

Transfer Of Antibiotic Resistant Genes between *Escherichia coli* and *Salmonella typhimurum*

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ABSTRACT

The research in this study tried to demonstrate the horizontal gene transfer of *Escherichia coli* with *Salmonella typhimurum*. It was believed that if after mating *Salmonella* and *E.coli* together *Salmonella* would receive *E.coli*'s genes that are resistant to Ampicillin. Different concentrations of Ampicillin was introduced to *Salmonella* to get a measurement of what concentrations *Salmonella* would survive in. The manipulated *Salmonella* was cultured on SS agar and tested negative for a lactose reading to show significant evidence that the bacteria was in fact *Salmonella*. This study is important because of the growing number of farmers giving their live stock antibiotic therapy for growth promotion.

Keywords: Horizontal gene transfer, *E. coli*, *Salmonella*

INTRODUCTION

Horizontal gene transfer is a process that occurs in many different ways, transposition, plasmid conjugation, bacteriophage transduction, and transformation. This is a common way to spread the different traits of bacteria from one type to another. (Rouxel, 1991).

The conjugation method transfers DNA in distantly related bacteria. The transduction method transfers DNA in bacteria that share cell surface receptors, so this is for only bacteria that are closely related. The transformation method can mediate the exchange of chromosomes and it commonly happens to naturally transformable bacteria. By using Horizontal gene transfer, bacteria can adapt to new environments from gaining new genes (Rouxel, 1991).

Transferring genes from one bacterium to another can be very dangerous. The hazards of horizontal gene transfer are the birth of new bacterial pathogens, the spread of drug and antibiotic resistances genes, and damage to the ecosystem. (Mae- Wan Ho, 1999).

The antibiotic themselves is one of the factors that contributes to rise of antibiotic resistance. The other is the type of resistant traits that are being selected. The resistance that *Salmonella typhimurum*. and *Escherichia coli* demonstrate is a reflection of their environments they live in. (Stuart B. Levy, M.D. 1998). *Salmonella* and *E. coli* infections are on the rise. There are about 800,000 cases of *Salmonella* infections a year and about 500 of those people die from their infection. There are about 73,000 cases of *E. coli* infections a year. Around 2000 of these people will be hospitalized and about 8.2% of the infected people will die according to the reports received from the Center for Disease Control and Prevention (CDC 2002).

E. coli and *Salmonella* are ideal subjects to study, because they share common traits. They both grow rapidly at 37c on nutrient agar and are gram

negative. They are rod shaped and motile. *E. coli*'s O antigens are similar to *Salmonella*'s O antigens which are found in the polysaccharide part of the lipopolysaccharide molecule in the outer layer of the cell wall (Bergey's manual, 1975).

By using high doses of Ampicillin I will attempt to transform *E. coli* and then culture the transformed *E.coli* with *Salmonella*. Then I will screen for Ampicillin resistance in the *Salmonella*. I will use *Shigella*, *Salmonella* agar to separate the *Salmonella* from the *E.coli*. SS agar expresses *Salmonella* distinct characteristics. If *Salmonella*'s genes mutate they may be resistant to Ampicillin.

MATERIALS AND METHODS

Nutrient broth was prepared in the following format. 8 grams of Nutrient broth mix and 1 liter of distilled water made 1000 ml of Nutrient broth. Test tubes were filled with 10 ml of broth each. The test tubes were autoclave for 45 minutes at 121c. After autoclaving the tubes they were placed in the incubator.

Nutrient agar was prepared in the following format. 3 grams of Bacto Beef Extract, 5 grams of Bacto peptone, 15 grams of Bacto agar and 1 liter of distilled water were mixed. The mix was when boiled for 1 minute to completely dissolve the mix (Difco Manual) This made 1000 ml of agar. 500 ml was poured into another flask and both flasks were autoclaved for 30 minutes at 121c

Ampicillin was mixed into one of the 500 ml of agar. 1 ml of Ampillican to every 100 ml of agar.

Gradient plates were made next. The plates were tilted at an equal angel. 20 ml of agar and Ampicillin mix was pour first and set for 24 minutes. Then the plate were turned around and tilted at the same angel as before. 20 ml of the plain agar was poured on top of the agar mix and set for 24 minutes.

The bacteria were re-hydrate. For the *Salmonella*, 1 ml of the re-hydrating broth was poured into the bacteria. The re-hydrated *Salmonella* was then poured into the rest of the re-hydrating broth and incubate for 24 hours at 37c. 5 ml of distilled water was poured into the tube with the *E. coli*. The *E.coli* was incubate at 37 c of 24 hours.

Both bacteria were cultured. Both bacteria were placed in the nutrient broth and incubated at 37 degrees for 24 hours.

The cultured *E. coli* was placed in to nutrient broth that was mixed with different concentration of Ampicillin. The concentrations were 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml, 0.8ml, 0.9ml, 1ml and 2ml. The same procedure described above was done with *Salmonella* and incubated at 37 degrees for 24 hours.

E. coli cultured with the Ampicillin and *Salmonella* that was not in the Ampicillin mix, were mated together placed in, and incubate for 24 hours.

1ml of mated bacteria was spread onto the gradient plates and incubated at 37 degrees for 24 hours.

SS agar was prepared in the following format. 60 grams of mix and 1 liter of distilled water was mixed and brought to a boil. The SS agar was not autoclaved. After the plates were filled the tops of plates were left off slightly for two hours, so the plates could dry out.

Using a velveteen disk the bacteria was transferred from the gradient plates to SS agar plates. Incubate for 24 hours.

RESULTS

All of the gradient plates had growth on them. The bacteria seemed to grow just as well in the lower concentrations (0.1ml) as in the higher concentrations (2ml). The *Salmonella* that was not mated with *E.coli* did not have any growth. There was also significant amount of growth on the SS agar plates. Some of the growth on the plates had black centers and were not clear, giving evidence that the colonies are not *Salmonella*. *Salmonella's* colonies should be translucent. The other colonies did look translucent. So a lactose fermentation test was preformed to conform the presence of *Salmonella*. Three tests were preformed one with *Salmonella*, *E.coli* and the unknown colony growth. *E.coli* showed a change in color from red to yellow, giving a positive lactose reading, but no gas was produced. Both *Salmonella* and unknown color stayed red, giving a negative lactose reading. The *Salmonella* produced gas, but the unknown did not. Slides were made to look at the structures of all the bacteria, so that the unknown could be morphologically characterized. The slides of The *E.coli* had long rods, the *Salmonella* were small rod shaped bacteria and the unknown bacteria were small but had a more circular

shape. The unknown culture was not cultured at the same time *Salmonella* and *E.coli* was. The age of unknown bacteria could be the reason it looked different from other bacteria. Further biochemical testing would be needed characterize the unknown bacteria.

DISCUSSION

A problem with this experiment is trying to identify the bacteria that have grown on the SS agar plates. The SS agar plates where recommended as a superior media for isolation for *Salmonella* and *Shigella* (DIFCO Manual) With out doubt there is bacteria that is resistant to the Ampicillin. The lactose test and the slides did not confirm that the bacteria is in fact Ampicillin resistant *Salmonella*. The bacteria had traits from both *E.coli* and *Salmonella*. A Gel Electrophoresis would be the next step to help identify the unknown bacteria. Antibiotic resistant bacteria is a problem the CDC is researching, because of the concern that soon there will not be a cure for bacterial infections, because of the use of antibiotics for growth promoters in livestock.

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