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# Antimicrobial susceptibility of *Leptospira* isolates from dogs and rats to 12 antimicrobial agents

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**Abstract.** This study determined the antimicrobial susceptibilities of 67 isolates of *Leptospira* from dogs (suspect canine cases: n=7 and stray dogs: n=6) and rodents (n=54) in Trinidad to 12 antimicrobial agents using broth microdilution and macrodilution techniques. Commonly used antimicrobial agents such as the penicillin G and ceftriaxone had relatively low minimal inhibitory concentrations (MICs) while doxycycline displayed a relatively higher value but was still considered to be effective. While imipenem was the most effective with low MIC values *in vitro*, sulphamethoxazole-trimethoprim had the highest i.e. least effective. Based on these results, the drugs commonly used in the treatment of leptospirosis (penicillin G, penicillin-streptomycin, doxycycline and ceftriaxone) in both humans and animals in Trinidad appear to have similar MICs and MBCs *in vitro* when compared with published reports. The serovar of *Leptospira* spp. and in most cases the origin of the isolates did not significantly (P>0.05) influence their susceptibilities to the antimicrobial agents tested.

## INTRODUCTION

Leptospirosis is a bacterial zoonosis of global importance however, it is endemic in tropical regions (Bharti *et al.*, 2003; Ricaldi & Vinetz, 2006) including Trinidad and Tobago where there is high rainfall (Levett, 2004). The organism is transmitted by direct and indirect methods (Faine, 1994). There are numerous reservoirs of leptospiral infection inclusive of rodents (Vanasco *et al.* 2003), dogs (Merson *et al.*, 2006) and cattle (Meites *et al.*, 2004) and these animals occasionally shed leptospires into the environment via the urine. To date, there are more than 300 serovars of which more than 230 are virulent (Kmety & Dikken, 1993). These virulent strains produce a wide range of clinical signs of disease which may range from mild influenza-like syndrome to more severe disease which may lead to death (Murray & Hospenthal, 2004b). Definitive diagnosis of leptospirosis is often unavailable or may be inordinately long. In the interim, the initial therapy of leptospirosis is usually empirical and based upon a broad differential diagnosis that includes leptospirosis and other causes of acute febrile illness in man (Murray & Hospenthal, 2004c). Leptospirosis in animals may manifest with clinical signs such as jaundice, anorexia and vomiting (Prescott *et al.*, 2002). Even when leptospirosis is confirmed, the choice of an optimal treatment regime is difficult for a variety of reasons. These include the fact that the relative virulence of pathogenic species and serovars is understudied especially in areas where the disease is endemic, the pathogenesis of the agent is poorly understood and finally the lack of funds (Pappas & Cascio, 2006). There are two (2) primary methods used for *in vitro* antimicrobial susceptibility testing of *Leptospira*, namely, the broth macrodilution (producing results of the

minimal inhibitory concentration by the broth macrodilution-$MIC_{mac}$ and minimal bacteriocidal concentration-MBC) and the microdilution technique (minimal inhibitory concentration by the broth microdilution-$MIC_{mic}$) (Murray & Hospenthal, 2004b,c). Currently, there is no standard method for assessing *in vitro* antimicrobial agents for anti-leptospiral activity, however, the broth microdilution method is more rapid than the traditional broth macrodilution method (Murray & Hospenthal, 2004b, c). At present, the drugs of choice for the treatment of leptospirosis in humans are penicillin G, doxycycline and ceftriaxone (Hospenthal & Murray 2003; Raptis *et al.*, 2006), while the drugs of choice for the treatment of dogs include amoxicillin and oral doxycycline (Langston & Heuter, 2003). In addition, for the treatment of leptospiraemia, penicillin G or ampicillin can be administered intravenously for 2 weeks and for leptospiruria doxycyline can be administered orally for 2 weeks (Birchard & Sherding, 1994). It should be noted that only a small number of clinical trials have been performed worldwide in humans, with one assessing the efficacy of ceftriaxone and penicillin G (Panaphut *et al.*, 2003). Another study investigated both doxycycline and azithromycin and demonstrated that both drugs were equally effective in the treatment of leptospirosis in 34 and 35 humans respectively (Phimda *et al.*, 2007). Also, a randomized study performed with penicillin, doxycycline and cefotaxime (Suputtamongkol *et al.*, 2004) and a placebo-controlled trial using penicillin G (Watt *et al.*, 1988) showed that all these antimicrobial agents were effective in the treatment of leptospirosis. *In vivo* testing performed in animals showed the efficacy of drugs such as azithromycin (Moon *et al.*, 2006), while another, comparing the efficacies of penicillins, cephalosporins and tetracyclines in hamsters, found cefotaxime and ampicillin to be the most effective (Alexander, 1986; Alt & Bolin, 1996). In Trinidad, it is possible for individuals to purchase antimicrobial agents 'over-the-counter' because there is no strict enforcement of regulation controlling the sale of these drugs (Parimi *et al.*, 2002). As a result, the possibility exists for people to treat themselves or their animals with antimicrobial agents which may be inappropriate for the treatment of leptospirosis. In addition, there is often a lack of compliance with dosages and duration of antimicrobial therapy, which may lead to the development of resistance to these commonly used antimicrobials. To assess the susceptibility of *Leptospira* isolates from several sources in Trinidad, a wide range of antimicrobial agents from different classes of antimicrobial drugs were used. Overall, a total of 67 isolates of *Leptospira* were tested against 12 antimicrobial agents by both the microdilution and macrodilution methods. The primary objective of the study was to use both techniques to determine the MICs and MBCs of the various antimicrobial agents against 67 isolates obtained from dogs (suspected canine cases and strays) and wild caught rats in Trinidad and Tobago.

## MATERIAL AND METHODS

Trinidad, the southernmost island in the Caribbean, covers an area of 4852 $km^2$. It is roughly rectangular in shape, with a length of 80 km and a width of 59 km. The humidity is high (85-87%), particularly during the rainy season (June to December). *Leptospira* isolates were obtained from the kidneys of stray dogs (n=6), blood and kidneys of suspected canine cases (n=7) and wild rodents (n=54) in Trinidad (Suepaul *et al.*, 2010). The serotypes and sources of *Leptospira* used in the study are in Table 1. These isolates were frozen at –70°C between isolation and the commencement of the testing when they were defrosted and subcultured only once to attain the density required for susceptibility testing. The serovars of the isolates were determined by serology using rabbit antiserum and monoclonal antibodies as earlier documented (KIT Biomedical Research, Royal Tropical Institute, 2006; Suepaul *et al.*, 2010). The leptospires were grown in EMJH medium, which was incubated for approximately 7 to

Table 1. Serovars of *Leptospira interogans* used in the study

| Serovars* of *L. interrogans* | No. of rodent isolates** | No. of suspected canine case isolates*** | No. of stray dog isolates**** | Total |
|---|---|---|---|---|
| *L. interrogans* serovar Copenhageni | 37 | 7 | 2 | 46 |
| *L. interrogans* Mankarso | 7 | 0 | 0 | 7 |
| *L. interrogans* Icterohaemorrhagiae | 1 | 0 | 0 | 1 |
| Inconclusive | 9 | 0 | 4 | 13 |
| Total | 54 | 7 | 6 | 67 |

*Using the 23 international panel of sera and the panel of 6 monoclonal antibodies
**Of a total of 54 isolates of *Leptospira interrogans*
***Of a total of 7 isolates of *Leptospira interrogans*
****Of a total of 6 isolates *Leptospira interrogans*

14 days at 30ºC. Growth was checked by dark field microscopy to ensure the adequate density and absence of contaminating bacteria. The bacteria were counted using the **MacFarland nephelometric method** described by KIT Biomedical Research, Royal Tropical Institute (2006). A total of 12 antimicrobial agents consisting of β-lactams (penicillin and amoxicillin), a third generation cephalosporin (ceftriaxone), an aminoglycoside (gentamicin), a semi-synthetic tetracycline (doxycycline), a fluoroquinolone (ciprofloxacin), a lincomycin (clindamycin), a polypeptide (polymyxin B), a macrolide (erythromycin) and combinations (penicillin-streptomycin and sulphamethoxazole-trimethoprim (SXT). Imipenem, an intravenous β-lactam used in humans was also included in the panel of antimicrobial agents tested in the study. It is pertinent to mention that amoxicillin and penicillin-streptomycin are commonly misused drugs in Trinidad (Parimi *et al.*, 2002). All antimicrobial agents were obtained from Sigma-Aldrich™ (St. Louis, Missouri) except for imipenem which was supplied by the USP Reference Standards, U.S. Pharmacopeia™ Rockville, Maryland. Stock solutions of antimicrobial agents were made by adding 70 mg of each antimicrobial agent to 70 ml of liquid Ellinghausen-McCullough-Johnson-Harris (EMJH) to produce a 1µg/µl (or in the case of penicillin,1000 U/ml) stock solution. One millilitre quantities of stock solution were aliquoted into 2.0 ml micro-centrifuge eppendorf tubes at -70ºC until required. For both the broth macro and microdilution the procedure of Murray and Hospenthal (2004b) was used. Positive and negative controls were set up for each batch. The batch was only valid if the positive control fell within one dilution of the expected value. Briefly, the microtitre plates were numbered from rows 1 to 12 and 100 µl of medium was placed into each well. Thereafter, serial two-fold dilutions of each antimicrobial agent were made with EMJH medium resulting in concentrations ranging from 100 µg/ml to 0.01 µg/ml (100 to 0.01 U/ml for penicillin G). Thereafter, 100 µl of 2 x $10^6$ *Leptospira* /ml was added to each of the wells, positive control wells, (which contained leptospires but no antimicrobial agent) except for the negative controls (which contained no leptospires) and to which 100 µl of EMJH was added instead. The mixture of leptospiral culture and antimicrobial agent was incubated for 3 days at 30ºC. On the third day, 20 µl of 10 X AlamarBlue® (Trek Diagnostics, Cleveland, Ohio) was added to each well. After two additional days of incubation (i.e. overall five days of incubation) at 30ºC, a change in colour from blue to pink was considered to indicate cell growth while an unchanged blue colour was determined to be evidence of no cell growth. The $MIC_{mic}$ was recorded as the lowest concentration used that did not result in a colour change from blue to pink. From a 1 µg/µl stock solution, a 200 µg/ml solution was made by the adding 200 µl of stock solution to 800 µl of liquid EMJH. Then 2 ml of the 200

µg/ml of antimicrobial solution was added to the first 2 ml Eppendorf tube in a series of tubes, each containing 1 ml of liquid EMJH. The antimicrobial concentration of the first tube was therefore 100 µg/ml followed by a 2-fold dilution. Successive duplicate serial two-fold dilutions were made using EMJH with concentrations ranging from 100 µg/ml to 0.01 µg/ml (100 to 0.01 U/ml for penicillin G). *Leptospira* isolates were then added to produce a final volume of 2 ml and a final concentration of $10^6$ leptospires per ml. Tubes were incubated at 30°C for seven days. The antimicrobial concentration contained in the Eppendorf tube with the lowest concentration without visual growth by the broth macrodilution technique was recorded as the $MIC_{mac}$. The MBC was determined by transferring 10 µl of content of each tube without visible growth to 2 ml of fresh EMJH medium. The lowest antimicrobial concentration that yielded no growth by visual inspection after 3 weeks of incubation at 30°C was adjudged to be the MBC.

**Data Analysis**

Statistical Package for Social Sciences (SPSS) version 17.0 was used to analyze the data collected and Kruskal-Wallis analyses were used to determine if the animal source and serovars observed varied in susceptibilities. The Wilcoxon test was also conducted to determine if the differences observed between the broth microdilution and broth macrodilution techniques were significant. All analyses were done to 5% level of significance.

## RESULTS

Table 2 displays the medians and ranges of MICs and MBCs of each antimicrobial agent utilized in the study. The highest medians displayed were to sulphamethoxazole-trimethoprim, gentamicin, polymyxin B and doycycline. Wide ranges of $MIC_{mic}$s were observed for antimicrobial agents such as ciprofloxacin, doxycyline and clindamycin. However, generally smaller ranges were observed for the $MIC_{mac}$s and MBCs. Isolates displayed relatively high $MIC_{90}$s and $MBC_{90}$s values (Table 3) against the following antimicrobial agents: gentamicin, sulphamethoxazole-trimethoprim, doxycycline, amoxicillin and ceftriaxone. The differences in the susceptibilities of isolates of *Leptospira* serovars to antimicrobial agents tested, using both techniques, were not statistically significant ($P>0.05$) as shown in Table 4. A comparison of the frequency of susceptibilities of isolates

Table 2. The medians and ranges of MICs obtained by the broth microdilution and macrodilution techniques as well as the MBCs values as determined by the broth macrodilution technique

| Antimicrobial agent | $MIC_{mic}$ | | $MIC_{mac}$ | | MBC | |
|---|---|---|---|---|---|---|
| | Median | Range | Median | Range | Median | Range |
| Ceftriaxone | 0.05 | 0.05–0.20 | 0.78 | 0.78–1.56 | 50 | 25–100 |
| Clindamycin | 0.05 | 0.01–0.78 | 0.1 | 0.05–0.20 | 3.13 | 1.56–6.25 |
| Penicillin-Streptomycin | 0.01 | 0.01–0.39 | 0.78 | 0.39–1.56 | 3.13 | 3.13–12.5 |
| Doxycyline | 3.13 | 0.01–6.25 | 3.13 | 0.78–50 | 100 | 25–100 |
| Amoxicillin | 0.05 | 0.05–0.10 | 1.56 | 0.78–12.5 | 6.25 | 0.78–100 |
| Polymyxin B | 3.13 | 0.05–6.25 | 3.13 | 1.56–50 | 100 | 100 |
| Erythromycin | 0.05 | 0.01–0.39 | 0.05 | 0.05–0.1 | 0.39 | 0.39–0.78 |
| Imipenem | 0.01 | 0.01 | 0.05 | 0.05 | 0.1 | 0.05–0.10 |
| Ciprofloxacin | 0.1 | 0.01–25 | 0.05 | 0.05–0.20 | 1.56 | 1.56–3.13 |
| Sulphamethoxazole-trimethoprim | 25 | 25 | 100 | 100 | 100 | 100 |
| Penicillin G | 0.1 | 0.05–0.1 | 1.56 | 0.78–1.56 | 3.13 | 3.13–6.25 |
| Gentamicin | 25 | 25 | 100 | 100 | 100 | 100 |

Table 3. The $MIC_{90}$s obtained by both the broth microdilution and macrodilution techniques as well as the MBC90s obtained by the broth macrodilution technique

| Antimicrobial agent | Microdilution | Macrodilution | |
|---|---|---|---|
| | $MIC_{90}$ | $MIC_{90}$ | $MBC_{90}$ |
| Ceftriaxone | 0.1 | 1.56 | 50 |
| Clindamycin | 0.39 | 0.2 | 3.13 |
| Penicillin-Streptomycin | 0.1 | 0.78 | 3.13 |
| Doxycyline | 6.25 | 6.25 | 100 |
| Amoxicillin | 0.05 | 6.25 | 50 |
| Polymyxin B | 3.13 | 50 | 100 |
| Erythromycin | 0.2 | 0.1 | 0.78 |
| Imipenem | 0.01 | 0.05 | 0.1 |
| Ciprofloxacin | 1.56 | 0.1 | 3.13 |
| Sulphamethoxazole-trimethoprim | 25 | 100 | 100 |
| Penicillin G | 0.1 | 1.56 | 6.25 |
| Gentamicin | 25 | 100 | 100 |

Table 4. P-values obtained by the Kruskal-Wallis analysis on differences in susceptibilities amongst serovars of *Leptospira* spp. to 12 antimicrobial agents

| Antimicrobial agent | Microdilution | Macrodilution | |
|---|---|---|---|
| | $MIC_{90}$ | $MIC_{90}$ | $MBC_{90}$ |
| Ceftriaxone | 0.1 | 1.56 | 50 |
| Clindamycin | 0.39 | 0.2 | 3.13 |
| Penicillin-Streptomycin | 0.1 | 0.78 | 3.13 |
| Doxycyline | 6.25 | 6.25 | 100 |
| Amoxicillin | 0.05 | 6.25 | 50 |
| Polymyxin B | 3.13 | 50 | 100 |
| Erythromycin | 0.2 | 0.1 | 0.78 |
| Imipenem | 0.01 | 0.05 | 0.1 |
| Ciprofloxacin | 1.56 | 0.1 | 3.13 |
| Sulphamethoxazole-trimethoprim | 25 | 100 | 100 |
| Penicillin G | 0.1 | 1.56 | 6.25 |
| Gentamicin | 25 | 100 | 100 |

of *Leptospira* spp., by origin (suspected canine cases, stray dogs and rodents) revealed statistically significant differences ($P<0.05$) in some (Table 5). The p-values of Wilcoxon test results on the comparison of the frequency of susceptibilities amongst isolates by the macrodilution and microdilution methods are displayed in Table 6. For the broth macrodilution technique, in 10 of 12 antimicrobial agents the differences in MIC values were statistically significantly ($P<0.05$) higher than found for the microdilution technique.

## DISCUSSION

The diagnosis of leptospirosis in dogs in Trinidad is most often based on clinical signs, particularly fever and jaundice. As a result, veterinarians often prescribe broad-spectrum antimicrobial agents to treat not

Table 5. P-values obtained by the Kruskal-Wallis analysis to determine significant differences in antimicrobial susceptibilities of isolates from different origins (suspect canine case, stray dog or rodent)

| Antimicrobial agent | P-values | | |
|---|---|---|---|
| | $MIC_{mic}$ | $MIC_{mac}$ | MBC |
| Ceftriaxone | 0.031[a] | 0.546 | 0.339 |
| Clindamycin | 0.037[b] | 0.003[c] | 0.803 |
| Penicillin-Streptomycin | 0.072 | 0.426 | 0.58 |
| Doxycyline | 0.024[d] | 0.013[e] | 0.118 |
| Amoxicillin | 0.058 | 0.169 | 0.621 |
| Polymyxin B | 0.066 | 0.389 | 1.00 |
| Erythromycin | 0.469 | 0.625 | 0.805 |
| Imipenem | 1.00 | 1.00 | 0.294 |
| Ciprofloxacin | 0.127 | 0.113 | 0.108 |
| Sulphamethoxazole-trimethoprim | 1.00 | 1.00 | 1.00 |
| Penicillin G | 0.00[f] | 0.00[g] | 0.21 |
| Gentamicin | 1.00 | 1.00 | 1.00 |

[a] Suspected canine cases displayed significantly higher $MIC_{mic}$ compared to other groups.
[b] Suspected canine cases displayed significantly lower $MIC_{mic}$ compared to the other groups.
[c] Suspected canine cases displayed significantly higher $MIC_{mac}$ compared to other groups.
[d] Stray dogs displayed significantly lower $MIC_{mic}$ compared to the other groups.
[e] Rodents displayed significantly lower $MIC_{mac}$ compared to the other groups.
[f] Rodents displayed significantly higher $MIC_{mic}$ compared to the other groups.
[g] Stray dogs displayed significantly higher $MIC_{mac}$ compared to the other groups.

only the possible leptospiral infection but also for other potential infectious diseases considered as differential diagnoses. To date there are no published studies on the antimicrobial susceptibilities of *Leptospira* spp. in Trinidad and Tobago. This information is essential considering the prevalent, inappropriate and uncontrolled use of antimicrobial agents in veterinary practice in the country where there is unrestricted access by animal owners to antimicrobial agents. The potential of leptospires as well as other pathogens to develop resistance to antimicrobial agents can therefore not be over-emphasized. Reports exist on the *in vitro* antimicrobial susceptibilities of *Leptospira* spp. (Murray et al 2004a). A publication by Ressner *et al.* (2008) describes the susceptibilities of isolates obtained from various geographical locations inclusive of Egypt, Thailand, Nicaragua and Hawaii. The authors reported that the traditionally used antimicrobial agents for the treatment of leptospirosis in humans: doxycycline had slightly higher $MIC_{90}$s than the other agents used. In the current study we also observed that doxycycline had higher $MIC_{90}$ and MBC values when compared to the other antimicrobial agents. Ressener *et al.* (2008) also suggested that there may be regional differences in $MIC_{90}$s which will explain the variations between our study and other published studies. A comparison of the medians and ranges of MICs obtained in the current study with published reports revealed many differences. A study by Murray & Hospenthal (2004c) reported on the susceptibilities of a wide variety of serovars of *Leptospira* spp. (obtained from USDA National Veterinary Services Laboratories, Ames, Iowa) to 24 antimicrobial agents using the broth microdilution technique. The authors documented the following $MIC_{90}$s for: doxycycline (1.56 µg/ml), penicillin G (6.25 µg/ml), amoxicillin (3.13 µg/ml), ceftriaxone (0.39 µg/ml), imipenem-cilastatin (≤0.01 µg/ml), erythromycin (≤0.01 µg/ml) and ciprofloxacin (0.2 µg/ml). In comparison, in the current study, we detected higher $MIC_{90}$ values for doxycycline (6.25µg/ml)

and ciprofloxacin (0.78µg/ml) whereas for penicillin G, amoxicillin and ceftriaxone the $MIC_{90}$ values were actually similar, varying by only one dilution in some cases. The variations among the values obtained in this study and others may be due to the variations in the geographical locations from which the isolates were obtained as well as possible strain to strain variations. Of interest is the finding by Hospenthal & Murray (2003) in another study which employed the macrodilution technique, obtained results with higher $MIC_{90}$ and $MBC_{90}$ values respectively for penicillin (3.13 µg/ml and 50 µg/ml) and ciprofloxacin (0.20 µg/ml and 25 µg/ml), but lower values for amoxicillin (0.20 µg/ml and 25 µg/ml), doxycycline (3.13 µg/ml and 25 µg/ml), erythromycin ($\leq$0.01 µg/ml and 0.20 µg/ml) and ceftriaxone (0.20 µg/ml and 0.39 µg/ml) than stated in this study. The differences observed amongst studies maybe attributed to strain-to-strain variations in antimicrobial susceptibility as well as the variations in the geographical locations of the isolates (Ressner *et al*., 2008). In addition, possible variations between laboratories cannot be ignored. It is however pertinent to mention that the theory of strain-to-strain variation in resistance to antimicrobial agents amongst leptospires has been disputed by Murray & Hospenthal (2004b) who reported that there were no significant variations in susceptibility from strain-to-strain and species-to-species of isolates studied. Murray & Hospenthal (2004c) also noted that the serovar-specific diagnosis of leptospirosis is seldom available, hence the antimicrobials used are generalized for leptospiral infection because the culture and sensitivity of the infecting strain is rarely available. However, in the current study we were able to compare the susceptibilities of various serovars of *Leptospira* spp. to antimicrobial agents and detected no statistically significant differences amongst serovars (by neither the broth microdilution nor the broth macrodilution techniques). This has therapeutic and economic significance as it indicates that the various serovars of isolates present in Trinidad have similar antimicrobial susceptibilities and as such a generalized treatment regime may be used. Although the isolates used in this study were obtained from different animals, inclusive of dogs (stray dogs and suspected canine cases) and rodents, the differences observed in susceptibilities were not statistically significant with some exceptions which cannot readily be explained. Chakraborty *et al.* (2010) conducted a similar study which investigated rats as a reservoir of infection. In that study doxycycline, ciprofloxacin, and erythromycin were found to be effective against the strains of *Leptospira* isolated from rats in Manila and the Philippines. We observed higher ranges of values for these antibiotics in rats in Trinidad which once again may be attributed to variations in geographical location, probable lab to lab and strain to strain variations. It was not a surprise that overall, the MICs for the antimicrobial agents tested by the macrodilution technique were considerably higher than those found for the microdilution technique. This phenomenon has been documented in the literature (Murray & Hospenthal, 2004b). The microdilution technique is considered more sensitive than the macrodilution technique since the latter technique relies on the observation of leptospires via dark field microscopy. This can be difficult especially when there are very few leptospires present, whereas, the microdilution technique detects metabolic activity which result in a colour change which is easily detectable (Murray & Hospenthal, 2004b). For chemotherapy, it should be noted that both the microdilution and macrodilution techniques are simple *in vitro* methods which can be used to test multiple antimicrobial agents against multiple *Leptospira* spp. (Murray & Hospenthal, 2004b). However, *in vitro* activity may not exactly mirror what occurs *in vivo* in the treatment of leptospirosis. The use of an animal model to determine, *in vivo*, the most effective antimicrobial agents for use against local isolates of *Leptospira* may therefore be imperative. Susceptibilities of *Leptospira* isolates to antimicrobial agents *in vivo* have been reported by others (Alexander, 1986; Alt & Bolin, 1996) and used in selecting antimicrobial agents in

the treatment of humans and animals (Hospenthal & Murray, 2003). It has also been documented (Ressner *et al.*, 2008) that serovars of *Leptospira* may display geographical variation in their susceptibility to antimicrobial agents. Of interest is the fact that previously reported ranges for the MIC and MBC of amoxicillin against isolates obtained from the National Veterinary Service Laboratory in the USA was 0.05-6.25 µg/ml and 6.25-25.0 µg/ml respectively (Kim *et al.*, 2006), these values are slightly lower than those obtained in this study where the corresponding values were 0.78-12.5 µg/ml and 0.78-100 µg/ml. The variations in the values suggest that antimicrobial resistance to amoxicillin maybe developing due to the fact that amoxicillin is a commonly abused drug due to the fact that it is readily available in Trinidad, even without a prescription at times and hence it is postulated that there may be local drug pressure occurring with respect to amoxicillin. The observed general high resistance detected to antimicrobial agents in our study may in fact be due to the unregulated use and abuse of antimicrobials in Trinidad and Tobago. Similarly, Ressner *et al.* (2008) also postulated about local drug pressure occurring in Egypt with respect to antimicrobials commonly used. It is concluded that the antimicrobial agents used in this study are effective against the isolates of *Leptospira* spp. studied. The antimicrobial agents commonly used in Trinidad such as doxycycline, penicillin, penicillin-streptomycin, amoxicillin and ceftriaxone appear to be effective from this *in vitro* study. However some antimicrobial agents specifically sulphamethoxazole-trimethoprim and gentamicin had relatively high MIC values suggesting that they may be less effective in the treatment of leptospirosis. Clinical trials can be performed to determine the true efficacy of these antimicrobial agents in the treatment of clinical leptospirosis.

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