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Determination of Susceptibility of Malaysian *Leptospira* Isolate to Antimicrobial Agents

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Abstract: One *L. hardjo* Malaysian isolate and five *Leptospira sp.* reference strain namely *L. pomona, L .icterohaemorrhagae, L. canicola, L. australis* and *L. hardjo* Prajitno were selected in this study. The *Leptospira* strains were tested for it susceptibility against five types of antibiotics. The antibiotics are tetracycline, oxytetracycline, penicillin G, streptomycin and erythromycin. All the strains were observed under dark-field microscope for their mortality after inoculation of 18 different concentration of antibiotic at 24, 48, 72, 96, 120, 144 and 168 h post inoculation. The study showed that tetracycline is the antibiotic of choice to treat leptospirosis because it is effective against most of the strains used in the study in very short time after inoculation. However, *L. canicola* was resistant against tetracycline but sensitive to penicillin G.

Keywords: *Leptospira sp.*, antibiotic, mortality

INTRODUCTION

Leptospirosis is a world-wide veterinary and human public health problem, caused by spirochetes belonging to the genus *Leptospira*. In Malaysia, it is recognized as important zoonosis as well as an important animal disease with substantial loss in production[1].The human disease is an acute febrile illness characterized by a broad range of clinical forms, the severities of which vary from mild to rapidly fatal[2]. Human are incidental, "dead-end" hosts because transmission from human to animals or to other human does not occur. Serologically, leptospiral infection has been detected in buffaloes, pigs, horses, cattle, goats, sheep, cats, dogs and rats in west Malaysia[1]. Cattle were shown to have high serological prevalence of leptospiral infection when compared to the other animal species[3]. Leptospiral infection was reported to cause depression, inappetance, abortion, stillbirth, retain placenta, weak progeny, mastitis and infertility in cattle[4]. Bahaman and Ibrahim[5] have isolated serovar *canicola, australis* and *javanica* from a herd of cattle in Malaysia. Recently, Khairani-Bejo[6] has isolated serovar *hardjo* from cattle in selected cattle farms in the State of Johor Malaysia. Currently, the antibiotics of choice for the treatment of leptospirosis are penicillin, streptomycin and tetracycline. In the present study, we determined the minimum inhibitory concentrations (MICs) of penicillin G, erythromycin, tetracycline, oxytetracycline and streptomycin against one *L. hardjo* isolate that was obtained from cattle urine.

MATERIALS AND METHODS

Bacteria strains: One Malaysian *Leptospira* isolate was obtained from cattle urine. Five *Leptospira sp.* reference strains namely *L. canicola, L. icterohaemorragiae, L. hardjo* Prajitno, *L. australis* and *L. pomona* were obtained from Royal Tropical Institute, Amsterdam, The Netherlands.

Antibiotics: Five types of antibiotic were used namely, penicillin G, erythromycin, tetracycline, oxytetracycline and streptomycin.

Susceptibility test: Antibiotic dilution was performed with 96-well, round-bottom microtiter plates. Positive control (bacteria without antibiotic) and negative control (medium only) were included in every plate. Antibiotic-containing wells included final concentrations of each drug, ranging from 512.0 to 0.004mg mL^{-1}. *Leptospira* inoculum was produced from cultures grown for 7 days at 30°C with organism counts approximately 10^6. Following the addition of a 100 μL inoculum to the antibiotic-containing and positive control wells, the plates were incubated at 30°C. Ten-micro liters (10μL) of fluid from each well were transferred into 2 mL of fresh JS medium at 24, 48, 72, 96, 120, 144 and 168 h after incubation.

The antibiotic concentration contained in the lowest-concentration well without visual growth was recorded as the MIC. Minimal Bactericidal Concentration (MBC) testing was performed by transferring 10μL of fluid from

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each well without visible growth into 2 mL of fresh JS medium. The lowest antimicrobial concentration that yielded no growth by visual inspection after 3 weeks of incubation at 30°C was documented as the MBC.

RESULT AND DISCUSSION

This study shown that, there was no different between MIC and MBC results. The MIC and MBC for all antibiotics against *L. hardjo* Malaysian isolate and *Leptospira sp.* reference strain was 0.004mg mL^{-1}. The current chemotherapy for the treatment of leptospirosis consists of using penicillin, streptomycin, or tetracycline. This study have shown that tetracycline, oxytetracycline, penicillin G, streptomycin and erythromycin exhibits weak bactericidal activity, even though it's MIC and MBC is low. It has also been shown that penicillin G is incapable of destroying organisms in the kidneys of animals experimentally infected with leptospires.

All tetracycline derivatives are excreted in the urine and bile in high concentrations. It would therefore be expected that *L. icterohaemorrhagiae* and *L. canicola* infection would be highly susceptible to tetracycline treatment. This was confirmed but early treatment is necessary in view of the structural damage which these organisms cause. In cattle, high dose with intravenous administration is indicated. *L. pomona* carriers in pigs have been cleared by oxytetracycline in the food at the rate of 500g per tonne. In small animals the tetracyclines, and in particular oxytetracycline because of the higher levels achieved when compared with chlortetracycline, are superior to other antibiotics but should be given as early after infection as possible and in maximum doses[6]. Empiric antimicrobial therapy must be comprehensive and should cover all likely pathogens in the context of the clinical setting. No controlled studies have been performed to evaluate the efficacy of antibiotics for leptospirosis in children, nor have specific doses been used consistently in reported case series. The following dosage guidelines are extrapolated from research on adult patients. Penicillin G the only drug proven effective in adults; interferes with synthesis of cell wall mucopeptide during active multiplication, resulting in bactericidal activity against susceptible microorganisms. Adult dose recommended are 1.5 million U intravenous for 7 days. Pediatric dose recommended 100,000 u/kg/d intravenous for 7 days. Precaution should be taken because impaired renal function.

Leptospiral infection occurs in all species and clinical recovery results from penicillin therapy in the case of *L. canicola* and *L. pomona*, but not *L. icterohaemorrhagiae*. It is indicated that a minimum of 10 mg per kg body weight every 12 h eliminates *L. pomona* from the urine of calves and cattle. Penicillin does not clear the organism from the urine. Streptomycin in doses 0.25 to 0.5 g has also proved effective in stopping the excretion of *Leptospira* in the urine of pigs weighing about 40 kg. In the dog, though penicillin gives clinical improvement, streptomycin is superior in both *L. canicola* and *L. icterohaemorrhagiae* infections, completely eliminating the former from urine: 40 mg per kg body weight in divided dose daily by intramuscular injection are recommended. Treatment should be continue for 4 to 6 days[6].

Penicillin is effective against *L. canicola* and *L. pomona* in this study, an advantage of penicillin is that they are well distributed to most tissues in the body. They reach therapeutic concentrations in most tissues, with exception of the eye globe and the brain. However in situations such as meningitis where the blood brain barrier becomes more permeable, penicillin may enter this compartment but still not reach significant therapeutic concentrations. *Leptospira hardjo* local isolate, *L. hardjo* Prajitno and *L. pomona* is sensitive to tetracycline in this study, the pharmacokinetics of tetracycline and oxytetracycline are once absorbed into the systemic circulation, they are distributed to most tissues and can reach significant concentrations in saliva and bronchial secrection[7]. The spirochetes multiply in organs, most commonly the central nervous system, kidneys, and liver. They are cleared by the immune response from the blood and most tissues but persist and multiply for some time in the kidney tubules. Infective bacteria are shed in the urine. Penicillin is usually excreted intact and unchanged; it retains bactericidal activity in the urine[7].

Streptomycin are effective against *L. pomona* in this study, the pharmacokinetics of streptomycin can only leave those cells by passive diffusion, so the dosage interval (time between doses) must be increased to allow streptomycin plasma concentrations to decrease enough to set up a concentration gradient that permits drug movement out of the cells and into the plasma. The kidney accumulates streptomycin via an active transport process. This accumulation is thought to contribute to the nephrotoxicity produced by large or frequent doses of streptomycin. Streptomycin is eliminated almost exclusively via glomerular filtration in the kidneys. This efficient elimination helps explain the short half-life of streptomycin (usually 1 to 2 h depending on the species) in animal with normal renal function[7]. *Leptospira australis* and *L. pomona* are sensitive to erythromycin in this study, it is well absorbed from the gut but the they requires an enteric coat to resist digestion by gastric acid. Plasma levels reach a peak in about 2 h and the drug is cleared with a half-life of 1-1.5 h. The drug reaches most

tissue, except the brain and only 20% is excrected in the kidney[8].

Antibiotic therapy in the early course of leptospirosis infection is efficient in shortening duration of the disease, reducing the time period for risks of contagion, and decreasing the severity of liver and kidney damage. Although the leptospires are susceptible to antibiotics such as penicillin and tetracycline in vitro, use of these drugs in the treatment of leptospirosis is somewhat controversial. Treatment is most effective if initiated within a week of disease onset. At later times, immunologic damage may already have begun, rendering antimicrobial therapy less effective. Leptospirosis is zoonotic, thus recommend precautions and proper hygiene, especially regarding exposure to contaminated urine.

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