Determination of Genetic Differences for Antibiotic Resistance in *Escherichia coli* using Oxytetracycline.

By

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Determination of Genetic Differences for Antibiotic Resistance in *Escherichia coli* using Oxytetracycline

Abstract

This study provides an insight as to how quickly antibiotic resistance can arise under selective pressure of antibiotics and antibiotic use directly precedes the rise of resistant pathogens. Rates at which resistance can arise and potentials for cross-resistance to similar antibiotics within the same class could be determined by evaluating antibiotic resistance displayed by *Escherichia coli(E.coli*) in feedlot cattle to antibiotics such as oxytetracycline. This research shows development of new molecular techniques in the determination of antibiotic resistance in broad spectrum antibiotics such as oxytetracycline.

Samples of fresh fecal piles were collected from a cattle feedlot that use antibiotics and another feedlot where antibiotics are used. In the laboratory, initially, each sample was streaked onto Eosin methylene blue agar and incubated. A second swab of each sample was streaked onto M-enterococcus agar and incubated. Isolated colonies were transferred from agar plates to Tryptic Soy Agar (TSA) slants and incubated prior to transfer to refrigeration for culture storage until utilization for testing. All the samples were then transferred from the TSA slants to Tryptic Soy Broth (TSB) and incubated. The antibiotic discs were evaluated based on Kirby-Bauer method and the diameters of the zones of inhibition were measured. The *E.coli* strains were grouped into three categories based on their measured characteristics. Oxytetracycline was chosen as antibiotic for the research because it was resistant to most of the strains. Antibiotic resistance determination was repeated for each of the resistant samples to the oxytetracline. The resistance levels were grouped into three categories. Miniprep was performed for DNA extraction and the products were sent to University of Nebraska-Omaha for sequencing. Computer analyses of the sequences were performed using five molecular analysis websites.

Blast analysis showed that all the sequences were more than 93% identical to the reference sequence, confirming that all the *E.coli* strains carry the drug resistance gene due to their maximum identity level to the reference sequence. Previous research has shown that the higher the raw score of the resistance gene, the more likely a strain would be resistant to an antibiotic. This appears to be supported by this study.

Keywords: Oxytetracycline, susceptibility, resistance, zones of inhibition, DNA sequence, Protein sequence

Introduction

To observe the different bacteria strains' susceptibility to oxytetracycline, this study provides an insight as to how quickly antibiotic resistance can arise under the selective pressure of antibiotics and that antibiotic use directly precedes the emergence of resistant pathogens. The rate at which resistance can arise as well as the potential for cross-resistance to similar antibiotics within the same class could be determined by evaluating the antibiotic resistance displayed by *Escherichia coli(E.coli*) in feedlot lot cattle to multiple classes of antibiotics; oxytetracycline in this case.

Antibiotic resistance is a pathogen's ability to possess certain characteristics that could ultimately render a drug useless when a certain regimen is used on the pathogen (6). Such characteristics might involve gene expression level or certain secretions that make the pathogen grow in the presence of the antibiotic. These characteristics help the pathogen survive the adverse effects of a drug. Antibiotic resistance is no longer simply an emerging menace in society, as it has transitioned into a universal pandemic threatening our very existence (6). However, continued and widespread use of antibiotics has led to the development of resistant strains (3, 8, 7, and 16). Furthermore, cross-resistance is of particular importance as it may allow pathogens to exploit resistance mechanisms against commonly prescribed antibiotic therapies used in humans. The development and introduction of new antibiotics into market has decreased drastically due to economic and regulatory constraints over the past decade, and while pharmaceutical companies explore higher profit areas of the drug market, little is being done to provide incentive for research and development of new antibiotics. Armed with the knowledge of how quickly bacterial populations grow and evolve coupled with advanced detection methods, it is not surprising that the incidence of antibiotic resistance is growing at a near exponential rate.

Mechanisms of Antibiotic Resistance

Mechanisms of antibiotic resistance occur in varying degrees of complexity. Efflux pumps offer a relatively mechanism for bacteria to resist destruction by antimicrobial agents by simply pumping the agent out of the intracellular space, thus not allowing the agent to reach its desired target or concentration. Another mechanism to provide simple resistance is for the bacteria to retard influx of the antibiotic. Bacteria also have a repertoire of more individualized forms of antimicrobial resistance, which includes amplification of the antibiotic target, target site alteration, and inactivation of the antibiotic itself (18).

Figure 1 Mechanisms of Antibiotic Resistance (18)

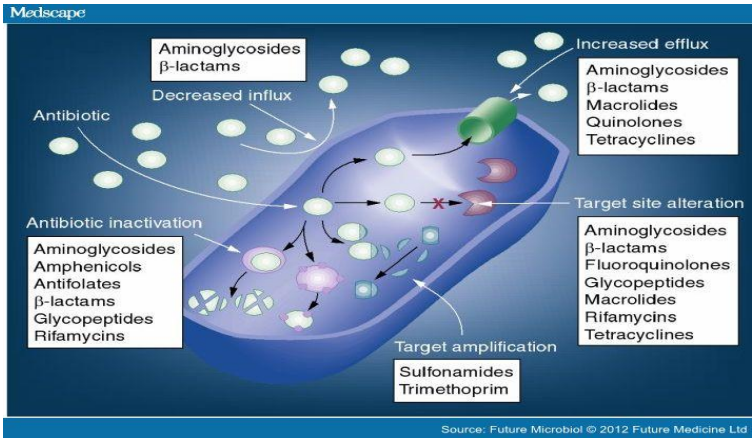

Figure 1 displays the mechanisms that bacteria may utilize to provide resistance to antibiotics. Under each mechanism, the classes of antibiotics resisted using each specific mechanism is listed (11).

Transfer of Resistance

Bacteria possess three primary mechanisms to aid in their transfer of resistance genes: transferring plasmids through conjugation, transduction with the aid of specialized viruses called bacteriophages, and transformation, which is the direct uptake of DNA from the environment (6).

Figure 2 Transference of Resistance (9)

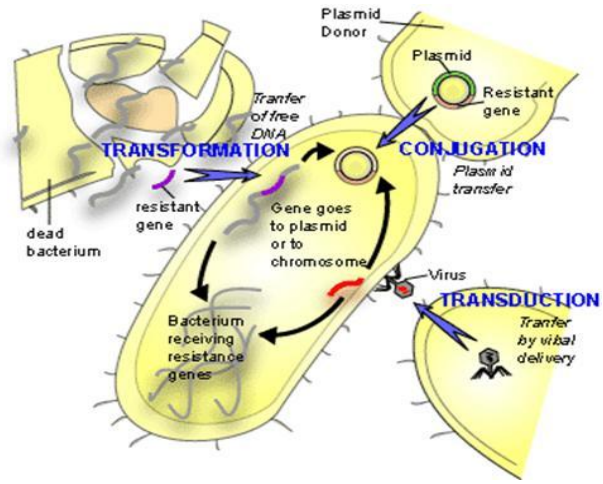

Figure 2 displays how bacteria go about transferring resistance genes by transformation, transduction, and conjugation (9).

Empiric Therapy and Resistance

For many years, antibiotic therapy has been widely performed empirically. Empirical therapy is not based on the scientific method but rather on observations and records (6). The use of empiric therapy can be an effective approach due to most infecting pathogens being susceptible to the therapies, and a majority of the patients having a strong immune system. However, the lack of identification of the causative bacteria can lead to the development of resistance in pathogens that are not susceptible to the antibiotic utilized, potentially initiating a cycle of antibiotic resistance into society (see Figure 3) (6).

Figure 3 Antibiotic Resistance Cycle (10)

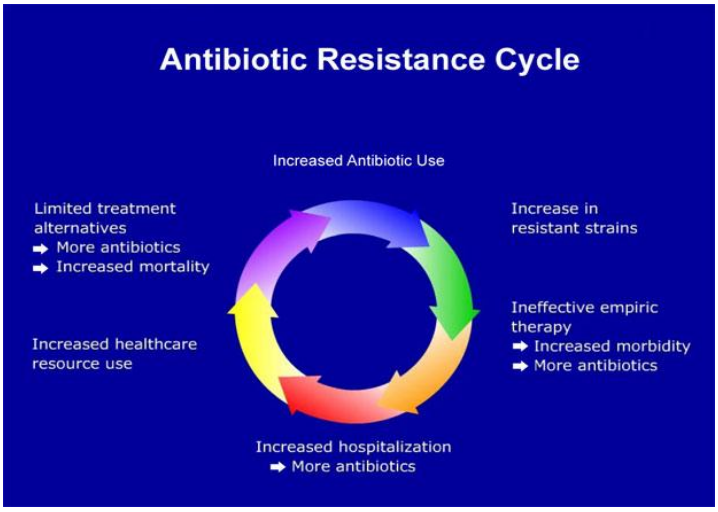

Figure 3 shows the cyclic pattern that can occur when increased numbers of antibiotics are being used leading to increased resistant bacteria and a failure of empiric therapy. This failure of empiric therapy in turn leads to increased use of antibiotics, increased healthcare costs, and higher morbidity and mortality rates (11).

The advancement of molecular techniques in determining antibiotic resistance has over-taken phenotypic assays in determination of antibiotic resistance. Previous researches have used phenotypic analysis and have also been the most common method of resistance determination. However, research has proven that phenotypic analysis is labor intensive, expensive and very time-consuming. Nucleic acid-based assays for the detection of resistance may offer advantages over phenotypic assays. The detection of the methicillin resistance-encoding mecA gene in staphylococci, rifampin resistance in *Mycobacterium tuberculosis*, and the spread of resistance determinants across the globe are examples (19). This research has shown the development of new molecular techniques, e.g. Polymerase Chain Reaction using molecular beacons and DNA chips which expands the possibilities for monitoring resistance as well as sequencing bacteria strains. By using molecular techniques, this research illustrates developments, such as the ability

to read longer sequences faster and cheaper, DNA sequencing within the capabilities of at least some diagnostic laboratories and is the method of choice for determining the resistance of human immunodeficiency virus to antiviral drugs. The advancement in molecular techniques has over-taken the arena of gene analysis.

Oxytetracycline is a one of the many tetracyclines, which are a class of antibiotics and comprise a distinctive family of substituted hydroxynaphthaline. The tetracycline family includes antibiotics such as doxycycline, oxytetracycline, and demeoclocycline, the tetrafamily obtain their name from its' structure which consist of four fused cyclic rings. Oxytetracycline inhibits cell growth by inhibiting translation. It binds to the 30S ribosomal subunit and prevents the amino-acyl tRNA from binding to the A site of the ribosome (17)

Table 1: Discovery of antibiotics and emergence of resistance **(4)**

Antibiotic	**Discovered**	**Introduced in clinic**	**Emergence of resistance**
Penicillin	1940	1943	1940(methicillin,1965)
Streptomycin	1944	1947	1947,1956
Chloramphenicol	1947	1949	1970
Tetracycline	1948	1952	1956
Erythromycin	1952	1955	1956
Gentamycin	1963	1967	1970
Vancomycin	1956	1972	1987
Ciprofloxacin	1969	1987	1992
Telithromycin	1997	2001	2003
Quinupristin and	1999	2000	2000
Daptomycin	1980	2003	2004

Table 1 shows the year that each major class of antibiotics was discovered, the period that it was introduced in clinic and the year when the first cases of resistance were observed. Resistance for tetracycline emerged since 1956.

All in all, this research can aid in the discovery of non- resistance antibiotics by studying the strains' resistance levels based on the applied treatment methods. The application of microbiology and molecular techniques will present the reasons why the *Escherichia coli* have such high or low levels of resistance to oxytetracycline. This result can aid the scientific world to have a better understanding of the mechanisms of survival of pathogens. Such a result can also

assist pharmaceutical companies to gain better strategies in developing drugs. Changes in the levels of expression of genes come about by mutation as well as horizontal gene transfer. Antibiotic resistance moves beyond the affected patient and gradually renders a drug useless (6). In conclusion, this research will be useful in understanding what details matter to the big picture, and to learn more about the important issues facing drug companies' decision-makers. My hypothesis is that the difference in level of resistance to oxytetracycline is correlated to the resistance gene of the DNA sequence and protein sequence of *Escherichia coli*.

MATERIALS/METHODS

Field Methods

In spring 2012, in my Techniques in Microbiology class, initially utilizing 78 samples obtained from fresh bovine feces, research was conducted to find the different levels of antibiotic resistance. The control group contained 26 samples obtained from the Brown Farm, located at highway FF near Diamond Missouri. The Brown Farm does not use antibiotics. The remaining samples were collected at Neosho Valley Feeders near Parsons, Kansas, with 26 samples originating from the feedlot pens and the remaining 26 samples were obtained from the hospital pens. The feedlot cattle have greater exposure to antibiotics than the cattle on the Brown Farm, and the Hospital pen group is actively being treated with antibiotics. The antibiotics currently being used at Neosho Valley Feeders are florfenicol, tulathromycin, tilmicosin, and tetracycline. Sample collection was distributed across the sample areas as to not obtain a majority of the samples from one pen. Samples were collected using two sterile swabs for each sample obtained by swabbing the fresh feces piles with both swabs and placing them in a collection tube containing phosphate buffered saline for transport back to the laboratory.

Laboratory Methods

Upon returning to the laboratory, one swab from each sample was streaked onto Eosin methylene blue (EMB) agar for lactose fermenting microbes and the remaining swab from each sample was streaked onto M-enterococcus agar for *Enterococcus spp*. The use of EMB agar was used to facilitate the isolation of *Escherichia coli* colonies by identifying colonies with a metallic green sheen and dark centers following twenty-four hours of incubation at 37°C. M-enterococcus agar was used to facilitate the isolation of *Enterococcus spp.* by identifying colonies with a maroon appearance. Isolated colonies were transferred from agar plates to Tryptic Soy Agar (TSA) slants and incubated for twenty-four hours at 37°C prior to transfer to refrigeration for culture storage until utilization for testing.

Twenty-four hours before beginning antibiotic susceptibility testing, all samples were transferred from the TSA slants to Tryptic Soy Broth (TSB) and incubated at 37°C to establish antibiotic discs and were placed according to standard procedure for antibiotic susceptibility testing. Initially, the antibiotics utilized for the susceptibility testing of *E. coli* were oxytetracycline, ceftriaxone, tulathromycin, sulfamethoxazole/trimethoprim, colistin, levofloxacin, gentamycin, and imipenem. Following inoculation of the Muller-Hinton agar and placement of the antibiotics, the plates were incubated for twenty-four hours at 37°C. After the twenty-four hours of incubation, the zones of inhibition created by the antibiotics were measured and recorded as susceptible, intermediate, or resistant according to the interpretation of inhibition zones of test cultures as shown in the table below. All individual samples used for antibiotic susceptibility testing were evaluated using triplicates and the zones of inhibition were recorded and analyzed for consistency before determining the susceptibility of each sample. Table two on

the next page displays the interpretation of the zones of inhibition of different antibiotics that were initially used. For example, in oxytetracycline, 14 mm or less is considered as resistant, 15 to 18mm is considered as intermediately susceptible and 19 or more is considered susceptible to the antibiotic.

Table 2: Interpretation of zones of inhibition of different antibiotics (20)

Antibiotic (and disc identifier)	**Disk potency**	**Inhibition zone diameter to nearest mm**		
		Resistant	Intermediate	Susceptible
Ciprofloxacin	5 µg	15 or less	16 to 20	21 or more
Colistin	10 µg	8 or less	09 to 10	11 or more
Doxycycline	30 µg	12	13-15	16
Erythromycin	15 µg	13 or less	14 to 17	18 or more
Gentamycin	10 µg	12	13-14	18
Methicillin (ME5)	5 µg	9	10-13	14
Nalidixic Acid (NA)	30 µg	13 or less	14 to 18	19 or more
Penicillin G (P)	10 units			
Polymyxin B (PB)	300 units	8 or less	9 to 11	12 or more
Streptomycin (S)	10 µg	11 or less	12 to 14	15 or more
Sulfadiazine	300 µg	12 or less	13 to 16	17 or more
Sulfaoxizole	300 µg	12 or less	13-16	17 or more
Sulfisoxazole	25 µg	12 or less	13 to 16	17 or more
Tetracycline (TE)	30 µg	14 or less	15 to 18	19 or more

After comparing the different levels of resistance of the different strains and the strengths of the different antibiotics, the resistant *Escherichia coli* strains and oxytracycline were chosen as the sole strains and antibiotic respectively for the research. The chosen grown strains in the broth were vortexed and spun to obtain the different resistant strains. After centrifugation, the resistant strains pellets were refrigerated at 0°C.

Miniprep

The resistant colonies were harvested for the resistance plasmid DNA to be sequenced. Extraction of DNA was carried out according to the PureYield Plasmid Miniprep System. This involved three major steps for each of the bacteria strains:

Preparation of lysate

i. 600ml of bacterial culture was added to a 1.5ml micro centrifuge tube.
ii. 100ul of cell lysis buffer (blue).
ii. 350ul of cold neutralization solution was mixed and thoroughly inverted
iv. The tube was centrifuged at maximum speed for 3 minutes.
v. The supernatant was transferred to a minicolumn without disturbing the cell debris pellet
vi. The minicolumn was placed in a collection tube and centrifuged at a maximum speed in a microcentrifuge.
vii. The flow through was discarded and the minicolum was placed in the same collection tube

Wash

xiii. 200ul of endotoxin removal wash was added to the minicolumn and then centrifuged at maximum speed for 15seconds.
ix. 100ul of column wash solution was added to the minicolumn and then centrifuged at a maximum speed in 30 seconds.

Elute

x. The minicolumn was transferred to a clean 1.5ml microcentrifuge tube, and then 30ul of Elution buffer or nuclease-free water was directly to the minicolumn matrix. This solution was left to stand for a minute.
xi. Lastly, the tube was centrifuged for 15 seconds to elute the plasmid DNA. The micro centrifuge tube with the eluted plasmid DNA was capped and stored at 20 degrees Celsius.

Sequencing

The DNA extracted from each of the bacteria strains were sent to University of Nebraska-Omaha for DNA sequencing.

Computer Analysis Methods

Reference Sequence Search

The reference sequence used for this research is the perfect sequence that is totally resistant to Oxytetracycline. From the National Center for Biotechnology Information (NCBI) database website, the reference strain sequence was obtained by selecting Nucleotide and searching for "tetracycline resistance gene," Over 400,000 hits show up. To be more specific in the search, "bacteria" and then *Escherichia coli* were clicked and by searching through over 500 hits, the sequence was obtained.

Blast

A blast search of the resistant DNA sequences that were received from the University of Nebraska-Omaha was performed on NCBI database to compare the sequences in the resistant bacteria to known reference sequences. The aim of the blast search is to confirm that the *E.coli* strains were resistant to oxytetracycline and have major similarities to the reference strain which is highly resistant to the antibiotic.

On the NCBI website, nucleotide was searched, blast and then nucleotide blast was clicked to show a page where the query DNA sequence was entered for each of eleven sequences. The sequence is entered for on this search engine then "blast "is hit. This search engine shows the query ID, the query sequence alignment scores, the molecular type, the total score and most importantly the maximum identity of the bacteria strain to the reference strain. This shows the similarities and differences of the bacteria strain to the reference sequence. This is repeated for all of the ten other different *E.coli* strains

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Cluster and Multiple Sequence Alignment (MSA) DNA Analysis

To show how closely related or different the various DNA *E.coli* strains are to each other as well as comparing each of them to the reference bacteria strains, the cluster and MSA website was utilized. The eleven DNA sequences were copied into the search box in the middle window, then DNA is selected then "Execute Multiple Alignment". On the results, this show how the DNA bases align and the percentage differences of each other.

At the bottom of these results, the NJ tree from the menu was selected and then execute. Four trees were obtained and the best one is further analyzed. The cladogram is used as a basis of comparison of evolutional features of the different sequences. The comparison is based on the exons of the sequences. This search method is repeated for only the reference sequence compared to each one of the sequences.

Sequence Manipulation Suite

This search engine converts of the messenger Ribonucleic Acid (RNA) to protein by a process called translation. From the DNA sequence of each of the bacteria strains, a specific beginning and ending is chosen from each of the sequences. The chosen raw sequence of each of the *E.coli* sequences is entered one at a time and this translates the DNA sequence to a protein sequence. The reading frame of the protein is obtained for each of the sequences including the reference strain.

Cluster and Multiple Sequence Alignment (MSA) Protein Analysis

In search of the protein differences of the various strains, the cluster and multiple sequence alignment protein analysis show how related or different the various *E.coli* protein strains are to each other as well as comparing each of them to the reference bacteria protein sequence. The eleven DNA sequences were copied into the search box, and "protein" is selected in the middle window and the "Execute Multiple Alignment" bottom is clicked. On the results, this show how the protein bases align as well as the percentage differences of each other. This website also yielded the cladogram for the amino acid sequences.

Motif Scan

On "myhits sib" protein domain site, the motif searches of the translated sequences were obtained. The motif scanning finds all known motifs that occur in each of the sequences. The protein sequences obtained from the cluster and multiple sequence alignment protein analysis is entered in the search box. In addition, the following are selected for the search: peroxide bases profiles, hamap profiles, prosite patterns, prosite profiles and more profiles. This resulting page shows if the protein sequence has genes such as major facilitator super family, sugar transport system, phosphor site such as protein kinase C phosphylation site, or undergone processes such as amidation and glycosylation. Most importantly, this search shows if the protein sequences contain a multidrug resistance protein (mtdG). The start and ending of the sequence, the length of the various effects on the sequence, the raw score, N-score and E-values are obtained from this site.

RESULTS

Antibiotics Analysis

Initially, analysis was performed to determine the antibiotics that were the least effective as well as the most effective. Respectively, tulathromycin, oxytetracycline, and colistin have the most resistance on the isolates of the tested *E. coli* samples. The most effective antibiotics against the *E. coli* samples obtained from all the groups were trimethoprim/sulfamethoxazole, levofloxacin, imipenem, ceftriaxone, and gentamycin respectively (figure 4).

Figure 4 Number of Resistant *E. coli* Isolates by Antibiotic

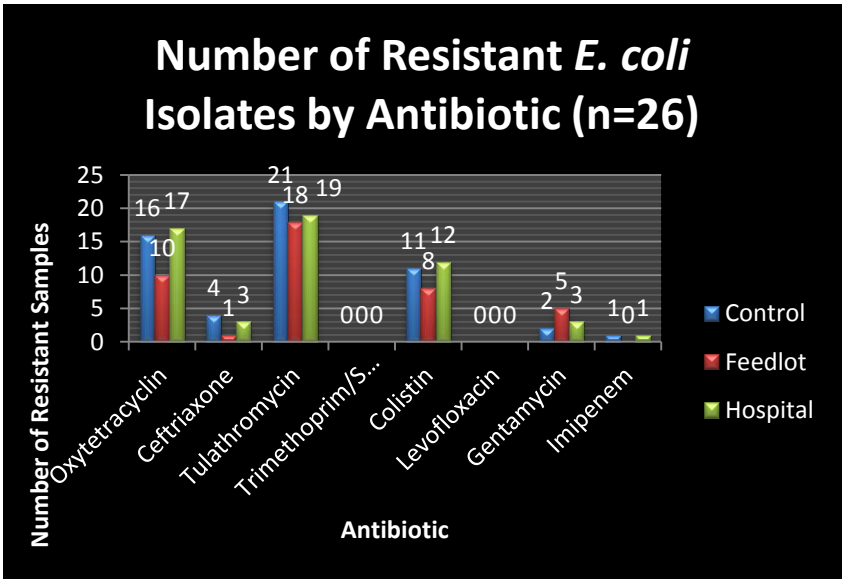


Figure 4 displays the number of isolates displaying resistance to each antibiotic tested against out of 26 isolates for each group.

Initially the oxytetracycline resistance was highest in the control group, followed by hospital pen group and the feedlot group had the lowest resistance for oxytetracycline.

Zone of Inhibitions

After choosing oxytetracycline as the sole drug for the research, as displayed on table 3, the resistant strains showed these different levels of resistance based on the evaluation of the diameter of the zones of inhibition. The diameter of the zones of inhibition is inversely proportional to the resistance level of the bacteria to oxytetracycline.

C stands for control

F stands for feedlot

H stands for Hospital pen

Table 3 *Escherichia coli* strains and their levels of resistance

Sample	Phenotypic Resistance
Reference	High
3H	High
3C	High
4H	High
11H	High
1OF	Intermediate
26H	Intermediate
21H	Intermediate
11C	Low
2C	Low
25F	Low

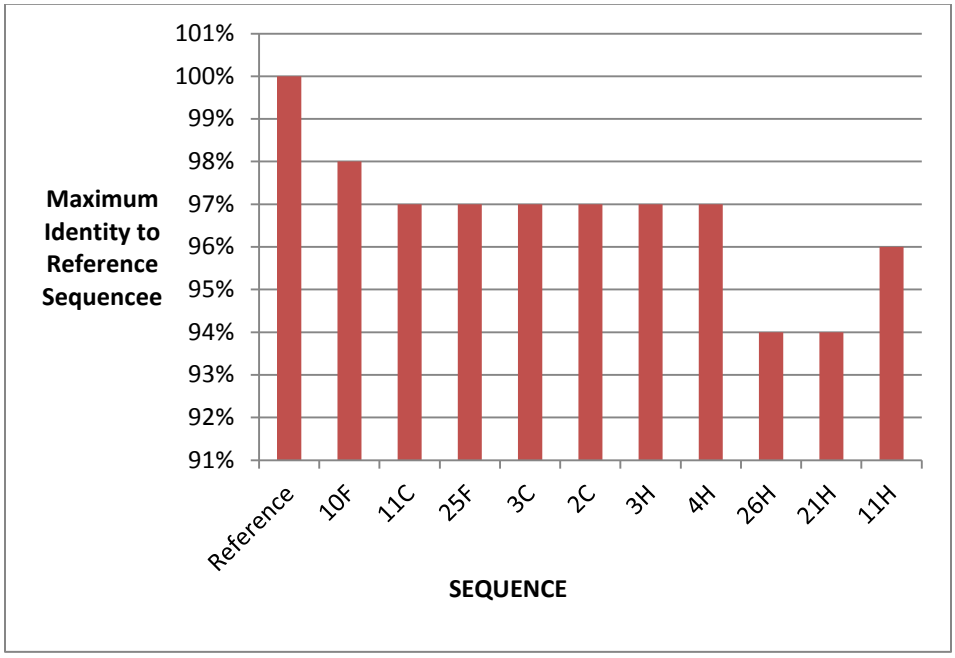
Computer Analysis Results

Blast

The blast confirmed that all the DNA sequences were very similar to the DNA reference strain proving that the bacteria strains are all resistant to oxytetracycline.

Figure 5 below shows the maximum identity of each of the bacteria strains sequences to the reference gene which is highly resistant to Oxytetracycline.

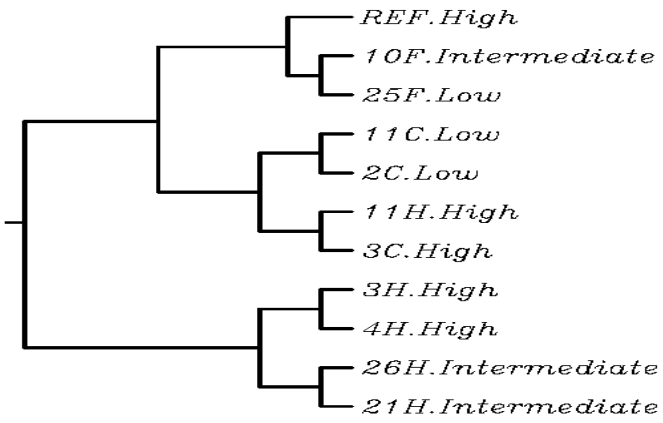
Figure 5 Graph of Maximum identity of bacria strains to Reference Strain



Cluster and Multiple Sequence Alignment (MSA) DNA Analysis

Figure 6 below shows the yielded cladogram of the evolutionary DNA sequences of the eleven *E.coli* strains on the cluster and multiple sequence alignment analysis.

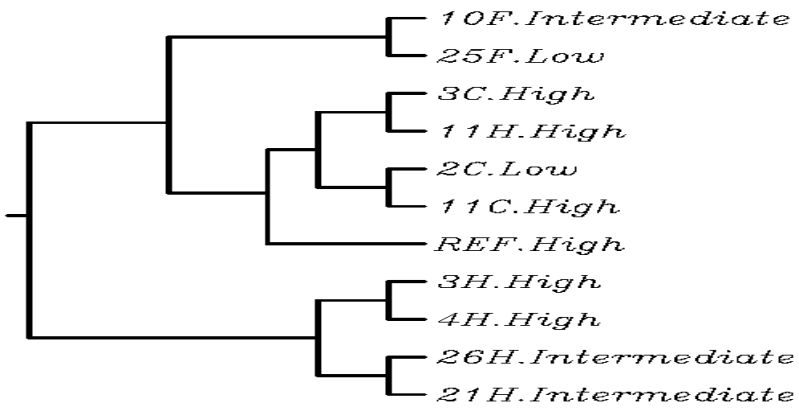
Figure 6 DNA Sequence Cladogram



Cluster and Multiple Sequence Alignment (MSA) Protein Analysis

Figure 7 below illustrates the cladogram of the protein evolutionary features and their phenotypic resistance.

Figure 7 Protein Sequence Cladogram



Motif Scan

An analysis was carried out on the translated sequences of each of the *Escherichia coli* strains on motif scan. The gene analysis showed that each one of the sequences contained the multiple drug resistance and major facilitator super family genes. Table 4 shows the average raw score of each resistance categories of the multidrug resistance gene and the major facilitator super family. The higher the average raw scores of the multi-drug resistance gene (MDTG) the higher the resistance level. The results showed that the *E.coli* that were detected as the highest resistance using techniques in microbiology had the highest raw scores, followed by the intermediate resistance bacteria and the low resistance level *E.coli* strains had the lowest raw scores.

Table 4 Raw scores showing trends of resistance

Resistance Levels	*E.coli* Sequence	MDRG Gene RAW Score	Major facilitator Super Family
High			
	3C	1831	1038
	11H	1774	932
	3H	1631	1074
	4H	1612	992
Average		**1712 +/-107**	**1009+/-61**
Intermediate			
	10F	1624	1098
	26H	1362	782
	21H	1362	781
Average		**1449 +/-151**	**887+/-183**
Low			
	11C	1001	1082
	2C	1712	1016
	25F	1525	1080
Average		**1412+/-368**	**1059+/-1409**

Table 4 displays the raw scores and the standard deviations that were obtained on motif scan for each of the analyzed sequences.

Figure 8 shows the MDTG matches map that were common for each of the bacteria strains

Legends 1, freq_pat:AMIDATION [?]; **2**, freq_pat:ASN_GLYCOSYLATION [?]; **3**,

freq_pat:CK2_PHOSPHO_SITE [?]; **4**, freq_pat:MYRISTYL [?]; **5**, freq_pat:PKC_PHOSPHO_SITE [?

(?) indicates there is a fifty percent chance that the gene might be present

Figure 9 shows an image of the gene analysis showing the specific position for each present gene

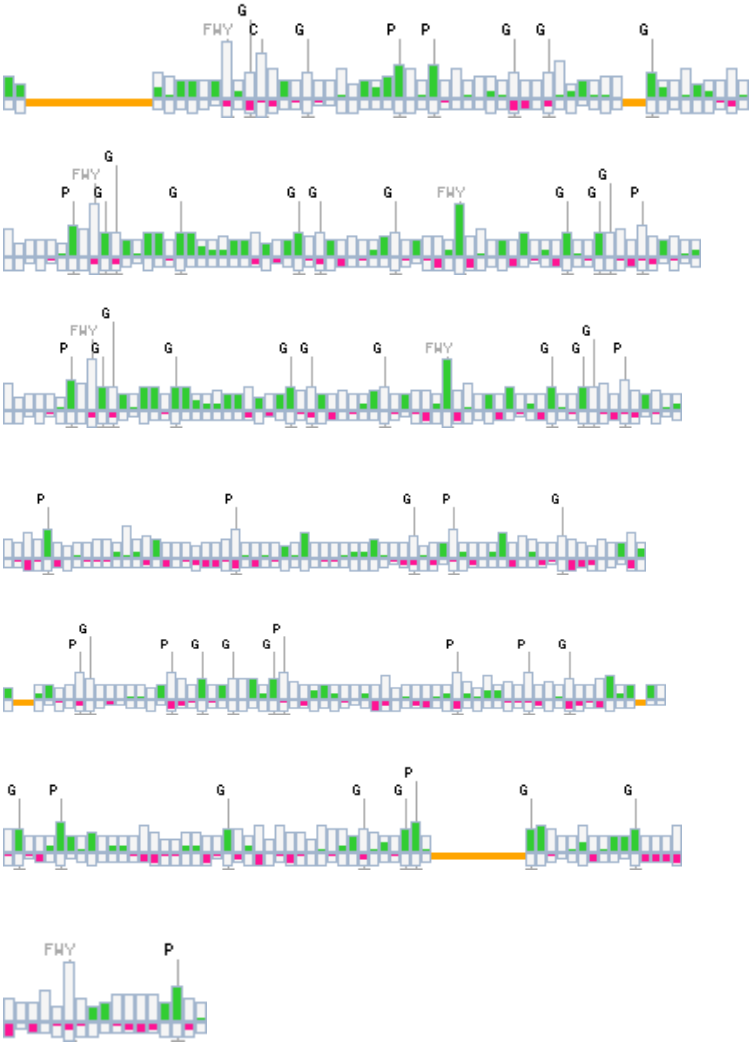
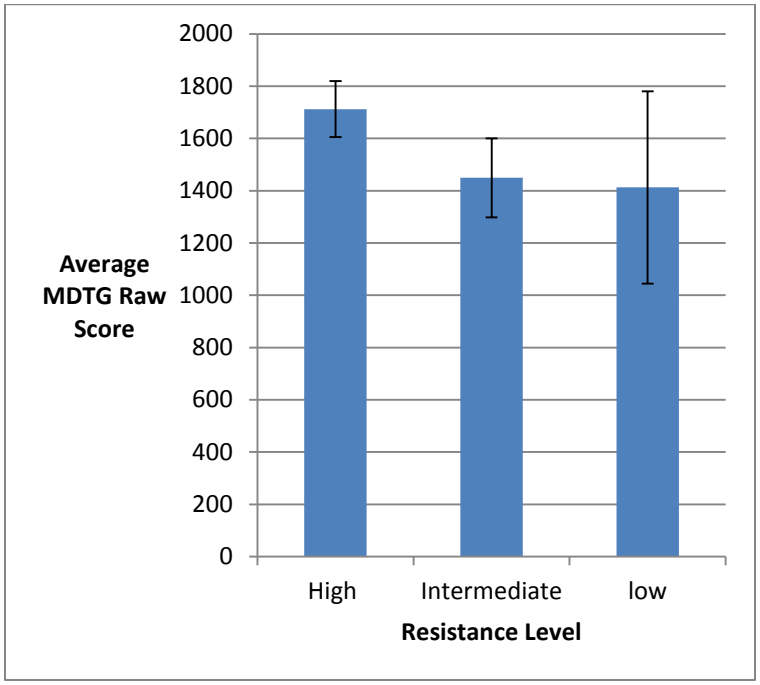


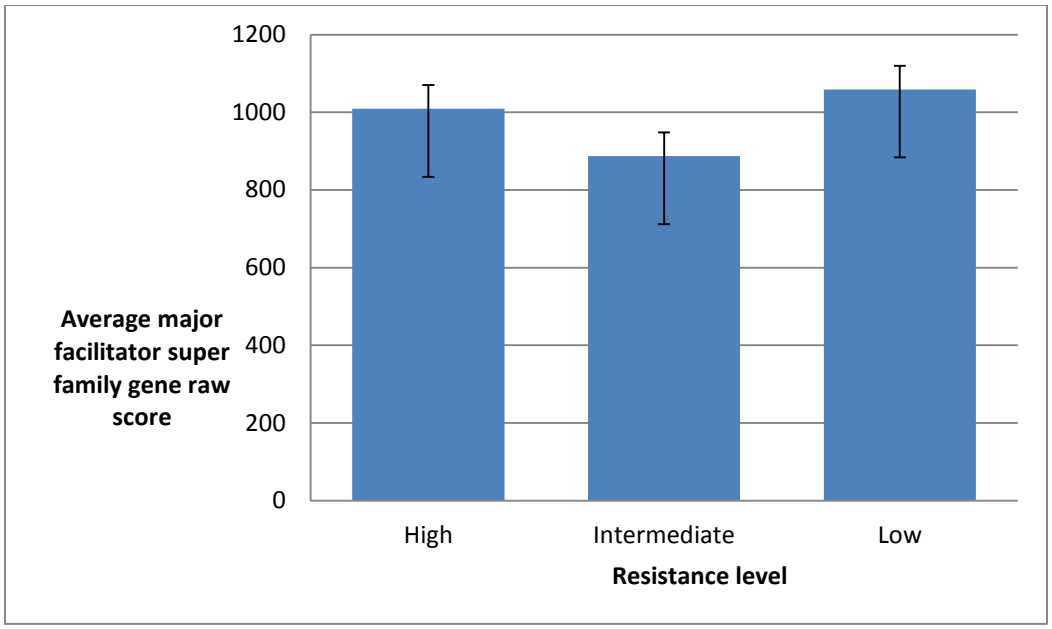
Figure 9 above shows an example of the graphical results that was obtained for each of the resistant *Escherichia coli* sequences on the motif scan website. It displays the specific position of the genes that are present such as the multidrug resistance gene, major facilitator super family gene and transport gene. The start and end of the amino acid sequence of the specific gene is indicated.

Figure 10 Graph shows the Multidrug Resistance Gene Average raw scores for each of the three groups.



The graph above shows the average raw scores of the bacteria strains in the three levels of resistance. The highly resistance bacteria strains got an average of 1712, intermediately resistance got an average of 1449 and the low resistance bacteria strains group got an average raw score of 1413. Research has proven that the higher the raw score, the more likely the bacteria strains are resistance to the antibiotic.

Figure 11 Graph shows the major facilitator super family gene raw score



The graph above illustrates the average raw scores in the three levels of resistance of the major facilitator super family genes in the strains. The highly resistance bacteria strains got an average of 1009, intermediately resistance got an average of 887 and the low resistance bacteria strains group got an average raw score of 1059. The low resistance genome got the highest raw score for the major facilitator super family gene.

DISCUSSION

Initially, on evaluating the overall results of the effectiveness of the gram negative *E.coli* antibiotics, the numbers of susceptible bacteria strains were higher than the number of resistant bacteria strains during the study. Maybe there are preventive strategies that are taken by the different feedlots to prevent the occurrence of resistance of the cattle's bacteria to the antibiotics. In the zones of Inhibition analysis of the resistant *Escherichia coli* strains, resistance was highest in the hospital pen group, followed by the feedlot group and the control group had the least resistance. This might be due to the previous antibiotics exposure of the cattle, as the hospital pen group was highly exposed to antibiotics more than the feedlot group. Weaker immune system might be a contributing factor for the Hospital pens' bacteria strains to be less resistant that the feedlot bacteria strains. The strains could develop various strategies to overcome the antibiotic impact.

The blast analysis showed that all the sequences were more than 93% identical to the reference sequence. This proves that all the samples in the study were resistant to oxytetracycline. The cluster and multiple sequence alignment analysis indicated that even though the *E. coli* isolates had resistance to oxytetracycline, that resistance was not statistically different between the isolates obtained between the control, feedlot, and hospital pens. Neither the cladogram of the DNA analysis nor the cladogram of the protein analysis showed any correlation in terms of the level of resistance of the different *E.coli* samples. This might be due to mutations that might occur in the individual *E.coli* strains as they adapt to harsh conditions and develop different survival strategies.

On the motif scan website, the average raw scores of the *E.coli* strains obtained proved that samples 3C, 11H, 3H and 4H had the highest resistance level with an average raw score of 1712 for the multidrug resistance gene and 1009 for the major facilitator super family genes; the raw score proved that samples 10F, 26H and 21H had an intermediate resistance level with a raw score of 1449 for multi-drug resistance gene and 887 for major facilitator super family. Samples 11C, 2C and 25F had the lowest raw scores for the multidrug resistance gene of 1412 and 1059 for major super facilitator family. Research has shown that the higher the raw score of the resistance gene of a bacteria strain, the more likely the strain will be resistance to an antibiotic. The raw score of the multi-drug resistance gene have confirmed that in using molecular techniques, resistance is directly correlated to the resistance gene of the protein sequence. As a result, these results support the hypothesis that the difference in the level of resistance to oxytetracycline correlate to the resistance gene and protein sequence.

In simplifying the resistance phenomenon, two major factors might contribute to this complication: the oxytetracycline, which is acting as a selective agent helps to propagate organisms that have the second factor: the resistance gene. Resistance to antibiotic drugs will always eventually arise as a result of the exposure to the selective pressure posed by antibiotic

use or an intrinsic change in organisms, examples mutations. If either the antibiotic or the resistance gene were not present, we would not face a resistance problem. These results have implications to the problems we are currently facing with the use, and in some case the abuse, of antibiotics not only in veterinary medicine, but in human medicine as well. Many of the resistance mechanisms that bacteria employ not only have consequences leading to the ineffectiveness of a particular antibiotic, but to the ineffectiveness of a whole class of antibiotics or even several classes of antibiotics. When bacteria employ these measures of resistance it greatly enhances the risk for a bacterial infection in a human to be resistant to many antibiotics used to treat humans simply because of the bacteria's prior exposure and acquired resistance mechanisms to similar antibiotics used in veterinary medicine.

Under the current crisis of resistance emergence, spread and the lack of new treatments for bacterial infections, it is only a matter of time before all of our current antibiotics are ineffective. To ensure the preservation of the effectiveness of our current antibiotics, drastic measures should be implemented not only in veterinary medicine practice, but in human medicine as well.

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