UNIVERSITI PUTRA MALAYSIA

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

CHEW YING YI

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A project paper submitted to the

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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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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

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

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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 towards

selected antibiotics. Laboratory strain of *Corynebacterium 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, sulfamethoxazole-trimethoprim and tetracycline but showed resistance to streptomycin and polymixin B. 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 daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

KEPEKAAN *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* TERHADAP ANTIBIOTIK BERASAL DARIPADA KES KAMBING YANG MENYEBABKAN LIMFADENITIS KASEUS

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ABSTRAK

Limfadenitis kaseus (CLA) merupakan penyakit jangkitan yang kronik oleh bakteria gram-positif yang dikenali sebagai *Corynebacterium pseudotuberculosis* yang menjangkiti haiwan, terutamanya ruminan kecil serta mengakibatkan kerugian. Antibiotik tidak dapat merawat penyakit ini sepenuhnya walaupun disebabkan oleh bakteria. Oleh itu, kemungkinan rintangan antibiotik terhadap antibiotik yang sedia ada harus dipertimbangkan. Kajian ini bertujuan untuk

mengenal pasti kepekaan bakteria tersebut terhadap antibiotik 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 antibiotik terpilih yang sedia ada, dengan kaedah mikropencairan, 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, sulfamethoxazole-trimethoprim dan tetracycline. Tetapi ia merintang terhadap streptomycin dan polymixin 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 menpunyai peptida kitaran (polymixin B).

Kata kunci: Limfadenitis kaseus, 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 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.

2.0 LITERATURE REVIEW

2.1 *Corynebacterium pseudotuberculosis*

2.1.1 Aetiological agent

Corynebacterium pseudotuberculosis belongs to the genus Corynebacterium, family Corynebacteriaceae, suborder Corynebacterineae (Guimaraes *et al*, 2011). The genus *Corynebacterium* belongs to the class of Actinobacteria, which also includes genera *Mycobacterium* and *Nocardia.* They are high guanine- cytosine (GC) gram positive and characterized by waxy cell envelope composition with presence of envelope containing mycolic acid, conferring alcohol and acid- fast staining properties. However, the genus *Corynebacterium* exhibits considerable heterogeneity (Ventura *et al.*, 2007) and comprises a collection of morphologically similar, irregular- or club-shaped non-sporulating aerobic microorganism (Burkovski, 2012). To date, it comprises of 88 species (Bernard, 2012) and more than half of these are connected to human and animal infection (Burkovski, 2012). Besides samples from human and animal, it can also be isolated from saline soil, or even the surface of smear-ripened cheese (Ventura *et al*, 2007).

According to Dorella (2006), *Corynebacterium pseudotuberculosis* is an important animal pathogen and considered as zoonotic agent (Burkovski, 2012). It is a facultative intracellular pathogen that exhibits pleomorphic forms, such as coccoids and filamentous rods, ranging in size from 0.5 μm to 0.6 μm by 1.0 μm

to 3.0 µm and non-sporulating, non-capsulated and non-motile bacterium with presence of fimbriae.

2.1.2 Distribution and Economic Impact

According to Baird *et al.* (2007), *Corynebacterium pseudotuberculosis* is the etiological agent of Caseous lymphadenitis (CLA) or cheesy gland (Dorella, 2006), a chronic, contagious bacteria disease (Williamson, 2001). This disease is found in sheep and goat production (Dorella, 2006) distributed throughout almost the whole world and found in all continents including Asia, Australia, Africa, Europe and Americas (Jeber *et al.*, 2016).

Farmers face economic consequences resulted from meat condemnation, reduced wool production, reduced sales of breeding stock, reduced milk yield, reduced reproductive efficiency as well as increased rates of culling and mortality (Stanford *et al.*, 1997).

2.1.3 Prevalence in Malaysia

The first case of CLA reported in Malaysia was in 1970 (Osman *et al*, 2012). According to Bahaman *et al.*(1989), a cross-sectional study of 3484 goats throughout Pennisular Malaysia was done and there was about 6.95% of the goats were positive for caseous lymphadenitis (CLA) using gel diffusion test. In Selangor, the prevalence of CLA was estimated at 28% in goats and 75% in farms (Ismail *et al*, 2012). According to Komala *et al.* (2008), 17% of the animals were

positive towards CLA using Agar Gel Precipitation Test (AGPT) and Enzyme Linked Immuno Asorbent Assay (ELISA) in Ipoh.

2.1.4 Sources of Infection and Form of Transmission

Clinically or subclinically infected animals serve as the main source of infection as they contaminate the soil, water, feed, equipment and environment via nasal secretion, faeces and pus from abscesses that drain spontaneously (Guimaraes *et al*, 2011).

Experiments done proved successful establishment of disease using inoculation routes of intradermal, subcutaneous, intravenous, intratracheal, intravaginal, and intra-lymphatic (Baird, 2007). In natural infection, transmission occurs primarily via contamination of inflicted superficial wounds with the bacteria from purulent discharges through traumatic events or even herd management activities (Williamson, 2001). New infection in the farm is usually due to introduction of new animal to the farm that are clinically or subclinically infected carrier animal (Ayers, 1977).

2.1.5 Virulence factors and pathogenesis

The microorganism is a facultative intracellular pathogen (Dorella *et al*, 2006). The organism moves into regional draining lymph node after infection through superficial skin wounds or abrasion. Bacteria multiply intracellularly and result in arise of lesions that vary with the initial number of organisms, the rate of

multiplication and accessibility of the lesion to the host's defense cells (Kuria *et al*, 2001). After penetrating into the host, the agent disseminates freely or within macrophages to lymph nodes and internal organs via afferent lymphatic system. Local reaction followed and degeneration and necrosis occurs. One of the virulence factors of the microorganism is the lipid cell layer that is pyogenic but not immunogenic which challenge the phagocytosis process. Presence of mycolic acid in the microorganism has contributed to the survivability of the microorganism in the environment. Besides that, phospholipase D (PLD) plays an important role in dissemination as it increases vascular permeability and it lyses mammal cell membranes causing microhaemorrhages and vascular lesions (Guimaraes *et al*, 2011).

2.1.6 Incubation period and clinical manifestation of CLA

CLA has incubation period ranging from 7 to 140 days (Kuria *et al*, 2001; Jeber *et al.*, 2016) and can become endemic in a herd or flock (Williamson, 2001). According to Williamson (2001), it is difficult to eradicate as it has poor therapeutic responses and its ability to persist in the environment.

Clinical signs of CLA are in two main forms: external form characterized by infection of subcutaneous tissue and abscesses in superficial lymph nodes (mandibular, parotid, superficial cervical, sub iliac, popliteal and mammary) of the body and visceral lymph nodes such as mediastinal, bronchial and lumbar lymph nodes and internal organ such as lungs, liver, kidneys, uterus and spleen are affected, with the former form of manifestation more commonly found in goats

and the later in sheep (Williamson, 2001; Jeber *et al.*, 2016). Internal involvement can be subclinical and but is also often associated with weight loss and ill thrift (Williamson, 2001). In some cases, the infection produces few obvious clinical signs in the animal, remaining unrecognized until a post-mortem examination has been done (Dorella, 2006). The disease has distinct clinical manifestations when the lesions become progressive (Jeber *et al.*, 2016). According to Williamson (2001), in external form, encapsulated mass in or near a peripheral lymph node enlarges slowly forming abscess and it matures and finally ruptures through a fistula. Infective purulent of thick, greenish-white, pasty consistency and odourless drains into the environment. The wound heals initially leaving a scar but often recur months to years later as failure of total elimination of the infection. New lesions sites can be developed during reactivation of disease after some period of time.

Mastitis has been reported in horses and cows which associated with visceral abscesses. In horses, there have been reports of abortions as well (Guimaraes *et al*, 2011).

2.1.7 Diagnosis

Presence of external abscesses on animal bodies highly suggest the disease (CLA) especially in endemic farm but bacteriology culture where sample can be obtained by swabbing on the purulent material from a draining abscess or aseptic aspiration from closed external abscess is necessary to differentiate the agent *C. pseudotuberculosis* from *Actinomyces pyogenes* that can cause abscess

(Williamson, 2001). Sample can also be collected at necropsy or during slaughter (Guimaraes *et al*, 2011). Diagnosis could be more challenging but radiology and trans-tracheal aspiration can be used in visceral abscesses that involve pulmonary. Other viscera abscesses will need visualization using sonography (Williamson, 2001).

Several serodiagnostic tests have been developed worldwide including serological neutralization for antitoxins, immunodiffusion in agar gel, indirect haemagglutination, complement fixation and hypersensitivity tests for diagnosis in sheep and goats. Immunoenzymatic tests (ELISA) using bacterial cells, toxins and PLD has been reported to be effective in CLA control and eradication programmes (Guimaraes *et al*, 2011). However, infection less than two weeks is not effective to diagnose via serology (Kuria *et al*, 2001).

Besides that, molecular techniques has also been adapted for the diagnosis of CLA. Polymerase chain reaction (PCR) including multiplex PCR could facilitate the diagnosis by differentiating the microorganism from other pathogens present in abscesses (Guimaraes *et al*, 2011).

2.1.8 Treatment, Prevention and Control

Antibiotic therapy as one of the treatment option appears to be inefficient. Drainage of abscesses followed by proper cleaning without contamination especially to the environment appear to be more useful. However, this treatment option has been limited due to the presences of internal abscesses (Guimaraes *et*

al, 2011). Before introducing new animals into a clean herd, isolation period at least 20 days followed by a BAT test is recommended (Kuria, *et al*, 2001).Common disinfectant, such as hypochlorite, formalin and cresol is effective to *Corynebacterium pseudotuberculosis* in the environment but organic materials such as faeces can interfere with the action of the agents, thus cleaning should always be done before disinfection. Disease eradication in endemically- infected farm should be done via culling those that show clinical signs or serologically positive animals, however, this is difficult to accomplish as the rapid dissemination of the agent and once environment is contaminated, the microorganism is hard to eliminate. Given that treatment is expensive and ineffective, immunization such as vaccination could be strategy for control and preventive measures (Guimaraes *et al*, 2011).

2.1.9 Antimicrobial Susceptibility Testing

The susceptibility pattern of *C. pseudotuberculosis* to antibiotics had shown differences among isolates obtained from various sources. In one of the study done using 25 strains of isolates *C. pseudotuberculosis* were susceptible to ampicillin, chloramphenicol, gentamicin, lincomycin, tetracycline, penicillin G and sulfamethoxazole- trimethoprim while most of the strains were resistant towards neomycin except for three strains (Muckle and Gyles,1982). All of the strains were resistant towards streptomycin. According to Judson and Songer (1991), *C. pseudotuberculosis* showed susceptibility to penicillins, macrolides, cephalosporins, tetracyclines, lincomycin, chloramphenicol, and rifampicin *in vitro* but resistant to aminoglycosides, nitrofurans, polymixins, nalidixic acid, and

cycloheximide. Wahad and Shigidi (2013) reported that resistance to nalidixic acid, colistin, novobiocin, penicillin, cloxacillin and streptomycin were found while nitrofurantoin, chloramphenicol, rifampacin, cotrimoxazole (sulfamethoxazole and trimethoprim), erythromycin, and ampicillin, methicillin, kanamycin, gentamicin and tetracycline were still sensitive. Penicillin resistance was also found in report by Garg *et al* (1985) as cited in Dorella *et al*. (2006). Abebe and Sisay (2015) reported sensitivity of norfloxacin, tetracycline, doxycycline HCL and kanamycin in some of the isolates while resistant to ampicillin, clindamycin and doxycycline HCL.

2.1.10 Public Health Concerns

First reported case of *C.pseudotuberculosis* in human was presented with localized lymphadenopathy, liver enlargement, fatique and muscular aches (Lopez *et al.,*1966*)*. Infection in human reported to be rare event (Mills *et al*. 1997) and mostly related to occupational exposure (Peel *et al*., 1997). Human infected with this microorganism would have painful purulent and necrotized skin wounds as well as abscessation of lymph nodes (Ganter, 2015).

3.0 MATERIALS AND METHODS

3.1 Bacterial isolation and identification

Laboratory strain isolate was activated using blood agar incubated at 37 ºC for 48 hours. Bacterial colonies showed whitish in colour and beta-hemolysis. Gram positive rods, clubbed end, sometimes pleomorphic, singly or paired were examined after gram staining. Bacteria cultured was confirmed for *Corynebacterium pseudotuberculosis* through biochemical test using catalase, urease, trehalose, glucose, nitrate reduction, sucrose, haemolysis and motility.

3.2 Antimicrobial susceptibility testing

3.2.1 Disc Diffusion Method

Pure colonies were inoculated into 3mL of nutrient (BHI) broth and incubated at 37 ºC for 48 hours using shakers. Bacterial suspension was compared with 0.5 McFarland standard turbidity. Bacteria was streaked using a sterile swab onto Blood Agar (BA) plates with three different directions. The antibiotic impregnated discs were applied on the surface of inoculated plates and stabilized using a sterile forceps where two discs were impregnated onto a plate. Antibiotics tested includes amoxicillin 10µg, amoxicillin with clavulanic acid 30µg, ampicillin 10µg, cephalexin 30µg, enrofloxacin 5µg, erythromycin 15µg, gentamicin 10µg, neomycin 30µg, oxytetracycline 30µg, penicillin G 10ui, polymixin B 300µg, streptomycin 10µg, sulfamethoxazole-trimethoprim 25µg, and tetracycline 30µg. The plates were then incubated aerobically for 48 hours at 37 ºC. The diameter of growth inhibition was measured in millimeters using calipers and recorded. Data were analyzed and reported as susceptible or resistant.

3.2.2 Broth Microdilution Method

Antibiotic solution was prepared by commercially available antimicrobial powder using water as diluent. Using sterile 96 well plates, 100 µl of different concentration of antibiotic solution was then filled with into each well as table shown below for neomycin, gentamicin, penicillin G and erythromycin (two-fold dilution) starting from column 3 of 96-well plates, column 1 as positive control while column 2 as negative control:

3	4	5	6	7	8	9	10	11	12
60	30	15	7.5	3.75	1.875	0.9375	0.46875	0.234375	0.117188
16	8	4	2	1	0.5	0.25	0.125	0.0625	0.03125
20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125	0.039063
20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125	0.039063

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

Bacteria suspension was adjusted to achieve turbidity equivalent to a 0.5 McFarland turbidity standard which results in a suspension containing approxiamately 1 to 2 x 10^8 CFU/mL. Optimally within 15 minutes of preparation, the adjusted inoculum suspension was diluted 1:150 to achieve approximately 5 x 10^ 5 CFU/mL of suspension. Each well was filled with 100µl adjusted bacteria suspension except for negative control. 96-well plates were then incubated for 24 hours at 37ºC. Each well was then cultured into blood agar plates to identify colonies growth and minimal inhibitory concentration (MIC) and minimal bactericidal concentration were determined.

4.0 RESULTS AND DISCUSSION

Table 2 shows zone of inhibition (mm) of selected antibiotics against *Corynebacterium pseudotuberculosis*.

Antibiotics	**Mean ± Std. Deviation**
Amoxicillin (AML)	44.0825 ± 2.96663
Amoxicillin with Clavulanic acid (AMC)	51.8750 ± 6.15166
Ampicillin (AMP)	42.3025 ± 5.11085
Cephalexin (CL)	43.0900 ±2.64520
Enrofloxacin (EN)	40.4375 ± 1.83754
Erythromycin (E)	44.1050 ±2.43429
Gentamicin (CN)	24.7950 ±2.50698
Neomycin (N)	23.2525 ±2.68254
Oxytetracycline (OT)	46.4150 ±2.97775
Penicillin G (P)	47.5300 ± 6.12193
Polymixin B (PB)	0.0000 ±.00000
Streptomyxin (S)	9.2200 ± 7.54819
Sulfamethoxazole-Trimethoprim (SXT)	23.2375 ± 3.29234
Tetracycline (TE)	46.8975 ± 3.09686

Table 2: Zone of inhibition (mm) of selected antibiotics against *Corynebacterium pseudotuberculosis*.

Figure 1 shows zone of inhibition of selected antibiotics against *Corynebacterium pseudotuberculosis*.

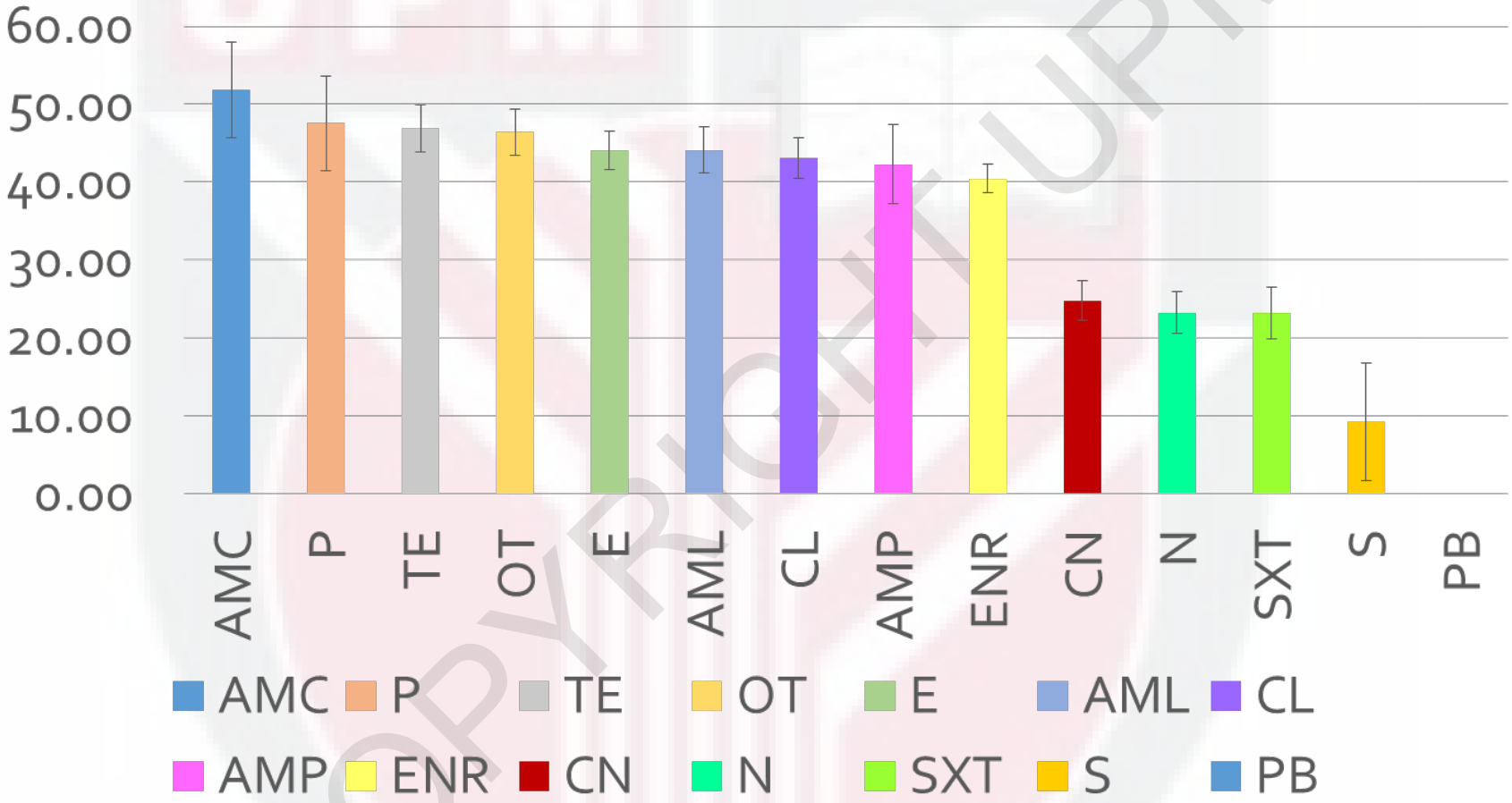

Figure 1: Zone of inhibition (mm) of selected antibiotics against *Corynebacterium pseudotuberculosis*

The susceptibility was analyzed using diameter of zone of inhibition according to the reference provided by National Committee for Clinical Laboratory Standards (NCCLS). From the study, the microorganism was susceptible towards amoxicillin, amoxicillin with clavulanic acid, ampicillin, cephalexin, enrofloxacin, erythromycin, gentamicin, neomycin, oxytetracycline, penicillin G, sulfamethoxazole-trimethoprim and tetracycline. These findings were in agreement with Muckle and Gyles (1982) and partially consistent with the findings of Judson and Songer (1991) where in this study, susceptibility was shown in penicillin groups (including aminopenicillin and ampicillin derivative), cephalosporins, aminoglycosides (except streptomycin), macrolides, fluoroquinolone and tetracycline group of antibiotics. According to the size of inhibition zone (in mm), the most effective antibiotics towards this microorganism would be amoxicillin with clavulanic acid, penicillin G and tetracycline (Figure 1 and Table 2). Even though gentamicin, neomycin and sulfamethoxazole-trimethoprim showed susceptibility in this study, their zone of inhibitions were respectively smaller compared to other antibiotics tested. As in the study done by Muckle and Gyles (1982), some isolates were reported to be resistant towards aminoglycosides and neomycin. In this study, *C. pseudotuberculosis* was resistant towards streptomycin. This is in agreement with Muckle and Gyles (1982) as well as Judson and Songer (1991). Antibiotics resistant towards polymixin B identified in this study is reliable with the support of the study done by Judson and Songer (1991). The susceptibility of ampicillin and penicillin varies from different studies but at least in this study, ampicillin and penicillin G found to be sensitive towards this microorganism.

Table 3 shows the minimum inhibitory concentration and minimum bactericidal concentration of selected antibiotics.

Antibiotic	*Minimal Bactericidal Concentration (MBC)*	*Minimal Inhibitory Concentration (MIC)*
Neomycin	1.875 µg/mL	0.9375 µg/mL
Gentamicin	0.25 µg/mL	0.125 µg/mL
Penicillin G	20 µg/mL	10 µg/mL
Erythromycin	20 µg/mL	10 µg/mL

Table 3: Minimal bactericidal concentration and minimal inhibitory concentration of selected antibiotics towards *C. pseudotuberculosis*

The minimal bactericidal concentration (MBC) for neomycin, gentamycin, penicillin G and erythromycin were 1.875 µg/mL, 0.25 µg/mL, 20 µg/mL, and 20 µg/mL, respectively. Minimum inhibitory concentration (MIC) for neomycin, gentamycin, penicillin G and erythromycin were 0.9375 µg/mL, 0.125 µg/mL, 10 µg/mL and 10 µg/mL, respectively (Table 3).

As being susceptible towards most of the antibiotics tested, the use of antibiotics in animals do not produce significant result in eliminating this disease due to inability to penetrate the thick encapsulated abscess. Moreover, *C.pseudotuberculosis* is an intracellular pathogen. Due to limited accessibility into the host cell, intracellular microbes are protected from many antimicrobial drugs.

Studies of the use of nanotechnology in medicine has been increasing in recent year and suggestion is made to consider the use of antibiotic-loaded nanoparticles in treating caseous lymphadenitis. One of the most favourable alternatives to

combat existing ineffective treatment is the use of antibiotic-loaded nanoparticles as it consists of multiple mechanisms simultaneously to deliver the drug to target tissues.

5.0 CONCLUSIONS

In this study, we found that strain of isolate in Malaysia is susceptible to amoxicillin with and without clavulanic acid, ampicillin, cephalexin, enrofloxacin, erythromycin, gentamicin, neomycin, oxytetracycline, penicillin G, sulfamethoxazole-trimethoprim and tetracycline but showed resistance to streptomycin and polymixin B *in vitro*. Minimum inhibitory concentration (MIC) for neomycin, gentamycin, penicillin G and erythromycin were 0.9375 µg/mL, 0.125 µg/mL, 10 µg/mL and 10 µg/mL, respectively. The minimal bactericidal concentration (MBC) for neomycin, gentamycin, penicillin G and erythromycin were 1.875 µg/mL, 0.25 µg/mL, 20 µg/mL, and 20 µg/mL, respectively.

6.0 RECOMMENDATIONS

Recommendations were made attributable to the limitations in this study where more strains of isolates origin from different sources (animal, species, farm, geographic regions) in Malaysia should be included. In addition, more antibiotics should be included to be studied in future research to be able to obtain a more complete database of antimicrobial susceptibility of *C.pseudotuberculosis* in Malaysia. The application of nanotechnology in treating this disease could be considered. Considering its antimicrobial resistance mechanisms in the host, treatment option using nanoparticles should be considered in future.

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