

Isolation and Identification of Antibiotic Resistant Bacteria from the Intestinal Flora of Feedlot Cattle and a Measure of Their Efficacy for Lateral Gene Transfer

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ABSTRACT

Lateral gene transfer is the process of the interspecies transfer of genetic material. It has been found that prokaryotic organisms, most notably bacteria, are especially proficient at lateral gene transfer. Some of the genes transferred between bacteria are R plasmids, plasmids coding for resistance to antibiotics. Veterinary antibiotics are known to play a role in the development of antibiotic resistant bacteria. One of the places where veterinary antibiotics are used is in feedlots. Due to injections with antibiotics and the use of feed laced with antibiotics, feedlots provide an excellent environment for antibiotic resistance development and transfer. In this study, I present further evidence of avenues of interspecies antibiotic resistance transfer. Using the antibiotic Tylosin, which is an additive in feed, antibiotic resistant bacteria were isolated from bovine fecal samples. The bacteria were isolated by using nutrient agar plates that contained a concentration of Tylosin similar to the overall concentration administered to adult cattle. *Escherichia coli* were found to be the isolated antibiotic resistant bacteria. A sample of *E. coli* was taken during the logarithmic growth phase and placed in a test tube containing nutrient broth and compatible bacteria in the same growth phase to act as the recipient of R plasmids. *E. coli* was found not only to exhibit marked resistance to Tylosin, but was capable of performing lateral gene transfer to *Staphylococcus epidermidis* with relative ease.

Keywords: Lateral Gene Transfer, Veterinary Antibiotics, Antibiotic Resistance, Feedlot

INTRODUCTION

In recent years, bacterial resistance to antibiotics has risen to the forefront of world health issues. Overuse of antibiotics has been identified as a leading cause for the development of bacterial antibiotic resistance. One of the areas in which antibiotics are used heavily is in cattle ranching; feedlots are especially concerning. Cattle are given feed laced with antibiotics as well as antibiotic injections to increase growth and stave off infection (Sarmah et al., 2006). This inundation of antibiotics has raised concerns regarding the development of multiple antibiotic resistant bacteria through fecal contamination (Parveen et al., 2005).

The overuse of veterinary antibiotics (VAs) inevitably selects for the survival of persister cells, "...cells that neither grow nor die in the presence of microbicidal antibiotics." (Keren et al. 2004). Bacteria resistant to the antibiotics being used are decidedly advantaged over bacteria without antibiotic resistance, allowing them to become more numerous than non-resistant strains. It is these bacteria that will pass into the environment to potentially spread antibiotic resistance.

In bacteria, the genes coding for antibiotic resistance are often stored in circular packets of non-essential DNA called plasmids. The plasmids containing resistance genes are termed R plasmids. R plasmids may be transferred to other bacteria through several methods; conjugation, transformation, and transduction (Madigan and Martinko 2006).

The mechanisms of R plasmid transfer may also serve as mechanisms of LGT, which "...is the process by which genetic material is transferred between distinct evolutionary lineages." (Kechris et al. 2004). This interspecies exchange of genetic material is common among prokaryotic life. "Prokaryotes are sexually promiscuous and can exchange genes across broad phylogenetic lines." (Madigan and Martinko 2006). LGT allows an avenue for the transfer of antibiotic resistance in bacteria, potentially from MAR bacteria arising from VA use in feedlots.

MATERIALS AND METHODS

Bacteria for this experiment were obtained from a local feedlot, McPherson Co. Feeders, McPherson, KS. Samples were collected from cattle pens containing sick and chronically sick animals. These cattle were given antibiotic injections and were being fed with feed laced with the antibiotic Tylan (Tylosin). A kit was prepared for the acquisition of samples. As scalpels were more readily available, they took the place of spatulas. The scalpels were wrapped in aluminum foil and autoclaved. Sample vessels used were screw-top vials. These were also autoclaved to insure sterility.

The samples were taken from recently excreted bovine feces. A total of ten samples were collected. Two sterile scalpels were allotted per sample collected. The top layer of feces, exposed to the

environment, was scraped off using the first scalpel. The second scalpel was then used to transfer a sample of uncontaminated fecal matter into the vials. Standard sterile technique was used throughout sample collection.

The antibiotic chosen for this experiment was Tylosin. Tylosin was chosen because the animals received the greatest exposure to it, being in their feed. To differentiate between antibiotic resistant bacteria and non-resistant bacteria, Tylosin was mixed with the nutrient agar (NA) the bacteria were to be plated on. A concentration of 1 mg Tylosin/L was decided on based upon the concentration of Tylosin administered to cattle. A serial 1:10 dilution of aqueous Tylosin was performed to arrive at a concentration of 1 mg/mL. One milliliter of the diluent was added to 1 L of agar cooled to -45°C to prevent degradation of the antibiotic.

A sample vial was chosen at random and 5 mL of nutrient broth (NB) was added. The sample was placed in a shaking incubator for 24 hours at 37°C . After incubation, 100 μL of fluid was taken and, using sterile spreading spatulas, spread over an agar plate containing Tylosin. The plate was incubated for two days at 37°C .

Bacteria chosen to be the recipients of R plasmids from the unknown sample were *Staphylococcus epidermidis* and *Bacillus coagulans*. These bacteria were chosen because they have growth requirements similar to the unknown bacteria and for their potential as pathogens. Specimens were ordered through Carolina Biological Supplies. *S. epidermidis* was cultured on NA while *B. coagulans* was cultured on tryptic soy agar (TSA) due to the fastidious nature of the bacteria. To ensure that these bacteria had no previous resistance to Tylosin, they were also plated on NA with Tylosin and, for *B. coagulans*, TSA with Tylosin. A control group was also formed where the bacteria were plated on their respective agar plates without the presence of antibiotics.

To promote LGT, samples were taken during the logarithmic growth phase. The unknown bacteria were placed in a test tube containing NB. One of the recipient bacteria was then chosen and placed in the aforementioned test tube. All attempts at LGT were performed in duplicate. The test tubes were incubated for 24 hrs. at 37°C . A 100 μL aliquot of broth was inoculated onto a NA plate with Tylosin to test for LGT of R plasmids.

The unknown bacteria were cultured on TSA and taken to Aperio Scientific for microbial identification.

RESULTS

Antibiotic resistant bacteria were found in the first randomly chosen sample from the feedlot. When plated on NA with Tylosin, colonies of one discernable morphology appeared. The colonies were cream colored, medium size, with convex

elevation, and showed signs of motility. Upon visualization with light microscopy, the bacteria were discovered to be of a rather indeterminate morphology, being either motile, large cocci, or small bacilli. A gram stain of the culture was performed and yielded definite gram negative results.

S. epidermidis and *B. coagulans* were tested for resistance to Tylosin. Colonies were plated on NA and TSA, both containing Tylosin. Neither species exhibited resistance to Tylosin as there was no microbial growth present.

B. coagulans was the first species to be used as a recipient for R plasmids from the unknown. A series of five attempts in duplicate at LGT with varying volumes of NB, ranging from 1-5 mL, yielded no evidence of LGT. The first and second attempts at LGT with *S. epidermidis* yielded no transfer of antibiotic resistance. Volumes of NB used for the first two attempts were 5 and 4 mL respectively, the incubation time was 24 hrs. A third attempt, with a NB volume of 3 mL and an incubation time of 24 hrs yielded four colonies discernable from the unknown. A gram stain of this colony showed gram positive cocci. When isolated and grown on NA with Tylosin, the antibiotic exhibited no ill effects on the growth of *S. epidermidis*.

Because of the unusual morphology of the unknown bacteria, a contract laboratory was hired to perform microbial identification. The unknown was cultured on TSA and taken to Aperio Scientific. Results of their tests showed that the unknown bacteria were, in fact, *Escherichia coli*.

DISCUSSION

This research was proposed to explore probable avenues of antibiotic resistance transfer. The results of this study show that antibiotic resistant bacteria are present in feedlots and that many may be able to perform LGT. As outlined by Maeda, et al. (2005), LGT is possible even among non-conjugative strains of bacteria. The use of VAs is widespread as is the rate of spread of antibiotic resistance as outlined by Sarmah, et al. (2006) and Parveen, et al. (2005).

The samples obtained for this study came directly from cow feces. Conditions in the feedlot are such that the cattle defecate where they live. This creates an excellent environment for bacteria to thrive in. As this study shows, antibiotic resistant bacteria are readily available from the fecal matter present in feedlots. The consequence of this is the possible spread of disease. It is not uncommon for cattle, living in conditions such as that of a feedlot, to receive cuts and scrapes on their legs or hooves. Often times these cuts may become infected. With antibiotic resistant bacteria present in the environment in such large quantities, it is probable that the bacteria infecting the cattle are antibiotic resistant themselves. Infection with such bacteria only makes treating the injured cattle more difficult,

costly, and time consuming.

With antibiotic resistant bacteria being released into the environment, the spread of antibiotic resistance to other bacteria and to other areas of the environment is perpetuated. Tylosin, the antibiotic used in this study, is of the macrolide class. It works by inhibiting the formation of the 50S subunit of the bacterial ribosome, thus stopping protein synthesis. Tylosin is very similar to macrolides developed for human use, such as Clarithromycin, Azithromycin, and Erythromycin (DiPiro et al., 2005). Antibiotic resistance to these commonly prescribed drugs passing through the environment now poses a threat to the human population as well.

Further study is needed into the spread of antibiotic resistance. Future studies may include a catalogue of assays concerning the different species of bacteria present and in what frequency they may be found. Research should also be performed to understand the scope of antibiotics the bacteria may be resistant. It would also be of value to investigate the efficacy of LGT exhibited by different antibiotic resistant bacteria.

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