COMPARATIVE STUDY OF FOUR FLUORINATED QUINOLONES IN SUSCEPTIBILITY FACTORS ON MICROORGANISMS.

by

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Submitted to the
Office of Honors Programs and Academic Scholarships
Texas A&M University
in partial fulfillment of the requirements for

1998-99 UNIVERSITY UNDERGRADUATE RESEARCH FELLOWS PROGRAM

April 15, 1999

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ABSTRACT

Comparitive Study of Four Fluorinated Quinolones in Susceptibility factors on Microorganisms. Ketul Patel, (Dr. Dawn Boothe) University Undergraduate Research Fellow, 1998-1999, Texas A&M University, Department of Veterinary Physiology and Pharmacology

Widely considered in the medical field as the last of the golden anti-microbial drugs, fluorinated quinolones provide a "last defense" for the attack on bacterial infections. The drugs are useful for treating gram negative and some gram positive infections. Among the advantages of fluorinated quinolones are oral dosing, resistance that develops slowly via mutation rather than plasmid mediated mutations and high bactericidal activity at low concentrations. All are quinolone carboxylic acid derivatives which inhibit bacterial DNA gyrase, a mechanism of action unique among the antibacterials. The drugs considered in this study were enrofloxacin, ciprofloxacin, difloxacin, and orbifloxacin. Enrofloxacin, difloxacin, and orbifloxacin are veterinary products used to treat dermal, respiratory and urinary infections in small animals. Ciprofloxacin, however, is a human drug used for similar infections. The human drug, ciprofloxacin, is often cited as effective against selected microorganisms in veterinary medicine, while the veterinary drug, enrofloxacin, is cited as resistant. As a result, veterinarians often use the human drug. Yet, the chemical structures of the drugs suggest that microorganisms should exhibit similar susceptibility patterns. This study hopes to prove an equal effectiveness of enrofloxacin and ciprofloxacin. Microbial susceptibility to drugs is measured by determining the MIC (Minimum Inhibitory Concentration). The MIC is measured based on the growth of the targeted bacteria in increasing concentrations of each drug following incubation under standard conditions. A method using microtiter plates containing broth was developed to measure concentrations of growth using colorimetric procedures. Comparisons in 100 bacterial organisms reveal subtle differences in the MIC's between the drugs (based on micromolar concentrations) showing relative effectiveness between the organisms. This study supports our belief that bacterial susceptibility does not differ among enrofloxacin and ciprofloxacin

available to the veterinary practitioner. This study also attempts to explain mechanisms of resistance in microorganisms found to be resistant to the four fluorinated quinolones. Using biochemical techniques, these organisms will then be used to analyze patterns of resistance on a cellular level with determination of why the organism is resistant. Searching for patters of resistance involves isolating the DNA, PCR reactions for amplification of the DNA, and bacterial transformation.

INTRODUCTION

Antimicrobials are compounds used to inhibit or kill microbes such as bacteria or fungi. Antimicrobial therapy began with the introduction of penicillin in clinical laboratories when Alexander Fleming discovered this first antibiotic in 1939. Since that time, many new antimicrobials have been identified or created and therefore, improved the lives of many. However, a new threat has emerged and affects even the new antimicrobials.

For centuries, the use of living matter to destroy infections and heal wounds has been used. However, it was not until after Louis Pasteur's observation of fungi's effect on an anthrax organism that the concept of living organisms used as therapy against other living organisms developed. Pasteur witnessed the antagonistic effect of other bacteria on anthrax. Paul Ehrlich, at the same time, coined the idea of selective toxicity. Through extensive research, he found that certain chemicals can have negative effects on some organisms and not others. Using bacteria and humans as an example, he found that some chemicals harm bacteria but not humans. Combining the two theories, Alexander Fleming, after doing research on *Penicillium notatum*, a common fungal contaminant of bacterial cultures, accidentally discovered a chemical product produced by the fungus that inhibited the growth of other bacteria. This product was then isolated and became what we know today as penicillin.

Penicillin is categorized as a beta-lactam antibiotic. Antibiotics are an antimicrobial compound secreted by one microbe that will inhibit another microbe. Penicillin inhibits the growth of the bacterial cell wall, which causes the cell to lyse, killing the bacterial cell. All cells in a colony are affected in the same manner and the

colony of bacteria in the patient is destroyed, thereby curing the patient of the infection. However, since each colony consists of millions of bacterial cells, there lies the threat of resistance developing in the bacteria through mutations. Mutations arise in the DNA of newly formed bacteria to encode for a number of different changes that may occur in the bacteria to create resistance. For example, in the case of penicillin, a mutation to create smaller porin channels through which the drug passes to enter the bacteria, may prevent the entry of penicillin and therefore, create resistance. Microorganisms may also develop beta-lactamases, enzymes which degrade the drug. The bacterium reproduces, and spreads its resistance to its offspring. It also can spread the genetic information to other bacterial organisms through plasmids. Soon, the entire colony becomes resistant to penicillin. The infection has now multiplied and drug therapy has failed. This often was the scenario after the second World War, when over-prescription of antibiotics increased the development of antimicrobial resistance. Because of the introduction of foreign diseases and infections and the convenience and safety of antibiotics, they were given excessively to patients suffering from bacterial infections. Unfortunately, they often were given in cases that seemed to be bacterial infections but were not. Obvious implications of this in appropriate use of antibiotics is the increased chance for bacterial mutations and therefore, increases risk of resistance. Once the bacteria become resistant, the drugs are ineffective. Therefore, since the creation of penicillin and the advent of bacterial resistance, the pharmaceutical industry has raced to find new, faster, and more effective drugs.

Antimicrobial drugs are classified by their chemical structure, source, or mode of action. Many are identified similar to penicillin, that is, as antibiotic compounds secreted

from microorganisms such as bacteria or fungi that inhibit the growth of other bacteria. Others reflect modification of this antibiotic (semisynthetic) and yet, others are synthesized in laboratories. They all largely affect crucial and pertinent functions of the bacterial cell, initiating biochemical and physiological changes. These functions include growth of the cell wall, replication, transcription and translation as well as many others. Penicillin and all beta-lactams, as stated earlier, functions by inhibiting the growth of the cell wall. Another group of antibiotics recently developed called the fluorinated quinolones inhibit a step in the replication process.

THE FLUORINATED QUINOLONE ANTIMICROBIAL

Introduction

Fluorinated quinolones were first introduced in the 1960's with the market of nalidixic acid, the first of the quinolones. All of these antibiotics have a four-quinolone ring structure and the same mode of action. The basic structure of the fluorinated quinolones can be found in Figure 1. Since the creation of nalidixic acid, new and more improved quinolones have been created through a series of structural modifications. Even though adverse effects of the drugs exist, such as nausea, abdominal discomfort, headache, and dizziness, they are rare and the antibiotics are commonly referred to as the "last of the golden antimicrobials" because of their safety and efficacy. These antibiotics are effective against both gram negative and select gram positive aerobic microorganisms, which covers a broad range of bacterial infections. Specifically, they are highly effective against gram-negative rods and coccobacilli, including *Pseudomona aeruginosa* and *Pasteurella species* organisms. Some gram-positive bacteria and acid-

Basic Structural Formula for Fluorinated Quinolones

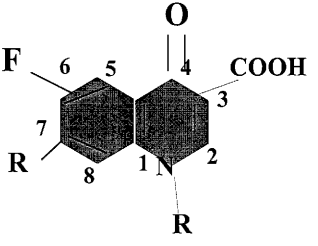


Figure 1: This figure represents the basic structure of the fluorinated quinolones. Fluorinated quinolones were first introduced in the 1960's with the market of nalidixic acid, the first of the quinolones. All of these antibiotics have a four-quinolone ring structure and the same mode of action. Since the creation of nalidixic acid, new and more improved quinolones have been created through a series of structural modifications. Even though adverse effects of the drugs exist, such as nausea, abdominal discomfort, headache, and dizziness, the antibiotics are commonly referred to as the "last of the golden antimicrobials" (Gilman at al, 1059). These antibiotics are effective against both gram negative and select gram positive aerobic microorganisms, which covers a broad range of bacterial infections (Ford, 14). Specifically, they are highly effective against gram-negative rods and coccobacilli, including Pseudomona aeruginosa and Pasteurella species organisms. Some gram-positive bacteria and acid-fast bacteria, such as Staphylococcus aureus and Mycobacteria species organisms, are also susceptible (Hooper, 1993). Fluorinate quinolones have offered clinicians orally and parenterally administrable compounds that cover a broad spectrum of bactericidal activity. The fluorinated quinolones are effective against a broad range of bacterial infections, including urinary tract infections, gastrointestinal infections, sexually transmitted diseases, respiratory tract infections, and osteomyelitis (Acar and Goldstein, 567). Another distinguishing characteristic of the quinolones is their ability to diffuse to the site of infection quickly and at large concentrations. Furthermore, the mechanism of action for the antibiotics is very unique.

fast bacteria, such as *Staphylococcus aureus* and *Mycobacteria species* organisms, are also susceptible. Fluorinated quinolones have provided clinicians drugs which can be administered orally and parenterally and which cover a broad spectrum of bactericidal activity. Another distinguishing characteristic of the quinolones is their ability to diffuse to the site of infection quickly and at large concentrations. The fluorinated quinolones are effective in a variety of body systems, including infections by susceptible bacteria in the urinary tract, gastrointestinal, reproductive tract (including sexually transmitted diseases), respiratory tract, and osteomyelitis.

Mechanism of Action

The mechanism of action for the antibiotics is very unique. To explain this, a look into the bacterial chromosome is needed. Bacterial DNA has a helical structure consisting of two strands. The two strands of DNA must separate for enzymes to replicate each strand and produce duplicate strands through a process called semi-conservative replication. A typical DNA strand in bacteria is circular and has a 1000-fold condensation within the cell. During replication, the circular DNA of bacteria replicates and transfers one copy to each daughter cell. Because of the 1000-fold condensation of DNA, called supercoiling, the chromosome must unwind for replication. Because of the circular structure of DNA, the DNA must be cleaved in order for unwinding to occur. The enzyme topoisomerase I is responsible for cleavage of the DNA strand allowing initiation of the replication process. Certain enzymes belonging to the topoisomerase I family cleave the DNA and separate the two strands. This, in turn, causes "overwinding" or excessive positive supercoiling around the point of separation. To relieve this mechanical obstacle, the topoisomerase II enzyme family introduces negative

superhelical twisting. Then, once replication is complete, the newly formed DNA strands must be reconnected and returned to its original structure also with the help of these enzymes. In particular, one type of topoisomerase II enzyme, DNA gyrase, induces negative supercoiling and decatenates DNA circles interlocked. DNA gyrase's mechanism of action can be followed in Figures 2 and 3. This is vital for replication procedures such as initiation, fork propagation, and termination. The enzyme is also used for transcription of certain operons and aspects of DNA repair, recombination and transposition. Important functions such as these are prime targets for antibiotic development.

In fact, DNA gyrase is a primary target of the fluorinated quinolones. Although most inhibitors of DNA replication bind to DNA and are too toxic to the host, fluorinated quinolones were developed to be safe and effective. Affinity of the host enzymes is $1/1000^{th}$ of that of bacterial enzymes, causing "selective toxicity" to the bacteria. They are claimed to be "exceptionally potent antibacterial agents" trapping DNA gyrase on DNA. They bind to the DNA gyrase enzyme and therefore, the enzyme is ineffective in resealing and winding the DNA after replication. Specifically, DNA gyrase has two gyrA subunits. It has been found through previous experimentation that the fluorinated quinolones bind to both of these subunits and inhibit the enzyme function. As a result, the chromosomal DNA remains fragmented and is eventually degraded by exonucleases. This bactericidal effect of fluorinated quinolones is effective in patients with impaired humoral or cellular immunity where bacteriostatic antibiotics are inadequate. A visual diagram of the mechanism of action of the fluorinated quinolones can be found in Figure 3. Fluorinated quinolones have also been found to decrease decatenation of interlocked

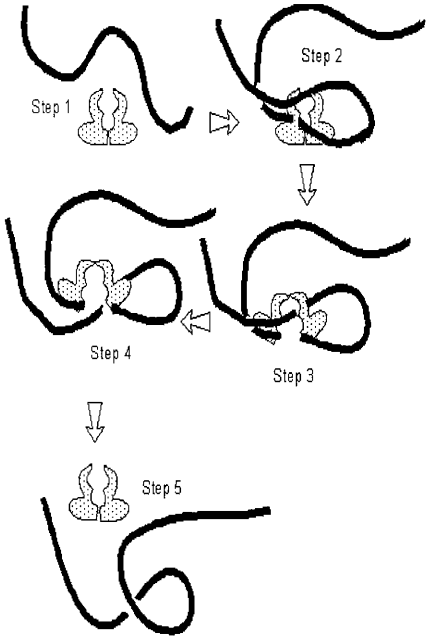


Figure 2: A visual diagram of the mechanism of action of DNA gyrase is found in this figure. A typical DNA strand in bacteria is circular and has a 1000-fold condensation within the cell. During replication, the circular DNA of bacteria replicates and transfers one copy to each daughter cell. However, because of the 1000-fold condensation, called supercoiling, the chromosome must unwind for replication. Bacterial DNA has a helical structure consisting of two strands. These two strands must separate for enzymes to replicate each strand and produce duplicate strands through a process called semiconservative replication (Lorian, 670). However, because of the circular structure of DNA, the DNA must be cleaved in order for unwinding to occur (Ford, 14). The enzyme topoisomerase I is responsible for cleavage of the DNA strand allowing initiation of the replication process. Certain enzymes belonging to the topoisomerase I family cleave the DNA and separate the two strands (Lorian). This, in turn, causes "overwinding" or excessive positive supercoiling around the point of separation. To relieve this mechanical obstacle, the topoisomerase II enzyme family introduces negative superhelical twisting. Then, once replication is complete, the newly formed DNA strands must be reconnected and returned to its original structure also with the help of these enzymes (Gilman et al, 1058). In particular, one type of topoisomerase II enzyme, DNA gyrase, induces negative supercoiling and decatenates DNA circles interlocked. This is vital for replication procedures such as initiation, fork propagation, and termination. The enzyme is also used for transcription of certain operons and aspects of DNA repair, recombination and transposition. Important functions such as these are prime targets for antibiotic development. This figure represents the function of DNA gyrase. The enzyme is shown as the grey objcet and the black line indicates the DNA strand.

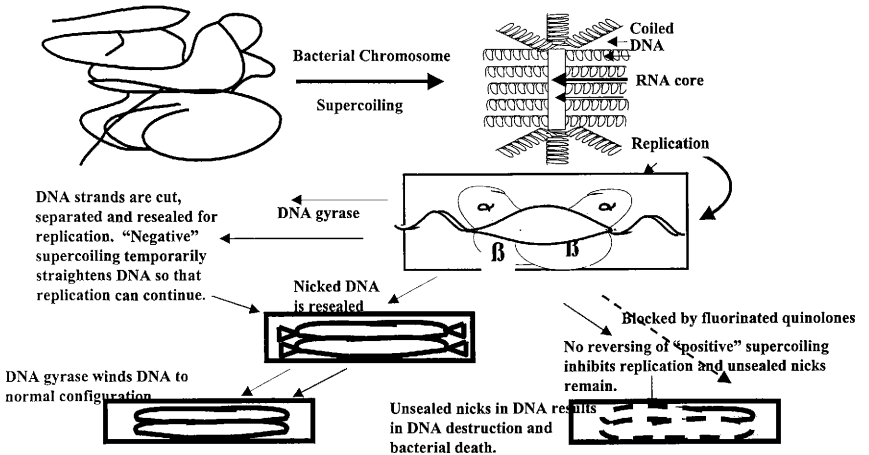


Figure 3: This figure illustrates the mechanism of action of the fluorinted quinolones. DNA gyrase is a primary target of the fluorinated quinolones. Although most inhibitors of DNA replication bind to DNA and are too toxic to the host, fluorinated quinolones were developed to be safe and effective (Schaechter et al, 43). They are claimed to be "exceptionally potent antibacterial agents" trapping DNA gyrase on DNA (Zhao, 13991). They bind to the DNA gyrase enzyme and therefore, the enzyme is ineffective in resealing and winding the DNA after replication. Specifically, DNA gyrase has two gyrA subunits (Gilman et al, 1058). It has been found through previous experimentation, that the fluorinated quinolones bind to both of these subunits and inhibit the enzyme function (Chi Truong, 85). As a result, the chromosomal DNA remains fragmented and is eventually degraded by exonucleases. This bactericidal effect of fluorinated quinolones is effective in patients with impaired humoral or cellular immunity where bacteriostatic antibiotics are inadequate (Ford 14). Fluorinated quinolones have also been found to decrease decatenation of interlocked DNA circles, impair segregation of replicated chromosomes into daughter cells, and interfere with transcription, DNA repair and recombination, in addition to inhibiting replication (Lorian, 670).

DNA circles, impair segregation of replicated chromosomes into daughter cells, and interfere with transcription, DNA repair and recombination, in addition to inhibiting replication.

Bacterial Resistance to Fluorinated Quinolones

Because resistance is a growing threat to fluorinated quinolones and other antibiotics, an insight into clinical cases of resistance may show how resistance patterns develop and propagate through bacterial populations. Only recently has previous investigations shown that resistance, although slow to develop, occurs through a number of ways in the fluorinated quinolones. First, a primary change in the chromosomal DNA sequence that codes for the subunits of DNA gyrase may occur. It has been discovered that if a double mutation in the chromosomal sequence that encodes for subunit gyrA occurs, resistance is conferred to the quinolone antibiotics. A different gyrA subunit may result in an inability of the antibiotics to bind to the enzyme, thereby reducing the antimicrobial effect. Another possible method of resistance within microorganisms is the increased efflux of the drug out of the cell. Once antibiotics accumulate in a bacterial cell and begin their effects, the bacteria defensively ejects the drug out of the cell. Yet, the amount of antibiotic rejected is usually not ample enough to stop the drug. However, a mutation in the regulatory genes that govern the bacterial permeability or efflux capacity can alter this amount of antibiotic emitted out of the cell. If an increase in the amount of drug ejected from the bacterial cell occurs, an increase in resistance is seen. Next, by reducing the levels of quinolone accumulation within the cell, the bacteria may also become resistant. This may be done, as discussed, by efflux of the drug out of the cell, or by degrading the antibiotic. A mutation may occur in the bacterial "immune"

system that allows the bacteria to detect and degrade foreign objects. This may lead to the detection and degradation of fluorinated quinolones and thus, reduce its amount in the bacteria.

Host factors and antimicrobial efficacy can be responsible for the effectiveness of fluorinated quinolones. First, the bioavailability of the drug will effect how must drug reaches the site of infection. Once a drug is administered orally, the drug is absorbed through the gastrointestinal tract. The amount of drug absorbed determines the level of bioavailability, which is the amount of drug at the site of infection. If large quantities of the drug are absorbed through the small intestinal mucosa, more of the drug can reach its destination. On the other hand, less absorption of the drug leads to lower efficacy. Host factors that determine the amount of drug absorption include environmental pH and temperature. These will also impact the site of infection. Because the pH at the site of infection is lower than other areas of the body and because these drugs are weak bases, the antibiotic may be altered (ionized) when introduced to the infection site. Also, the increased temperature that usually accommodates a bacterial infection may prove to have beneficial effects on the fluorinated quinolones. Inflammation at an infectious site usually means an increase in blood flow to the area, which would also increase the amount of drug flow to the area and therefore, immediate results. However, accumulating inflammatory debris and fibrous tissue may be deposited in abscesses and this may have detrimental effects. If the area of infection has become too inflammed, it would be more difficult for the drug to reach the infection and therefore, reduce drug penetration. Decreased blood flow may also decrease oxygen, rendering the site anaerobic and decreasing the effect of antimicrobials as well as the host immune system.

BACKGROUND

The four fluorinated quinolones of interest in this study were enrofloxacin, ciprofloxacin, orbifloxacin, and difloxacin. All of the antibiotics, with the exception of ciprofloxacin, are licensed to be used in veterinary clinics against susceptible microorganisms. Ciprofloxacin has been approved for usage in humans but not in animals. The author would like to acknowledge the supplier for these antibiotics, Bayer Corporations, Fort Dodge Animal Health, and Shering-Plough Animal Health.

Enrofloxacin is better known as Baytril® to the practicing veterinarian. Its chemical structure can be found in Figure 4. Enrofloxacin, as stated, is a veterinary drug that has been in used for over ten years.

The only human drug in our study was ciprofloxacin. Refer to the chemical structure in Figure 5. It was received in the form of a monohydrochloride monohydrate salt, ciprofloxacin hydrochloride, which is how it is supplied for in vitro diagnostic use. Its molecular weight is 385.8 grams and is highly water soluble at 3.5 g per 100 ml at the temperature of 25°C. Cipro® Diagnostic Powder was stored at the recommended temperature of below 30°C. Created solely for human purposes, it is not licensed to be used by veterinarians even though usage on animals does occur.

Similar to enrofloxacin, orbifloxacin and difloxacin are veterinary drugs. They are effective against animal bacterial infections. Their structures can be found in Figure 6. Unlike enrofloxacin and ciprofloxacin, these two antibiotics were recently developed and therefore, little attempt has been made to study them. Information concerning susceptibility patterns and effectiveness of the drugs is yet to be determined with the help of this study.

Enrofloxacin

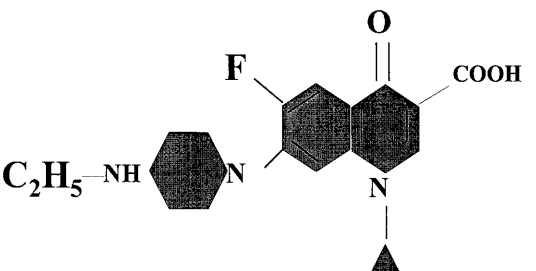


Figure 4: This is the chemical structure of enrofloxacin. Enrofloxacin is better known as Baytril® to the practicing veterinarian. Enrofloxacin, as stated, is a veterinary drug that has been used for over ten years now.

Ciprofloxacin

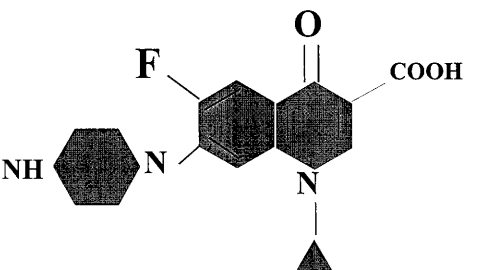


Figure 5: This figure visually shows the chemical structure of ciprofloxacin. The only human drug in our study was ciprofloxacin. It was received in the form of a monohydrochloride monohydrate salt, ciprofloxacin hydrochloride, which is how it is found for in vitro diagnostic use. Its molecular weight is 385.8 grams and is highly water soluble at 3.5 g per 100 ml at the temperature of 25°C. Cipro® Diagnostic Powder was stored at the recommended temperature of below 30°C (Package insert, 1998). Created solely for human purposes, it is not licensed to be used by veterinarians even though usage on animals does occur.

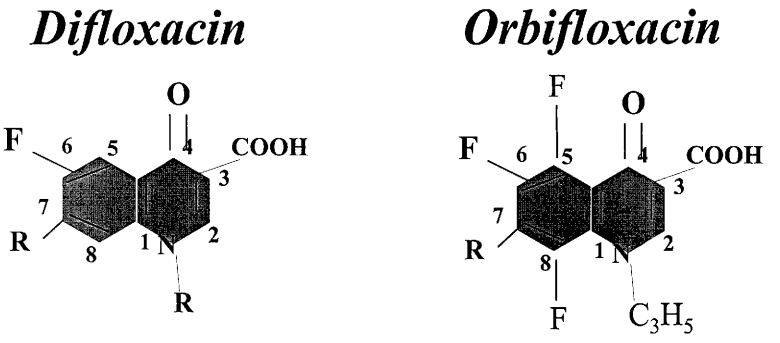


Figure 6: This figure illustrates the chemical structures of difloxacin and orbifloxacin together. Similar to enrofloxacin, orbifloxacin and difloxacin are veterinary drugs. They are effective against animal bacterial infections. Unlike enrofloxacin and ciprofloxacin, these two antibiotics were recently developed and therefore, little attempt has been made to study them. Information concerning susceptibility patterns and effectiveness of the drugs is yet to be determined with the help of this study.

The effectiveness of drugs can be compared based on susceptibility patterns. Susceptibility patterns help show how drugs are effective against bacteria with varying concentrations of drug. A test typically used for susceptibility pattern determination is the tube or plate dilution method. This test provides a quantitative comparison of antimicrobials relating the amount of drug necessary to inhibit microbial growth. The quantifiable number is called the minimum inhibitory concentrations (MIC) and is unique for each drug against each microorganism. The MIC is the lowest concentration of drug needed to inhibit visible growth of the organism. It can be determined by first, creating tube or plate dilutions of the drug in serially decreasing concentrations. Then, an equal and standard number of organism is inoculated in the tubes or the plate. After a standard incubation period, the tube or plate well with the lowest concentration and no visible growth is determined to be the MIC. For example, assume an infection with *Pseudomonas spp*. A tissue sample collected from the site of infection will be sent to the lab and the organism will be isolated. It will be exposed to increasing concentrations of several drugs. If the lab studies find that the MIC of enrofloxacin is 0.5 μg/ml, then this is the concentration at which no observable growth is seen in the laboratory environment. This, then would be the concentration of enrofloxacin to achieve in tissues that are infected. If the concentration of drug at the site is lower, at 0.25 μg/ml for instance, then bacterial growth will not be inhibited at the site of infection. In the test tube containing 25 μg/ml, growth of the organism will be seen. In contrast, if the concentration of the drug is higher, at 1 μg/ml, then bacterial growth will not be seen. The MIC value has practical purposes for the veterinarian. It allows the clinician to identify at which concentration the drug is effective in vitro, and this can be used as an estimation for

administratable doses. Note that the MIC only indicates the point at which the bacterial growth is inhibited. The minimum bactericidal concentration (MBP) is the minimum point at which the drug not only inhibits but also kills all of the bacteria. It can be determined by growing the tubes or plate wells with no visible signs of bacterial growth on agar plates. The plate with the smallest concentration that yielded no growth when inoculated on a blood agar plate contains the MBC.

From the MIC data, an applicable drug concentration can be calculated by comparing the MIC value to the breakpoint MIC value. The breakpoint MIC is the concentration reached safely within the patient after administration of the recommended dosing regimen. This value is unique to each host and drug but is not unique to each organism. In other words, a breakpoint MIC of 2 μg/ml for enrofloxacin in cats holds true for all organisms and all cats administered enrofloxacin. Every time the cat is given the recommended dosing regimen, it will achieve a 2 μg/ml concentration of enrofloxacin in vivo. For an organism to be considered susceptible to a drug, its MIC ideally is less than the breakpoint MIC. For example, the MIC of enrofloxacin against a certain strain of *Pseudomonas aeruginosa* in a dog may equal 0.5 μg/ml. The breakpoint MIC for enrofloxacin in a dog is 2 μg/ml, meaning that the recommended dose when administered to the patient will achieve 2 μg/ml. This is four times greater than the MIC of the organism causing infection, and thus, enrofloxacin should be effective against this organism. Therefore, veterinarians, by knowing the breakpoint MIC and the MIC of the bacteria, can conclude of how effective a certain antimicrobial is against a specific bacterial strain can be seen.

Breakpoint MIC values are crucial in determining susceptibility patterns in antimicrobials by comparing the values to MIC values. Susceptibility patterns refer to the organism's response to a specific drug concentration. This concentration is the MIC. If the MIC of an antimicrobial is less than the breakpoint MIC, then the bacteria is labeled as susceptible or S. In this case, the drug was effective. The bacteria is categorized as resistant or R if the MIC of the infecting organism is greater than the breakpoint MIC. This would result in the organism surviving even after the recommended amount of drug is given. The call for a new drug would be in order in these cases. Breakpoint MIC values are literature values taken from the Physicians Desk Reference, but can also be found in professionally fixed labels or the laboratory providing the microbiological data.

This experiment attempts to compare the efficacy of the four fluorinated quinolones mentioned: enrofloxacin, ciprofloxacin, orbifloxacin, and difloxacin. MIC values from various clinical bacterial cases were determined for a broad range of microorganisms and then compared to determined efficacy patterns.

HYPOTHESIS AND OBJECTIVES

The objectives for the experiment are three-fold. First, MIC ranges will be determined for each microorganism and each drug. These will be found by determining the highest and lowest MIC values and organizing them into a range. Furthermore, MIC_{90}'s (the MIC at which 90% of the organism are inhibited) of selected bacteria causing infection in veterinary patients for ciprofloxacin, enrofloxacin, orbifloxacin, and difloxacin will be determined. The selected bacteria for which MIC_{90} values are found are those in which ten or more different species were collected. Next, a comparison of the MICs of infecting bacteria for all four fluorinated quinolones will be made. This will be done by determining the proportions of organisms that are resistant versus susceptible to each drug. Finally, since the organisms were received from clinical cases across the nation, a demographic comparison will be made. Consideration will be given to where the organisms were collected from, the type of host, and the bacterial infection from which the organisms were found in.

As evident in the chemical structures, enrofloxacin and ciprofloxacin are very similar. Both antibiotics exhibit similarities in function, mechanism, and structure, with the only structural difference being an extra methyl group on enrofloxacin. Therefore, because of the similarities between the two drugs, our hypothesis is that both drugs are equally effective. We suspect they will exhibit similar susceptibility patterns. However, our expanded hypothesis is that resistance patterns will also be similar across all the fluorinated quinolones.

MATERIALS AND METHODS

Since this project works closely with infectious bacteria, a strict procedure of sterile laboratory technique must be closely followed. A procedure involving utmost care and management of the microorganisms was developed considering the guidelines set forth by the National Committee on Clinical Laboratory Standards (NCCLS). These guidelines were strictly followed and incorporated into every aspect of the procedure. In addition, aseptic technique was prescribed for techniques not only directly dealing with the organisms, but also with the rest of the procedure. All equipment used was autoclaved to achieve sterility. The procedure spans a number of days since isolation and growth of bacterial colonies requires multiple days. Furthermore, to ensure accuracy, drug solutions must be used the same day they are created. This would assure accurate readings because the drugs are prevented from reacting with the media they are prepared in over time and are unable to lose efficacy. Because consistency is very important, each drug was created in the same manner and treated equally. In fact, because the procedure was repeated numerous times, the procedure was conducted uniformly, rigorously adhering to the procedure carefully.

Upon agreement with Antech Diagnostics and Antech East Diagnostics, Inc. located in California and New York, respectively, bacterial organisms were received in transportable culturette tubes (BBL Culturette Systems®), via Airborne Express next day delivery service. Culturettes are standard transporting mechanisms for bacteria colonies. The device has a cotton swab that is used to pick up a bacterial colony. The swab is place back into the culturette device and the yellow end containing bacteria supporting media is crushed to release the growing media for the bacteria. The bacterial cultures are able to

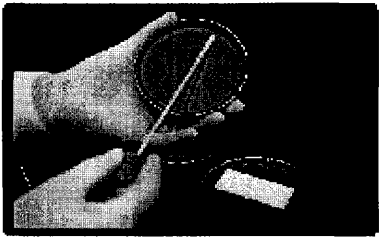


Figure 7: This figure illustrates a culturette tube, a blood agar plate, and the technique for streaking. Culturettes are standard transporting mechanisms for bacteria colonies. The device has a cotton swab that is used to pick up a bacterial colony. The swab is place back into the culturette device and the yellow end containing bacteria supporting media is crushed to release the growing media for the bacteria. The bacterial cultures are able to survive in the tubes for at most, three to four days without inducing harmful effects to the bacteria. Once the culturettes arrived, they were checked to see if the end was crushed and the media released. Once this was confirmed, they were streaked for isolation on (SIZE) blood agar plates. Blood agar plates are used to grow a broad range of gram negative and gram positive bacteria. It is partially made of sheep blood to provide extra nutrients for hard-to-grow and slow-growing bacteria.
With these blood agar plates, the organisms are streaked for isolation. The cotton swab of the culturette is removed and streaked, in a smooth, even manner, across half of the plate. Then, the swab is cut and placed in a tryptose agar bath. An inflammed loop is used to isolate the colonies by streaking through the already streaked portion of the plate and continuing to spread the bacteria onto the unstreaked part of the plate. Only half of the unstreaked portion of the plate is used, essentially only using one quarter of the plate. The loop is then placed in the flame again to sterilize it. Then, the rest of the plate is streaked with the same procedure as before, to isolate individual colonies. The plate is then placed in an incubator of temperature 37° C for 24 hours. The cotton swabs are placed in tryptose agar tubes to allow for sustained growth on the swab in case no growth is seen on the plates. They are also incubated at the same temperature for 24 hours.

survive in the tubes for at most, three to four days without inducing harmful effects to the bacteria. Once the culturettes arrived, they were checked to see if the end was crushed and the media released. Such organisms were not studied because of the risk of bacterial contamination. Organisms were streaked for isolation on (SIZE) blood agar plates. Blood agar plates are used to grow a broad range of gram negative and gram positive bacteria. The agar contains sheep blood to provide extra nutrients for hard-to-grow and slow-growing bacteria.

With these blood agar plates, the organisms are streaked for isolation. Figure 7 illustrates this concept. The cotton swab of the culturette is removed and streaked, in a smooth, even manner, across half of the plate. Then, the swab is cut and placed in a tryptose agar bath. An inflamed loop is used to isolate the colonies by streaking through the already streaked portion of the plate and continuing to spread the bacteria onto the unstreaked part of the plate. Only half of the unstreaked portion of the plate is used, essentially only using one quarter of the plate. The loop is then placed in the flame again to sterilize it. Then, the rest of the plate is streaked with the same procedure as before, to isolate individual colonies. The plate is then placed in an incubator of temperature 37° C for 24 hours. The cotton swabs are placed in tryptose agar tubes to allow for sustained growth on the swab in case no growth is seen on the plates. They are also incubated at the same temperature for 24 hours.

The next day, stock solutions of the four fluorinated quinolones are made using sterile glassware and aseptic techniques. First, five milligrams of each drug was accurately weighed using an analytical balance and dissolved in ten milliliters of methanol to give a 500 μg/ml stock solution, labeled stock solution A for discussion

purposes. Each of the drugs is dissolved in this same manner to ensure accurate readings. Once the drugs are dissolved, a 100 μg/ml stock solution (stock solution B) is made using Mueller-Henton broth. Mueller-Henton was pre-made beforehand using a powdered stock The broth is a liquid medium for bacterial growth. The equation used to determine how to prepare the 500 μg/ml stock solution is:

Equation 1 $C_1 * V_1 = C_2 * V_2$

where C_1 equals the initial concentration of stock solution A (500 μg/ml), V_1 is the unknown volume to be taken from stock solution A (X), C_2 is the desired concentration in stock solution B (100 μg/ml), and V_2 is the desired volume of Mueller-Henton broth stock solution B (5 mls). Using the formula, one milliliter of the methanol stock solution A is taken and added to four milliliters of Mueller-Henton broth. This results in a 1:5 dilution of stock solution A. Five milliliters were created to ensure enough of the stock to produce multiple dilutions. Once this solution is mixed, the initial tube dilution is made. Using the same formula, it was determined that 640 μL of stock solution B added to 1360 μL of Mueller-Henton broth would produce the first desired dilution concentration, which was 32 μg/ml. From this initial dilution, subsequent dilutions decreased by one-half. This meant that to produce the next dilution of 16 μg/ml, one milliliter of the first dilution was taken and added to one milliliter of Mueller-Henton broth. Continuing this process nine times results in all dilutions for one drug. The same procedure is used for all four fluorinated quinolones.

Once the dilutions have been made, the sterile microtiter plate (Nunc® V96 Polypropylene plate) can be created. Refer to Figure 9 for a diagram of the plate. The microtiter plate contains 96 wells, each capable of holding 150 μl. With one plate per

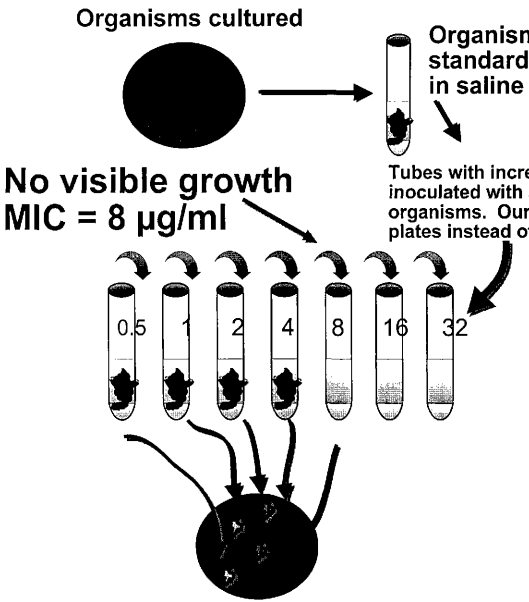


Figure 8: A test typically used for susceptibility pattern determination is the tube or plate dilution method (Boothe 1998). This test provides a quantitative comparison of antimicrobials relating the amount of drug necessary to inhibit microbial growth. The quantifiable number is called the minimum inhibitory concentrations (MIC) and is unique for each drug against each microorganism. The MIC is the lowest concentration of drug needed to inhibit visible growth of the organism (Boothe, 1998). The MIC value has practical purposes for the veterinarian. It allows the clinician to see at which concentration the drug is effective in vitro, which can be used as an estimation for administratable doses. It can be determined by first, creating tube or plate dilutions of the drug in serially decreasing concentrations. Then, an equal and standard number of organism is inoculated in the tubes or the plate. After a standard incubation period, the tube or plate well with the lowest concentration and no visible growth is determined to be the MIC.

drug, four plates are created. Each dilution is placed horizontally across the plate, beginning with the 32 μg/ml concentration and ending with the 0.0625 μg/ml concentration. Every row is filled horizontally in this manner. The last column is for the negative blank. In these well, 120 μl of Mueller-Henton broth is pippetted. The last row will contain the positive blank.

For preparation of the microorganism, the streaked plates are removed from the incubator. One isolated colony is removed and a standard solution is made to mediate the same number of organisms for each plate. This standard is made by inoculating the colony in two milliliters of saline solution using a cotton swab. The standard is then checked for a standard 0.5 Macfarland turbidity. The Macfarland system is used to characterize turbidity in bacterial suspensions. The more cloudy the suspension, the more the bacterial colonies present and the greater the Macfarland unit. The lower the unit indicates a clearer solution and a lower number of bacterial colonies. A 0.5 Marfarland turbidity was used because standard MIC determining procedures require this standard. For all the organisms to be tested for MIC determination, a 0.5 Macfarland saline standard was made. Then, again according to standard MIC determining procedures, one milliliter of this solution was diluted in 24 milliliters of sterilized water. This uniformly suspended 1:24 dilution was then added to the microtiter plate in equal quantities as the drug. Therefore, 120 μl of this solution was added to each well horizontally. Each row contained a different microorganism, using seven rows. In the final row, plain Mueller-Henton broth was added to create the positive control. This control was tested for no growth since only the drug and the broth were added. The negative control, however,

was used to indicate growth of the microorganisms as it contained only the broth and the organism.

Once the plate is created, they are covered with parafilm to avoid evaporation of the contents in the heated incubator. The plates are then placed in the same incubator used before to grow the microorganisms, at 37°C and for approximately 24 hours. After the incubation time, the plates were removed and read by visually observing the MIC in the microtiter plate using a mirror. Once the parafilm is removed, the plates were placed on a stand with a positioned mirror at the base. By looking at the mirror, readings were taken from observing below the plate. The recorded value of MIC was the well with the lowest concentration of no visible growth. These MIC values will be compared according to the genus and species of the microorganism. The percentage of resistant organisms will be found and comparisons on the types of hosts and geographical place of origin of the infections will be made.

RESULTS

The results are categorized into different tables. The first table simply shows the MIC values found for each organism to each fluorinated quinolone. The number of organisms tested was 63, all of which were either gram negative (n = 46) or gram positive (n = 17). The different organisms were *Escherichia coli* (n = 13), *Proteus mirabolis* (n = 6), *Pseudomonas species* (n = 16), *Staphylococcus species* (n = 8), *Streptococcus species* (n = 9) and miscellaneous isolates consisting of *Klebsiella pneumoniae, Actinomyces, pyogenes, Bacillus sp., Enterobacter sp., Bordetella bronchiseptica, Pasteurella multocida*, and *Serratia sp.* (n = 11). Also, of the 63 microorganisms collected and studied, most were collected from dogs (n = 43), some from cats (n = 7), some from birds (n = 6), and some from horses (n = 4). Sites of location of bacterial infection can also be compared using Table 1. Bacterial organisms were taken from urinary tract infections (n = 18), from ear infections (n = 9), from nasal infections (n = 4), and from other infections, such as tracheal, joint, or hip (n = 8) with the rest unindicated. The geographical locations of the bacterial infections can also be compared. More microorganisms were collected from the western half of the United States (n = 8) than from the eastern half (n = 21). Some were collected from either the Bryan/College Station community or the Houston community (n = 8). The rest of the organisms were not indicated geographically as to where the sample was taken. The susceptibility patterns were determined by comparing the experimentally determined MIC value with the breakpoint MIC for each antibiotic. If the experimental value is higher than the breakpoint value, the organism is categorized with an R for resistance. An S is designated for organisms that have experimental MICs lower than the breakpoint MIC.

Designations of S and R are given to the MIC values in Table 2. Table 3 shows the MIC ranges of the bacteria and MIC_{90} values of select bacteria. The MIC_{90} of Escherichia coli for ciprofloxacin was 0.0625, $\leq$0.031 for enrofloxacin, 0.25 for difloxacin and 0.125 for orbifloxacin. Table 4 shows the percent of resistant organisms overall and for each antimicrobial.

Table 1: Microorganism MIC results with the four fluorinated quinolones.

Genus	species	Origin	Animal	Location	Results (µg/ml): Enro	Cipro	Orbi	Di
Enterobacter	sp.	College Station, TX	Avian	Nasal	<0.0625	<0.0625	0.125	
Bordetella	bronchiseptica	College Station, TX	Canine	Tracheal	<0.0625	<0.0625	<0.0625	
Klebsiella	pneumoniae	College Station, TX	Equine	Joint	0.0625	<0.0625	<0.0625	
Klebsiella	pneumoniae	College Station, TX	Avian	Nasal	0.0625	<0.0625	<0.0625	
Actinomyces	pyogenes	College Station, TX	Canine	Nasal	<0.0625	<0.0625	0.0625	
Bacillus	sp.	Southern CA	Canine	N/A	0.03125	0.0625	0.125	0.25
Enterobacter	aerogenes	Fayetteville, GA	Canine	Colon	<0.03125	0.03125	0.0625	0.03125
Escherichia	coli	N/A	Feline	Urine	<0.03125	<0.03125	<0.03125	<0.03125
Escherichia	coli	N/A	Canine	Urine	<0.03125	<0.03125	<0.03125	<0.03125
Escherichia	coli	N/A	Canine	Urine	<0.03125	<0.03125	<0.03125	<0.03125
Escherichia	coli	N/A	Canine	Feces	<0.03125	<0.03125	<0.03125	<0.03125
Escherichia	coli	California	Avian	Feces	<0.03125	<0.03125	<0.03125	<0.03125
Escherichia	coli	California	Canine	Urine	<0.03125	<0.03125	<0.03125	<0.03125
Escherichia	coli	California	Canine	Urine	<0.03125	<0.03125	0.03125	0.0625
Escherichia	coli	Fayetteville, GA	Canine	Colon	0.03125	0.03125	0.03125	0.125
Escherichia	coli	Marietta,GA	Canine	Urine	<0.03125	<0.03125	0.03125	0.0625
Escherichia	coli	N.Charles-ton, SC	N/A	Mandibular pin	0.0625	0.0625	0.125	0.25
Escherichia	coli	Frazer, PA	Canine	Hip	<0.03125	<0.03125	<0.03125	<0.03125
Escherichia	coli	College Station, TX	Canine	Prostate	4	4	>16	>16
Escherichia	coli	Reisters-town, MD	Canine	N/A	0.03125	<0.03125	0.125	0.0625
Klebsiella	pneumoniae	Sante Fe, NM	Canine	Urine	0.5	0.0625	1.0	2.0
Klebsiella	sp.	N/A	Avian	N/A	0.0625	0.03125	0.5	0.125
Pasteurella	multocida	N/A	Feline	Nasal	0.0625	0.0625	<0.03125	<0.03125
Proteus	mirabolis	N/A	Canine	Feces	0.03125	<0.03125	0.25	0.25
Proteus	mirabilis	California	Canine	Ear	0.015625	0.015625	0.0625	0.0625
Proteus	mirabilis	NY	Canine	Ear	0.25	0.25	1	0.5
Proteus	mirabolis	Fayetteville, NC	Canine	N/A	<0.03125	<0.03125	0.03125	<0.03125
Proteus	mirabolis	Brooklyn, NY	Canine	Ear	0.015625	0.015625	0.03125	0.03125
Proteus	mirabolis	Southhold, NY	Canine	Tracheal	<0.03125	<0.03125	<0.03125	<0.03125
Pseudomonas	aeruginosa	N/A	Canine	Ear	0.125	<0.03125	0.5	0.125
Pseudomonas	aeruginosa	Maiyaro-neck, NY	Canine	Ear	<0.03125	<0.03125	0.125	0.0625
Pseudomonas	aeruginosa	Fayetteville, GA	Canine	N/A	<0.03125	<0.03125	0.125	0.0625
Pseudomonas	aeruginosa	Brooklyn, NY	Feline	Ear	0.125	0.125	0.5	0.25
Pseudomonas	aeruginosa	Garden City, NY	Canine	Vaginal	<0.03125	<0.03125	<0.03125	<0.03125
Pseudomonas	aeruginosa	Houston, TX	Avian	N/A	0.015625	0.015625	0.03125	0.015625
Pseudomonas	aeruginosa	N/A	Canine	N/A	0.0625	0.125	0.25	0.25
Pseudomonas	aeruginosa	N/A	Canine	N/A	0.25	0.125	0.5	2
Pseudomonas	sp.	N/A	Feline	N/A	0.125	0.0625	0.25	1
Pseudomonas	sp.	N/A	Canine	N/A	0.125	0.0625	0.25	1
Pseudomonas	sp.	N/A	Canine	N/A	0.5	0.5	1	4
Pseudomonas	sp.	N/A	Canine	N/A	0.125	0.125	0.25	1
Pseudomonas	sp.	N/A	Canine	N/A	0.125	0.0625	0.25	1
Pseudomonas	sp.	N/A	Canine	N/A	0.125	0.0625	0.25	0.5
Pseudomonas	sp.	N/A	Canine	N/A	0.125	0.0625	0.25	1

Genus	species	Origin	Animal	Location	Results (µg/ml): Enro	Cipro	Orbi	Di
Pseudomonas	aeruginosa	St. Paul, MN	Canine	Urine	I	1	4	2
Serratia	sp.	Greenwich village, NY	Feline	Alveoli	<0.03125	<0.03125	<0.03125	<0.03125
Staphylococcus	intermedius	N/A	Canine	Ear	0.0625	0.125	0.25	0.125
Staphylococcus	aureus	California	Avian	N/A	0.125	0.125	0.25	0.25
Staphylococcus	intermedius	California	Canine	Vaginal	0.03125	0.03125	0.5	0.5
Staphylococcus	intermedius	Marietta, GA	Canine	Ear	<0.03125	0.03125	0.25	0.25
Staphylococcus	intermedius	Glenn Dale, MD	Feline	Tympanic bullae	0.03125	0.03125	0.125	0.25
Staphylococcus	intermedius	Lebanon, NJ	Canine	Osteomyelitis of femur	<0.03125	<0.03125	<0.03125	<0.03125
Staphylococcus	sp.	N/A	Avian	N/A	8	8	>16	>16
Staphylococcus	sp.	N/A	Avian	N/A	1	1	1	0.5
Streptococcus	sp.	N/A	Canine	Urine	0.125	0.5	1.0	0.25
Streptococcus (Beta)	sp.	Fayetteville, GA	Canine	N/A	<0.03125	<0.03125	<0.03125	<0.03125
Streptococcus (Beta)	sp.	N/A	Canine	N/A	0.03125	<0.03125	0.25	0.0625
Streptococcus (Beta)	sp.	N/A	Canine	Ear	0.125	0.125	0.5	0.25
Streptococcus	equi	Albequerque NM	Equine	Uterine	<0.03125	<0.03125	<0.03125	<0.03125
Streptococcus	equi	Central TX	Equine	Uterine	<0.03125	<0.03125	0.03125	0.0625
Streptococcus	sp.	N/A	Canine	N/A	0.5	1	2	2
Streptococcus (Gamma)	sp.	Camden, SC	Equine	N/A	0.03125	0.0625	0.5	0.25
Streptococcus (Gamma)	sp.	Madison, AL	Feline	N/A	0.0625	0.0625	0.125	0.5

Table 2: The in vitro susceptibility patterns of microorganisms.

GENUS	SPECIES	Results (μg/ml): Enro	Cipro	Orbi	Di
BACILLUS	SPECIES	0.03125 S	0.0625 S	0.125 S	0.25 S
ENTEROBACTER	AEROGENES	<0.03125 S	0.03125 S	0.0625 S	0.03125 S
ESCHERICHIA	COLI	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
ESCHERICHIA	COLI	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
ESCHERICHIA	COLI	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
ESCHERICHIA	COLI	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
ESCHERICHIA	COLI	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
ESCHERICHIA	COLI	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
ESCHERICHIA	COLI	<0.03125 S	<0.03125 S	0.03125 S	0.0625 S
ESCHERICHIA	COLI	0.03125 S	0.03125 S	0.03125 S	0.125 S
ESCHERICHIA	COLI	<0.03125 S	<0.03125 S	0.03125 S	0.0625 S
ESCHERICHIA	COLI	0.0625 S	0.0625 S	0.125 S	0.25 S
ESCHERICHIA	COLI	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
ESCHERICHIA	COLI	4 R	4 R	>16 R	>16 R
ESHERICHIA	COLI	0.03125 S	<0.03125 S	0.125 S	0.0625 S
KLEBSIELLA	PNEUMONIAE	0.5 S	0.0625 S	1.0 S	2.0 S
KLEBSIELLA		0.0625 S	0.03125 S	0.5 S	0.125 S
PASTEURELLA	MULTOCIDA	0.0625 S	0.0625 S	<0.03125 S	<0.03125 S
PROTEUS	MIRABOLIS	0.03125 S	<0.03125 S	0.25 S	0.25 S
PROTEUS	MIRABILIS	<0.03125 S	<0.03125 S	0.0625 S	0.0625 S
PROTEUS	MIRABILIS	0.25 S	0.25 S	1 S	0.5 S
PROTEUS	MIRABOLIS	<0.03125 S	<0.03125 S	0.03125 S	<0.03125 S
PROTEUS	MIRABOLIS	0.03125 S	0.03125 S	<0.03125 S	<0.03125 S
PROTEUS	MIRABOLIS	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
PSEUDOMONAS	AERUGINOSA	0.125 S	<0.03125 S	0.5 S	0.125 S
PSEUDOMONAS	AERUGINOSA	<0.03125 S	<0.03125 S	0.125 S	0.0625 S
PSEUDOMONAS	AERUGINOSA	<0.03125 S	<0.03125 S	0.125 S	0.0625 S

GENUS	SPECIES	Results (µg/ml): Enro	Cipro	Orbi	Di
PSEUDOMONAS	AERUGINOSA	0.125 S	0.125 S	0.5 S	0.25 S
PSEUDOMONAS	AERUGINOSA	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
PSUEDOMONAS	AERUGINOSA	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
PSUEDOMONAS	AERUGINOSA	0.0625 S	0.125 S	0.25 S	0.25 S
PSUEDOMONAS	AERUGINOSA	0.25 S	0.125 S	0.5 S	2 S
PSUEDOMONAS	SP	0.125 S	0.0625 S	0.25 S	1 S
PSUEDOMONAS	SP	0.125 S	0.0625 S	0.25 S	1 S
PSUEDOMONAS	SP	0.5 S	0.5 S	1 S	4 R
PSUEDOMONAS	SP	0.125 S	0.125 S	0.25 S	1 S
PSUEDOMONAS	SP	0.125 S	0.0625 S	0.25 S	1 S
PSUEDOMONAS	SP	0.125 S	0.0625 S	0.25 S	0.5 S
PSUEDOMONAS	SP	0.125 S	0.0625 S	0.25 S	1 S
PSUEDOMONAS	AERUGINOSA	1 S	1 S	4 R	2 S
SERRATIA	SP.	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
STAPHYLOCOCCUS	INTERMEDIUS	0.0625 S	0.125 S	0.25 S	0.125 S
STAPHYLOCOCCUS	AUREUS	0.125 S	0.125 S	0.25 S	0.25 S
STAPHYLOCOCCUS	INTERMEDIUS	0.03125 S	0.03125 S	0.5 S	0.5 S
STAPHYLOCOCCUS	INTERMEDIUS	<0.03125 S	0.03125 S	0.25 S	0.25 S
STAPHYLOCOCCUS	INTERMEDIUS	0.03125 S	0.03125 S	0.125 S	0.25 S
STAPHYLOCOCCUS	INTERMEDIUS	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
STAPHYLOCOCCUS		8 R	8 R	>16 R	>16 R
STAPHYLOCOCCUS		1 S	1 S	1 S	0.5 S
STREP.	SPECIES	0.125 S	0.5 S	1 S	0.25 S
STREP. (BETA)		<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
STREP. (BETA)		0.03125 S	<0.03125 S	0.125 S	0.0625 S
STREP. (BETA-HEMOLYTIC)	SPEC.	0.125 S	0.125 S	0.5 S	0.25 S

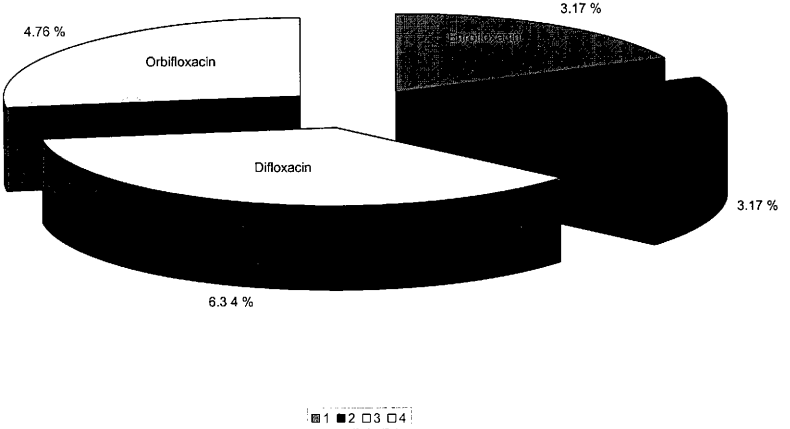
GENUS	*SPECIES*	Results (µg/ml): Enro	Cipro	Orbi	Di
STREPTOCOCCUS	*EQUI*	<0.03125 S	<0.03125 S	<0.03125 S	<0.03125 S
STREPTOCOCCUS	*EQUI*	<0.03125 S	<0.03125 S	0.03125 S	0.0625 S
STREPTOCOCCUS		0.5 S	1 S	2 R	2 S
STREPTOCOCCUS (GAMMA)		0.03125 S	0.0625 S	0.5 S	0.25 S
STREPTOCOCCUS (GAMMA)		0.0625 S	0.0625 S	0.125 S	0.5 S

Table 3: MIC ranges and MIC_{90} determinations of the microorganisms studied.

Staphylococcus spp	(9) ≤0.031-8	(9) ≤0.031-8	(9) ≤0.031->16	(9) ≤0.031->16
Staphylococcus aureus	(1) 0.125	(1) 0.125	(1) 0.25	(1) 0.25
Staphylococcus intermedius	(5) ≤0.031-0.125	(5) ≤0.031-0.0625	(5) ≤0.031-0.5	(5) ≤0.031-2
Streptococcus spp.	(9) ≤0.031-1	(9) ≤0.031-0.5	(9) ≤0.031-0.5	(9) ≤0.031-2
Escherichia coli	(13) ≤0.031-4 0.0625 *	(13) ≤0.031-4 ≤0.031 *	(13) ≤0.031->16 0.25 *	(13) ≤0.031->16 0.125 *
Klebsiella spp.	(2) 0.031-0.0625	(2) 0.0625-0.5	(2) 0.125-2	(2) 0.5-1
Proteus spp	(6) ≤0.031-0.25	(6) ≤0.031-0.25	(6) ≤0.031-0.5	(6) ≤0.031-1
Enterobacter spp	(1) ≤0.031	(1) ≤0.031	(1) 0.031	(1) 0.125
Bacillus spp.	(1) 0.0625	(1) 0.031	(1) 0.25	(1) 0.125
Pseudomonas spp.	(20) ≤0.031-1 0.5 *	(20) ≤0.031-1 0.5 *	(20) ≤0.031-4 2 *	(20) ≤0.031-4 0.5 *
Pseudomonas aeruginosa	(9) ≤0.031-0.125 0.125 *	(9) ≤0.031-0.25 0.125 *	(9) ≤0.031-2 0.25 *	(9) ≤0.031-0.5 0.5 *
Pasteurella multocida	(1) 0.0625	(1) 0.0625	(1) ≤0.031	(1) ≤0.031
Serratia spp.	(1) ≤0.031	(1) ≤0.031	(1) ≤0.031	(1) ≤0.031
MIC Breakpoint (μg/ml)[2]	≤ 2 μg/ml	≤ 2 μg/ml	≤ 2 μg/ml	≤ 4 μg/ml

MIC_{90} values are indicated with a *. The number in parenthesis indicates the number of isolates for each organism.

Table 4: Patterns of resistance in bacteria against the fluorinated quinolones



DISCUSSION

Our results showed data did not differ among the drugs, as was expected. The MIC determinations of ciprofloxacin and enrofloxacin showed no significant differences. Therefore, we can conclude, similarly to our hypothesis, that because enrofloxacin and ciprofloxacin are very similar in structure, they are also similar in susceptibility patterns. Since both drugs showed the same susceptibility patterns, we can conclude that enrofloxacin can and should be used by veterinarians. Because enrofloxacin is safer for small animals, our studies indicate that enrofloxacin remains the better choice for bacterial infections in veterinary cases. As seen in Table 4, our percent of resistant organisms show that susceptibility patterns among the organisms studied did not vary from ciprofloxacin and enrofloxacin. Susceptibility, however, generally decreased for orbifloxacin and difloxacin compared to ciprofloxacin and enrofloxacin. This suggests that based on this in vitro data, enrofloxacin remains the best of the three veterinary fluorinated quinolones.

The range of MIC's and some MIC_{90}'s of each antibiotic was developed for comparative purposes and is shown in Table 6. Comparing these two important characteristics is a thorough way of organizing and comparing efficacy among bacterial populations. Although only relevant to clinical cases if the organism is generally always resistant, our data shows that no organism is more resistant than the other. Our procedure using a microtiter plate proved to be very effective in determining MIC. The plate was clear in showing visible growth and minimum concentrations such as the plate in Figure 9.

Because our results show that an insignificant number of bacteria have different MIC's for ciprofloxacin and enrofloxacin, we can conclude that both drugs have the same susceptibility patterns. The numbers for MIC and the resistant patterns for the bacteria were consistent throughout for both drugs as shown in Table 1. Both orbifloxacin and difloxacin exhibited similar patterns of susceptibility and MIC's. However, MIC's for both of these drugs seemed to be higher than MIC's for ciprofloxacin and enrofloxacin. A greater number of organisms categorized as susceptible to ciprofloxacin and enrofloxacin were resistant to orbifloxacin and difloxacin. This led us to conclude that ciprofloxacin and enrofloxacin do not differ in bacterial susceptibility patterns and that orbifloxacin and difloxacin are less effective than ciprofloxacin and enrofloxacin. Thus, enrofloxacin, based on our in vitro data, is the best choice for veterinary use.

ACKNOWLEDGEMENTS

I would like to take a moment to give credit to where it belongs. First and foremost, I would like to thank and honor the Clinical Pharmacology lab for having me work in the laboratory for the past two and a half years. I would like to especially thank Dr. Boothe for always encouraging me and giving me support. And for always putting up with my character flaws, procrastination being one of them. She has greatly influenced me in the past years I have conducted research at the Clinical Pharmacology Lab and has always been there for me through personal problems and research hurdles. I greatly thank her for all she has done for me and will always honor the advice she has given me. Next, I could not have even gotten through the past two years without the help and encouragement of Dr. Albert Boeckh. He has been a great motivating force in my life and my career at the Clinical Pharmacology Lab. He has done more for me than I can even imagine and has always lend a helping hand. Thanks Albert! Finally, I would like to thank the Clinical Microbiology Lab, specifically Dr. Bruce Simpson, Kim Andrews and Rashel Mikulec. They have helped me from day one when I first picked up a blood agar plate and made smiley faces with my organism. Although I still am not perfect, they have patiently seen me through many ordeals and trouble I have encountered on this project. And all three have been really great friends as well. They have always been nice to me and tolerated listening to my problems. Thank you. More acknowledgements are in order to Antech Diagnostics and Antech East Diagnostics, Inc. Also, great appreciation is given to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL).

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