



HORIZONTAL GENE TRANSFER OF DRUG RESISTANCE GENES BETWEEN *SALMONELLA* AND *ESCHERICHIA COLI*

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Abstract: The physiological process that takes in the living organism is coded by a section of the DNA, the gene. Even resistance to antimicrobial by bacteria is conferred by resistant gene. Resistant *Salmonella* spp. and susceptible *E. coli* was used to study drug resistance gene transfer, which can be transferred by conjugation, transduction, or transformation. Materials used are, DST media, inoculating loops and sterile swabs. Furthermore, eight antibiotics were used in the study. The susceptibility and resistance of the *E. coli* and *Salmonella* spp. was first confirmed. Diagnostic sensitivity test of the *E. coli* under three conditions (*E. coli*+ Dead *Salmonella* spp., *E. coli*+ live *Salmonella* spp., *E. coli*+ DNA) were done. Results from the study confirm that the susceptible bacteria, *E. coli* was able to acquire resistance genes from resistant *Salmonella* spp. under the three conditions. Results proved that the most efficient means of acquiring resistance genes is through conjugation. It is recommended antibiotics that alter the cell wall as the most effective in capping resistance.

Key Words: Gene, HGT, Antibiotics, Resistance, Susceptible

INTRODUCTION

All the process occurring in the living organism is dictated by a section of the DNA called the gene. Even resistance to antimicrobial by bacteria is conferred by resistance gene. Many previously susceptible bacteria have acquired resistance and rendered many antibiotics ineffective. This is a major concern in the medical field following increasing cases of untreatable diseases that were previously treatable. This has been attributed to the ability of non-resistant bacteria to acquire resistant genes from the resistant strains. In addition, study need to be carried out to show that bacteria "resistant gene" from the resistant bacteria can be transferred from bacteria to bacteria of the same species and different species as well. Resistant *Salmonella* spp. and susceptible *E. coli* were used in this study on the mechanisms of interspecies resistance gene transfer.

A gene is section of the DNA encoding particular polypeptide chain or functional RNA such as an rRNA, tRNA or small non-coding RNA. It can also be defined as a unit of heredity in a living organism. It normally resides on a stretch of DNA that codes for a type of protein or for an RNA chain that has a function in the organism. All living things depend on genes, as they specify all proteins and functional RNA chains. Genes hold the information to build and maintain an organism's cell and pass genetic traits to offspring, although some organelles (eg. mitochondria) are self-replicating and are not coded for by the organism's DNA (Pearson, 2006). The modern definition of a gene which states that a gene is a locatable region of

genomic sequence, corresponding to a unit of inheritance, which is associated with regulatory regions, transcribed regions, and or other functional sequence regions (Pennisi, 2007). On the other hand, gene transfer can be divided into two: Horizontal Gene Transfer and vertical gene transfer. Vertical gene transfer occurs when an organism receives genetic material from its ancestor, e.g., its parent or a species from which it has evolved. While Horizontal Gene transfer, is any process in which an organism incorporates genetic material from another organism without being the offspring of that organism (Todar, 2008).

Bacterial resistance is the capacity of bacteria to withstand the effects of antibiotics or biocides that are intended to kill or control them. Multiple resistance (MR) or "multi-resistance" is a term used when a bacterial strain is resistant to several different antimicrobial classes (Todar, 2008). "Cross-resistant" bacteria are those that have developed survival methods that are effective against different types of antimicrobial molecules with similar mechanism(s) of action. Bacteria can transfer bits of genetic material to other bacteria, and when genetic information coding for several unrelated resistance mechanisms is transferred in a single event and expressed in the new bacterial host it is referred to as "co-resistance" (Todar, 2008).

A resistant gene transfer in bacteria is actually the horizontal gene transfer where different bacteria

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of the different species (one having developed resistant gene) transfer resistant gene to the one having not developed resistance to antibiotics. Several mechanisms have evolved in bacteria, which confer them with antibiotic resistance. These mechanisms can chemically modify the antibiotic, render it inactive through physical removal from the cell, or modify target site so that the antibiotic does not recognize it. The most common mode is enzymatic inactivation of the antibiotic. An existing cellular enzyme is modified to react with the antibiotic in such a way that it no longer affects the microorganism. An alternative strategy utilized by many bacteria is the alteration of the antibiotic target site, (Talaro, 2008).

Recent studies provide increasing evidence of adverse human health consequences due to the occurrence of resistant microorganisms. Infections that would otherwise not have occurred. Use of antimicrobial agents in humans and animals affect the health, placing those on help at increased risk of certain infections. For example people treated using antimicrobial drugs for unrelated reasons, for instance treatment of an upper respiratory tract infection, are at increased risk of infection with *Salmonella* that are resistant to the antimicrobial agent. This increased risk can be expressed if the *Salmonella* were not resistant or if the person had not been taking the antimicrobial agent for the unrelated reason. In a recent review over one million cases of *Salmonella* infection each year in the United States, it was estimated that antimicrobial resistance in *Salmonella* may result in about 30 000 additional *Salmonella* infections leading to about 300 hospitalizations and 10 deaths (WHO, 2004)

Horizontal gene transfer was first described in Japan in a 1959 publication that demonstrated the transfer of antimicrobial resistance between different species of bacteria. In the mid-1980s, Syvanen predicted that lateral gene transfer existed had biological significance. It was initially thought that Horizontal gene transfer occurs only between same species. However, it is increasingly becoming clear that Horizontal gene Transfer has been very important in the evolution of many bacteria species. (Wholvertson, 2008)

The development of resistance is inevitable following the introduction of new antimicrobial. Initial rates of resistance to new drugs are normally approximately 1%. However, modern uses of antimicrobial have caused a huge increase in the number of resistant bacteria. In fact, within 8-12 years after widespread use, strains resistant to multiple drugs become widespread. Multiple drug resistant strains of some bacteria have reached the proportion

that no antimicrobial are available for treatment. (Todar, 2008)

Several mechanisms are developed by bacteria in order to acquire resistance to antimicrobial. All require either the modification of existing genetic material or the acquisition of new genetic material from another source. Horizontal gene transfer (HGT) is a process whereby genetic material contained in small packets of DNA can be transferred between individual bacteria of the same species or even between different species. Conjugation occurs when there is direct cell-cell contact between two bacteria (which need not be closely related) and transfer of small pieces of DNA called plasmids takes place. This is thought to be the main mechanism of HGT. Acquisition of the resistance gene has been attributed to the increasing number of the resistant bacteria and the ability to acquire the resistance gene. Several researches focus on the increasing number of the bacterial resistance and have little concern on the efficiency of the horizontal gene transfer mechanisms, (Falagas & Blizotis, 2007)

The objective of this study was to find out inter species gene transfer between resistant *Salmonella* and susceptible *E. coli* and to determine the state at which susceptible *E. coli* acquire more resistance genes from the resistant *salmonella* spp.

MATERIALS AND METHODS

Area of Study

University of Eastern Africa, Baraton is located in Nandi County in the western highlands of the Rift Valley province in Kenya. The University is located at 00.25653° N and 35.08250° E at an altitude of 1970 meters above sea level. The *Salmonella* sp used was previously isolated from the University dairy Farm. The farm has dairy cattle and chicken, which serve the university population and the neighborhood. The area is dowered with cold and rainy seasons as well as hot and dry ones. The experimental aspects of this study were carried out in the microbiology laboratory of department of Biological Sciences.

Acquisition of the Samples

The multidrug resistant (MDR) *Salmonella* isolates used in this study were previously obtained from the dairy farm using enrichment, selective and differential media. The susceptible *E. coli* species that was used is a commercially acquired strain used in the laboratory.

The Horizontal Gene Transfer experiments

The commercially obtained *Salmonella* spp. and the *E. coli* were handled as follows: In the present study there was a working culture and the stock culture for the entire experiment. The commercially obtained *E. coli* was

resuscitated and then aseptically, using sterile swap, it was inoculated into the Tryptic Soy Broth. The inoculates were incubated at the temperature of 37.5 degree Celsius for 24hrs. *Salmonella spp* was also resuscitated and aseptically inoculated into the TSB and incubated for 24hrs at 37.5 degree Celsius. The antimicrobial susceptibility patterns of the two bacterial species were determined on Diagnostic Susceptibility Test agar using the following antibiotics: Ampicillin, Tetracycline, Nitrofuranto, Nolidixic Acid, Streptomycin, Cotrimoxazole and Gentamicine. Endo Agar and the Typtic Soy Broth. The experiment first tested the susceptibility of the *E. coli* and *Salmonella spp.* against eight antibiotics. A lawn of *E. coli* and *Salmonella spp* was made on the different DST plates using two different sterile swaps for each species of bacteria. Then an octo disc with the eight antibiotics was aseptically placed on top of the lawn on plate of *Salmonella spp.* The same was done to *E. coli*. The two plates were left for 24hrs in an incubator. Finally, their susceptibility test results were recorded.

Horizontal Gene Transfer experiment then followed. This experiment dealt with the two bacteria in three states. The three states are explained below.

The first part was the culture of *E. coli* and live *Salmonella spp* in DST. The two was cultured in DST because susceptibility test was to be carried out. Therefore, that gene exchange by the process of conjugation would take place between the two bacteria. The proper aseptic technique was complied with in all the inoculations. Pure culture of the *E. coli* was then be obtained from the mixture using Endo agar. This was achieved using the quadrant streak method and the S shape at the middle showed a pure colony in which it appeared as shiny colonies of *E. coli* on the agar surface. One colony was then picked using the sterile inoculating loop. The picked colony was inoculated into TSB and incubated for 24hrs. This was then picked using sterile swap, which was used to make a lawn on DST, and susceptibility was carried out following the procedure explained above. The results were recorded for the *E. coli* suspected to have acquired resistance genes through process of conjugation and results recoded.

The second part was done by mixing live *E. coli* with *Salmonella* killed by suspending a colony of *Salmonella spp.* with 1ml distilled water, vortexing for 15 seconds, and then placed in hot water bath for 3-4 minutes. The *E. coli* was then cultured in TSB for 24 hours at 37°C. Then when the growth was realized in 24hours, a colony of *E. coli* suspected to have acquired the resistance gene from *Salmonella spp.* underwent the Susceptibility Test again to see if it would have acquired resistance to the eight antibiotics. Once again, the results were recorded on the notebook.

Then what followed this was extraction of the DNA from *Salmonella spp.* This was done using a simple method. A colony of *Salmonella spp* was selected; inoculate into the 1ml distilled water using inoculating loop, then vortexed for 15 seconds, followed by placing the vortexes into hot water bath for 5 minutes. The mixture was then centrifuged for 20 minutes. The supernatant was collected which contains *Salmonella spp* DNA. The DNA collected was then be mixed with *E. coli* in broth, allowed to grow and acquire the DNA. Finally, a lawn was made on DST and octodisc was aseptically placed on the made lawn on DST, susceptibility test was carried and results recorded after 24hrs of incubation

In this experiment, there was a control experiment in which Calcium chloride, *Salmonella spp* DNA and *E. coli* was cultured in broth. Cad2 increases the affinity of *E. coli* to acquire DNA from *Salmonella spp.* This was to act as control experiment. Also the susceptibility Test was carried out in which this control experiment was used as reference against the other three.

RESULTS

E. coli before gene transfer was susceptible to all the antimicrobial drugs used, namely ampicillin, gentamicin, cotrimoxazole, sulfamethoxazole, streptomycin, nalidixic acid, nitrofuranto, and tetracycline (see table 1 below). *Salmonella spp* was resistant to all these antimicrobials.

Table 1: Antimicrobial susceptibility patterns of *E. coli* and *Salmonella spp* before gene transfer experiment

Bacteria Antibiotic	A	G	Co	Sx	S	Na	NF	T
<i>E. Coli</i>	S	S	S	S	S	S	S	S
<i>Salmonella spp.</i>	R	R	R	R	R	R	R	R

After the gene transfer experiments, the results were such that the *E. coli* that was mixed with dead *Salmonella* and that that mixed with live *Salmonella* acquired the seven genes each out of the eight genes in study (see table 2 below).

Table 2: Antimicrobial susceptibility results of *E. coli*

Bacteria/Antibiotic	A	G	Co	Sx	S	Na	NF	T	ACQD GENES
<i>E. coli</i> + Dead <i>Salmonella spp</i>	R	I	R	R	R	R	R	S	7
<i>E. coli</i> + live <i>Salmonella spp.</i>	R	R	R	R	R	R	R	S	7
<i>E. coli</i> + DNA	R	S	R	R	R	S	R	R	6
<i>E. coli</i> + CaCl ₂ + DNA	R	S	R	I	S	S	R	S	3

A-Ampicillin, **T-**Tetracycline **NF-**Nitrofuranto **Na-**Nolidixic Acid **S-**Streptomycin **Co-**Cotrimoxazole

G-Gentamicine. **R-** Stands for resistance **I-** Stands for Intermediate **S-**Susceptible.

DISCUSSION

Inter-species Gene transfer

Horizontal gene transfer is common among bacteria, even amongst very distantly related ones. This process is a significant cause of increased drug resistance when one bacterial cell acquires resistance and quickly transfers the resistance genes to many species. As Jain et al., (1999) put it: Increasingly, studies of genes and genomes are indicating that considerable horizontal transfer has occurred between prokaryotes. The phenomenon appears to have had some significance for unicellular eukaryotes as well. As Baptiste et al., (2005) observe, additional evidence suggests that gene transfer might also be an important evolutionary mechanism in protist evolution.

Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. Genes can be transferred between bacteria in a horizontal fashion by conjugation, transduction, or transformation. Thus a gene for antibiotic resistance which had evolved via natural selection may be shared. Evolutionary stress such as exposure to antibiotics then selects for the antibiotic resistant trait. Many antibiotic resistance genes reside on plasmids, facilitating their transfer. If a bacterium carries several resistance genes, it is called multi resistant (Raghunath, 2008).

In this study, the antibiotic resistance genes from the *Salmonella Spp* were used as selection factor to indicate the possibility of the inter-species gene transfer among bacteria. The laboratory evidence from the study shows that the antibiotic resistance genes were transferred from *Salmonella Spp* to *E. coli*. Table 1 shows the susceptibility test of the *E. coli* before the Horizontal gene transfer experiment was done.

The data collected and recorded represent susceptibility test of the *E. coli*. The susceptibility test was measured using a ruler in which the unit of measurement was in millimeters. Using the reference chart, it indicates that if the diameter of the circle around the antibiotic is <13mm, then the bacteria is susceptible to the antibiotic. Moreover, less than 13mm, means the bacteria is resistant to the antibiotic. And if the diameter is approximately 13 it's neither resistant nor susceptible, therefore it's intermediate.

The data above shows the susceptibility test of *E. coli* alone when subjected to the eight antibiotics. The results show that *E. coli* is susceptible to all the antibiotics. As mentioned earlier, the commercially obtained *E. coli* was susceptible. Likewise to the *Salmonella spp*, it shows that it's resistant to the eight antibiotics which was under the test.

This study also focused on the *E. coli* plus the dead *Salmonella spp*. *Salmonella* was killed by inoculating a colony into distilled water then placed in the boiling water bath for 3 minutes. This was then later mixed with live *E. coli*. DST was then obtained and a lawn made. Then incubate for 24hrs. The results are shown in table 2 above. *E. coli* was able to acquire all the resistant genes from the dead *Salmonella spp* except the genes for Tetracycline Resistance. And it could partially acquire resistance gene against Gentamicine. This means to some extent, not all the resistance gene can be transferred. The Tetracycline antibiotics are protein synthesis inhibitors, inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex. They do so mainly by binding to the 30S ribosomal subunit in the mRNA translation complex. This could not allow the transfer of the resistance gene from the dead *Salmonella spp*. to the *E. coli* because *E. coli* was experiencing the protein synthesis problem (Raghunath, 2008).

In addition, the study involved the culture of the two bacteria alive. Pure colony of *E. coli* and *Salmonella Spp* was obtained and aseptically using a inoculating loop, the two was inoculated into a TSB media allow to grow for 24hrs and then pure culture of *E. coli* was then isolated using Endo agar which appear as a shiny colonies. Then Susceptibility test of *E. coli* suspected to have acquired genes through conjugation was carried out in a DST agar. The results are shown on table 2. Once again, the tetracycline gave negative results. *E. coli* was susceptible to it following the same reason explained above. Gentamicin did not allow the transfer of resistance gene through conjugation. This is due to its mechanism of action by also disrupting the integrity of bacterial cell membrane apart from mismatching protein synthesis. The other drugs could micromanaged to disrupt the bacteria cell wall and the conjugation could take place.

Finally, DNA of the *Salmonella spp* was extracted. A pure colony of *Salmonella spp* was picked and inoculated into 2ml distilled water. After it was placed in hot water bath for 3-4 minutes and centrifuged for 20 minutes. The upper supernatant (DNA) was collected and using pipette, it was transferred into a TSB containing live *E. coli*. It was then allowed to grow for 48hrs. After which susceptibility test was carried out to inspect if transformation occurred the results shown on table 2as explained above. Thus, the resistance transferred. The results in the table above shows that Gentamycin and Nolidixic acid could not comply with expectation. The Gentamycine mode of action is well explained above which justify the reason why the results not positive. The Nolidixic acid mode of action is the cause of the failure for the failure of DNA cooperation. Nolidixic

acids inhibit DNA gyrase activity and induce formation of a relaxation complex analogue. Treatment of the complex causes a double strand break in the DNA substrate and the resulting linear molecule is covalently bound to protein. DNA gyrase is an essential bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA. Gyrase belongs to a class of enzymes known as topoisomerases that are involved in the control of topological transitions of DNA.

In the Control experiment, a culture of the *E. coli* in the environment of CaCl_2 which gave the *E. coli* more ability to be competent and incorporate high number of DNA into its system.

The experiments shows that the inter species gene transfer is possible among the different species of bacteria. This is shown in the pattern of the antibiotic resistance (See table 2) as shown in the table of the results.

Efficiency of the Horizontal Gene transfer

This discussion covers the three states and antibiotic mode of actions. Both these two factors influence the acquisition of the Resistance Gene and thus affecting the efficiency. There is need to study the mechanisms of action of the eight antibiotics which was used in this study.

Cotrimoxazole, which is 100% ineffective, the integrative medical group, says that these antibiotics inhibit production of folic acid by binding to the enzyme responsible for making folic and blocking the enzyme from making folic acid. It inhibits the bacterial enzyme much more than the human enzyme. The frequency of development of bacterial resistance to Co-trimoxazole is lower than it is to either of the components alone. Resistance in gram-negative bacteria is associated with the presence of R-factors, which can be transferred to susceptible microorganisms by conjugation. The co-administration of the drugs slows the absorption of Sulphamethoxazole. The half-lives of Trimethoprim and Sulphamethoxazole are 16 and 10 hours, respectively (Natural Medicines, 1996).

From the table 2 above it is very clear that the slowness of the Cotrimoxazole is the main reason for its dysfunctions. Since it takes 10 to 16 hours to function well in the system, the gene responsible for its resistance was already pick-up by the susceptible *E. coli*.

Ampicillin is also 100% ineffective. The data in the table 2 indicates that during the study the antibiotic could not be able to control the *E. coli*. Growth the data

shows all resistance(R). The mechanisms of actions determined its malfunctioning. It stops bacteria from multiplying by preventing bacteria from forming the walls that surround them. The walls are necessary to protect bacteria from their environment and to keep the contents of the bacterial cell together. Bacteria cannot survive without a cell wall. Nevertheless, Ampicillin resistance is usually a result of an enzyme (beta lactamase) that breaks down the ampicillin. The origin of the enzyme in certain resistant mutants is a mystery (Baker, 2006). This agree with the findings from this study that *E. coli* posses enough beta lactam enzyme in plenty that it was able to protect itself from the Ampicillin. But in the fast place before treatment, it could not protect itself from the Ampicillin, this clearly shows that *E. coli* preferred Genes from the resistant *Salmonella spp* or might have acquired linked genes with the other genes.

In the case of Tetracycline was 75% effective. This inhibits the bacterial growth by stopping protein synthesis. They have been widely used for the past forty years as therapeutic agent in human and veterinary medicine (Roberts et al., 1994) From the data record in the table 2, it very clear that it is our drug of choice only DNA + Live *E. coli* could resist its functioning. The way it function has made it antibiotic of choice, less resistance was encountered since its way of action affect the protein synthesis in which no bacteria can resist that. The results indicate that resistant gene for Tetracycline was transferred to *E. coli* during the study.

Gentamicine is 50% effective. Its protein synthesis inhibition is its mechanism of action. However, it is not fully known. They interfere with the proofreading process, causing increased rate of error in synthesis with premature termination (Levison, 2009) this is indicated on the table 2. Its interference with proofreading caused one intermediate resistance though more susceptible. This means actually one of the drugs of choice. Though its mechanisms of action are not clearly known, there is DNA stability from the information on the table 2.

Nitrofuranto works by damaging bacterial DNA. Its 100% ineffective. The result from the table two shows that it was not possible to control the *E. coli*. This will depend on the position of the gene coding for the nitrofuranto. There are higher chances that the gene coding its resistance entered into *E. coli* before it began to work on the DNA disruption.

Streptomycin is 25% effective and a protein synthesis inhibitor. It binds to the S12 Protein of the 30S subunit of the bacterial ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S

subunit. This prevents initiation of protein synthesis and leads to death of microbial cells. Before it binds to the protein subunits, the resistance genes had already been incorporated into the *E. coli* DNA. That is the reason why resistance was recorded in all except one. CaCl_2 seem to raise streptomycin mode of action.

Nolidixic acid is 50% effective, it can be effective or not. Its mechanisms of action is almost same with the others, it only works by DNA disestablish. Which can be seen from the table 2 above, the dead *Salmonella spp.* and the live one could not expose their DNA that is the reason why resistance was recorded. The other one exposed their DNA resulting to their digestion by the antibiotic.

The most effective means of the Resistance Gene transfer between two bacteria is when one possessing the resistance gene is dead. In the table 2 of the results shown above *E. coli* and *Dead Salmonella spp.* showed the highest number of the resistance gene transferred. Only tetracycline antibiotic was able to destroy the *E. coli*. The reason why tetracycline was the only antibiotic of choice is due to its mode of action. It works by binding to the 30S subunit of microbial ribosomes. They inhibit protein synthesis by blocking the attachment of charged aminoacyl-tRNA. Thus, they prevent introduction of new amino acids to the nascent peptide chain. The action is usually inhibitory and reversible upon withdrawal of the drug. Resistance to the tetracycline's results from changes in permeability of the microbial cell envelope. In susceptible cells, the drug is concentrated from the environment and does not readily leave the cell. In resistant cells, the drug is not actively transported into the cell or leaves it so rapidly that inhibitory concentrations are not maintained. Therefore, all these mechanisms of actions was maintained all the through the experiment. This means when one is infected with microbes and expect to be administered tetracycline, the correct concentration must be maintained for successful elimination of the microbes in the body system. The resistance to this antibiotic comes in when improper dose is administered resulting to the success of the bacteria in developing classical expression against the drug.

The antibiotic mode of action is very important when it comes to the resistance. In the case of the culture of the live *Salmonella spp.* and *E. coli*, the study found out that some antibiotics disrupting the cell membrane affect the conjugation of the bacteria. Since when the two bacteria are, alive the only mode of resistance gene transfer is through conjugation could not occur. Conjugation is the extension of the cell wall by the two bacteria creating a channel where resistance genes can be acquired. When the cell wall is

disrupted, no resistance was observed in the culture. This is very clear in the case of other drugs where their mode of action is not something to do with the cell wall interference.

Effectiveness of the antibiotics depends entirely on the mode of action. If the antibiotic mechanism of killing is by interfering with protein synthesis, there are high chances of this drug being resisted, because the bacteria can be able to acquire resistance gene through conjugation. This study prefers the antibiotics, which disturbs the functioning of the cell wall.

According to the present study where DNA extracted and cultured with live susceptible *E. coli* shows that those antibiotics in which their mode of action interfere with the nucleic acid synthesis and functioning was very successful in elimination of the bacteria. Otherwise, their functioning can be completely be limited by bacteria's conjugation means of acquiring resistance genes.

CONCLUSION

The present study clarifies that *E. coli* acquired the resistance gene from the resistant *Salmonella spp.* The alternative hypotheses, which Stated the cause of antibiotic resistance in *E. coli* is due to the acquisition of the resistance gene from the antibiotic resistant *Salmonella spp.* is supported. Bacteria can be able to acquire the resistance through conjugation, picking it from environment and from another dead resistant bacteria-transformation. The most efficient means of acquiring genes is through conjugation and the least means is through transformation. Therefore this study recommends cell wall interfering antibiotics.

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