnation, farmer can face huge economic losses due to infection or death on farms as well as from increased culling rates (Williamson, 2001). On top of that, most of the farmers do not have veterinary services, lack of sanitary control and limited awareness of separating affected animal (Osman, *et al.* 2012). This could further

XIV

increases the risk of infection in the farm. Moreover, this disease can be transmitted to human and considered as zoonotic(Burkovski, 2012).

Clinical manifestation of CLA are mainly in two forms: external form, with the involvement of inflammation of subcutaneous layer and abscess formation in superficial lymph nodes (mandibular, superficial cervical, parotid, and mammary); internal form, where the visceral lymph nodes (mediastinal, bronchial and lumbar) and internal organs such as lung, liver, kidney, spleen as well as uterus are affected with abscesses (Williamson, 2001). CLA, even though caused by a bacteria, use of antibiotics do not produce significant result in eliminating the disease (Guimaraes*et al*, 2011). The possibility of antibiotics resistance of *C. pseudotuberculosis* should always be considered. Therefore, the objectives of this study is to identify the antimicrobial susceptibility of *C. pseudotuberculosis* of selected antibiotics as well as to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *C. pseudotuberculosis* towards selected antibiotics.

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