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# Resistance of *Salmonella typhimurium* to Ethanol Based Hand Sanitizer

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***RESISTANCE OF S. TYPHIMURIUM******Abstract:***

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Salmonellosis is one of the most common food infections identified in medical microbiology. *Salmonella typhimurium*, a pathogenic bacterium that produces this food infection is found to be a problem in the United States and Canada (CDC 2015) (Public Health Agency of Canada 2014). It causes certain symptoms ranging from mild discomfort to death and depending on the individual's overall health. *S. typhimurium* is known to develop specific resistance mechanisms that allow it to withstand harsh environments produced by certain antibiotics and antibacterial agents. Antibacterial agents such as ethanol based hand sanitizers are common in today's society and can be found in almost any food industry, hospital or daycare facility. Identifying the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was required in order to determine the resistance of *Salmonella typhimurium*, Ames strain to ethanol based Advanced Gel Purell®. The inoculation of a series of 1:2 dilutions aided in determining the MIC while plating a lawn of bacterium helped identify the MBC resulting in two distinctive concentrations. The estimated concentration for MIC resulted in 6.3% (MIC<sub>6.3</sub>) while MBC presented a concentration of 25% (MBC<sub>25</sub>).

Key words: Salmonellosis, *Salmonella typhimurium*, Ames strain, Resistance, Dilutions, Ethanol based hand sanitizer, Minimum inhibitory concentration, Minimum Bactericidal concentration.

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**RESISTANCE OF *S. TYPHIMURIUM******Introduction:***

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*Salmonella typhimurium* is an important species of pathogenic bacterium. Discovered by Dr. Salmon and his colleague, these bacterial cells are flagellated, gram-negative and bacillus shaped organisms (Zinsser 1936). The *Salmonella* genus is a member of the family Enterbacteriaceae which is composed of bacteria that are related phenotypically and genotypically (Todar 2012). When ingested, *S. typhimurium* causes a common form of food infection known as salmonellosis (Jackson and Smyth 1964). Being ubiquitous in our environment, this particular pathogen has the potential to be found in places like: food industries, hospitals, daycare facilities, in the intestines of swine, cattle, poultry, in uncooked and/or undercooked food such as eggs, meat and even contaminated fruit and water. As the infection is newly acquired in each instance, every patient may respond differently post infection. Symptoms may range from mild discomfort to death, depending on the age of the individual and their health prior to infection. Currently, *Salmonella* infections are known to be problematic in North America. Salmonellosis occurs in the United States with an estimated 1,000,000 annual illnesses, 19,000 hospitalizations and 380 deaths (CDC 2012). These occurrences are commonly associated with contamination of food and water. Due to the high amount of cases and lack of health care, the U.S. Department of Agriculture (USDA) has estimated that *Salmonella* infections cost \$2.65 billion per year with an average cost of \$1,896 per case (Center for Infectious Disease Research and Policy 2010). Foodborne infections like salmonellosis are far more common in Canada. The Public Health Agency of Canada estimates about 4 million Canadians suffer from this food related infection each year. Salmonellosis is the second most frequently reported foodborne infection in Canada (Public Health Agency of Canada 2014).

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Due to the increasing rate of illnesses from bacterial infection, it becomes important to establish a line of defense mechanisms for these pathogens (Mazzola et al. 2009). The use of antibacterial agents helps to control bacterial populations. In order for this to occur, such agents must interfere with the reproduction and growth of the bacterium.

Antibacterial agents are categorized into disinfectants, antiseptics and antibiotics (APUA 2014). “Biocide” is a general term that is used to describe a broad spectrum of chemical agents like disinfectants and antiseptics (McDonnell and Russell 1999). Disinfectants are described as agents used to eliminate nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) and are specifically used on surfaces of inanimate objects, while antiseptics are characterized as biocides that destroy or inhibit the growth of microorganisms in or on living tissue (e.g., hand soap) (McDonnell and Russell 1999).

Worldwide, the most commonly used disinfectant or antiseptic agent is alcohol. Due its well-known antimicrobial properties, alcohol is increasing its popularity and therefore has become the active ingredient in most antimicrobial products, for commercial use (e.g., hand sanitizers).

Other antiseptic agents that may serve as active ingredients in hand sanitizers include:

chlorohexidine, chlorine, hecachlorophene, iodine, chloroxylenol (PCMX), benzalkonium chloride and triclosan (Yazdankhah et al. 2006). Hand sanitizers are sold in the form of a gel, foam, liquid, mist or wipe. They are typically used as a substitute for hand washing with soap and water because it is a much quicker and easier process. Researchers have discovered that diluting alcohol with water to 70%-60% is an appropriate concentration for antiseptics and disinfectants (Reynolds, Levy and Walker 2006). 100% alcohol makes the product inefficient for microbial elimination, since alcohol is known to evaporate rapidly once spread on surfaces or tissue. Therefore, concentration of the substance must correlate with exposure time in order to

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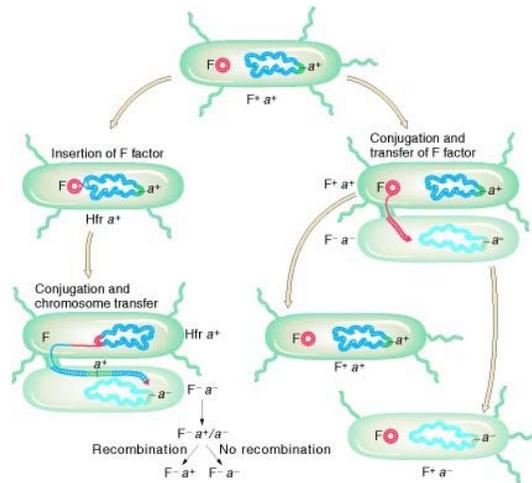
eliminate a significant portion of any microbial population (Occupational Safety and Health Branch Labor Department 2007).

Typically, antimicrobial agents are meant to interfere with bacterial reproduction and growth. However, when commercial products like hand sanitizers are over used, this can result in bacterial resistance. Further problems may arise when individuals become reliant on these products. Some pathogens are more resistant to certain biocides than others, depending on their virulent factors (Russell 1998). Resistance mechanisms vary from pathogen to pathogen and some are able to exhibit more than one mechanism. If the population of microorganisms is exposed to a high concentration of an antimicrobial agent, susceptible cells will be killed. Some cells in the population may possess a degree of natural resistance which is known as innate resistance while some may have acquired resistance through mutation or genetic exchange (Bower and Daeschel 1999).

Innate resistance is a chromosomally controlled property associated naturally to microorganisms (Anderson and O'Toole 2008). Innate resistance can slightly differ between the species and strains that are located in identical environmental conditions and antimicrobial concentrations (Davidson and Harrison 2002). Mechanisms of innate resistance include impermeable cellular barriers which prevent the entry of an antimicrobial agent. This is seen in gram-negative bacteria due to the outer membrane exterior of the cell wall which consists of lipopolysaccharides and protein (Kaiser 2012). Other innate mechanisms include efflux pumps and inactivation of antibacterial agents. Efflux pumps secrete antibacterial agents from the cell while antibacterial inactivation is caused from enzymatic activity (Bower and Daeschel 1999).

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During acquired resistance, bacterial cells change genetically through mutation or the direct gain of genetic material from plasmids (Russell 1991). Mutations are rare and spontaneous changes which are thought to occur in about one in one million to one in ten million cells where the genetic mutations yield various types of resistance (APUA 2014). Some mutations are able to influence bacteria to make the enzymes that inactivate any antimicrobial agents while other mutations alter the target that the agent attacks (APUA 2014). Bacteria can also acquire resistant genes by undergoing a process known as conjugation. Bacterial conjugation is the transfer of genetic material (e.g., plasmids) between two or more cells by direct cell to cell contact or a bridge like connection (Griffiths et al. 2000). The process of conjugation can be seen in Figure 1. Another way bacteria can acquire resistant genes are through viral transduction (APUA 2014).



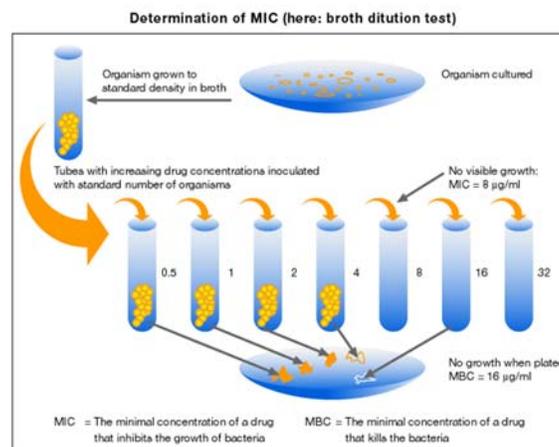
<http://www.ncbi.nlm.nih.gov/books/NBK21942/>

**Figure 1:** The conjugational cycle of *E. coli*

The purpose of this research is to test the resistance of *Salmonella typhimurium* to Advanced Gel Purell<sup>®</sup>, a leading brand of ethanol based hand sanitizer. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) will help establish the

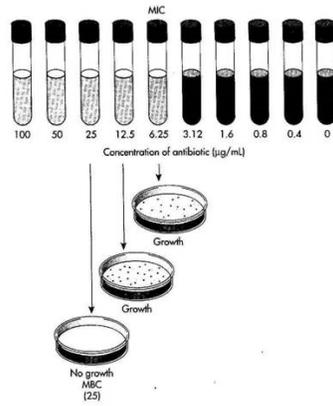
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resistance of *S. typhimurium* in this agent. The MIC is defined as the lowest concentration of antimicrobial agent that inhibits visible growth of a microorganism (Andrews 2001). This can be established through careful observations of the broth. If broth changes from clear to cloudy post incubation then growth of the microorganism occurred. In order to determine the MIC, bacterial cells must be inoculated and grown in mixtures of different antimicrobial concentrations and broth, as seen in figure 2. The minimum bactericidal concentration (MBC) is the lowest concentration of an antimicrobial agent that prevents any growth of a microbe after being sub-cultured onto antibiotic-free media (Andrews 2001). The MBC is determined by observations, if colonies form on the surface of the antibiotic-free agar then the microbe has survived that concentration (figure 3).



[http://animalhealth.bayer.com/ah/fileadmin/images/baytril/food\\_animals/NT\\_3\\_2\\_2\\_1\\_1.gif](http://animalhealth.bayer.com/ah/fileadmin/images/baytril/food_animals/NT_3_2_2_1_1.gif)

**Figure 2:** Minimum inhibitory concentration (MIC) observed when growth inhibition occurs in broth

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[http://intranet.tdmu.edu.ua/data/kafedra/internal/micbio/classes\\_stud/en/pharm/prov\\_pharm/ptn/Microbiology%20with%20basis%20immunology/2/Lesson%204.%20Microbial%20antagonism.files/image023.jpg](http://intranet.tdmu.edu.ua/data/kafedra/internal/micbio/classes_stud/en/pharm/prov_pharm/ptn/Microbiology%20with%20basis%20immunology/2/Lesson%204.%20Microbial%20antagonism.files/image023.jpg)

**Figure 3:** Minimum bactericidal concentration (MBC) observed when no growth occurs on plate

**RESISTANCE OF *S. TYPHIMURIUM*****Materials and Methods:**

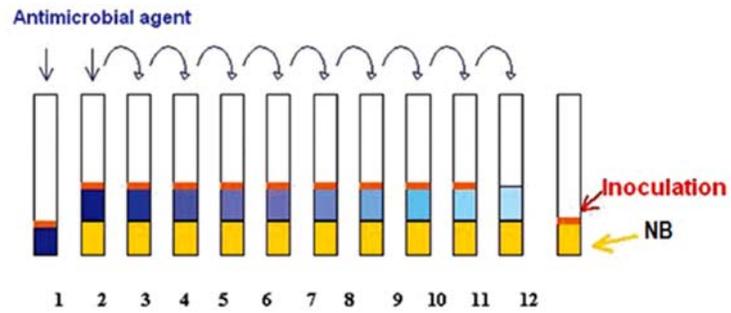
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**I. Bacteria Culture and growth:**

BD™ Difco™ Dehydrated Culture Media: Wallerstein Laboratory Nutrient Broth (No.:B247110) and BD™ Bacto™ Dehydrated Agar (No.B214050) was prepared for this experiment (Fisher Scientific 2013). Two mL of BD™ Difco™ Dehydrated Culture Media: Wallerstein Laboratory Nutrient Broth was dispensed into 13 Fisherbrand™ Heavy well Borosilicate disposable test tubes. Master plates were created by growing *S. typhimurium* on NA plates at 37°C for 24 hours.

**II. Sensitivity Testing:**

A series of 1:2 dilutions was prepared using Advanced Gel Purell® (70% ethyl alcohol). The thirteen different concentrations of Advanced Gel Purell® were 100%, 50%, 25%, 12.5%, 6.3%, 3.2%, 1.6%, 0.8%, 0.4%, 0.2%, 0.1%, 0.05% and 0%, as seen in Figure 4. These concentrations would translate to 70%, 35%, 17.5%, 8.8%, 4.4%, 2.2%, 1.1%, 0.6%, 0.3%, 0.14%, 0.07%, 0.03%, and 0% of ethyl alcohol. Following the appropriate aseptic technique, each concentration was inoculated with *S. typhimurium*, Ames strain. Once inoculated, the tubes were placed in an incubator shaker at 37°C overnight (Babu et al. 2011). The minimum inhibitory concentration (MIC) was determined empirically, post incubation. An aliquot of 100µl (from each concentration) was spread as a lawn onto the surface of the nutrient agar Fisherbrand™ 100x15 mm petri dishes (University of Missouri-St. Louis 1999). Each concentration was assessed with 2 replicates and plates were then incubated at 37°C for 24 hours (Babu et al. 2011). The minimum bactericidal concentration (MBC) was directly observed.

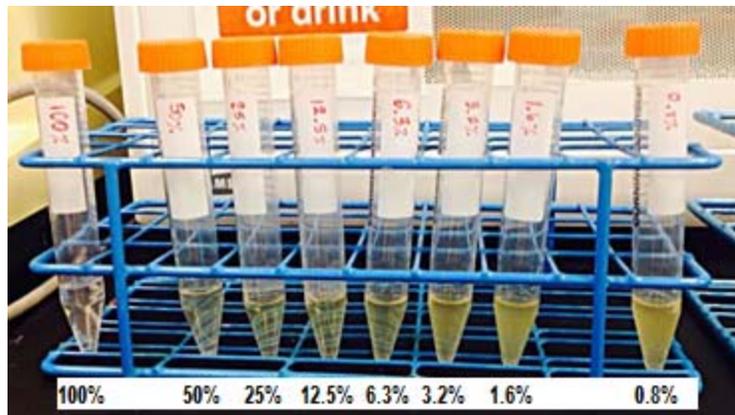
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<http://www.scielo.br/pdf/bjps/v45n2/v45n2a08>

Figure 4: Preparations of 1:2 dilutions of Advanced Gel Purell® (70% ethyl alcohol)

**RESISTANCE OF *S. TYPHIMURIUM*****Results:****I. Minimum inhibitory concentration (MIC), *Salmonella typhimurium* Ames strain:**

1:2 dilutions were prepared with Advanced Gel Purell®. Thirteen concentrations were inoculated with a consistent amount or loopful of *S. typhimurium* collected from master plates. 100%, with no nutrient broth and 0% (all broth and no hand sanitizer) were the controls for this experiment. Figure 5 and 6 together reveal negative growth in nutrient broth from 50% to 6.3% and positive growth from 3.2% to 0%. The lowest concentration of no visible growth is at 6.3%, therefore the minimum inhibitory concentration is 6.3% (MIC<sub>6.3</sub>).



**Figure 5:** Advanced Gel Purell® concentrations from 100% to 0.8%. No growth of *S. typhimurium* from 100% to 6.3% (MIC<sub>6.3</sub>) and positive growth from 3.2% to 0.8%

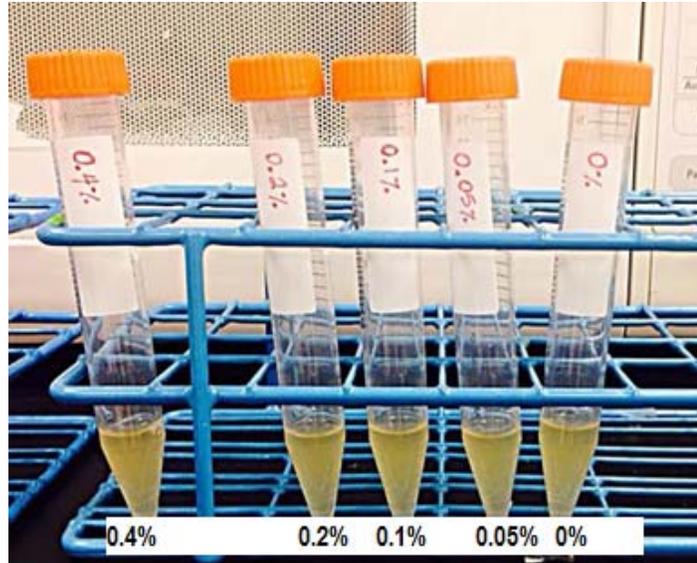
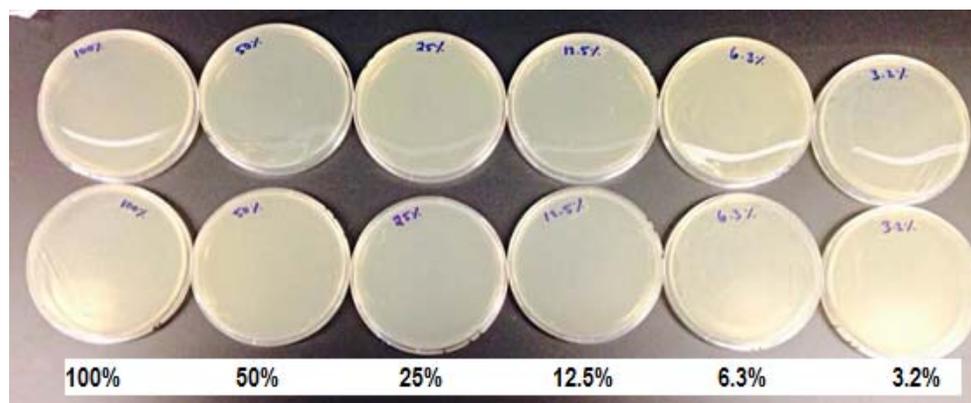
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Figure 6: Advanced Gel Purell® from 0.4% to 0% with positive growth of *S. typhimurium* in each concentration

**RESISTANCE OF *S. TYPHIMURIUM*****II. Minimum bactericidal concentration (MBC), *Salmonella typhimurium* Ames strain:**

After determining the MIC, all 13 concentrations were plated and grown in the incubator at 37°C, overnight. Each concentration contained replicates of two to increase accuracy, as seen in Figures 7 and 8. Negative growth was seen in 25% of both replicates and in one replicate from 12.5%, Figure 9. The other replicate from 12.5% contained 10 colonies of *S. typhimurium*. Concentrations from 6.3% to 0% presented full lawn growth thus; no colonies were properly seen in Figure 10. The lowest concentration that exhibited no growth on the plates of both replicates was 25% (MBC<sub>25</sub>).



**Figure 7:** Minimum bactericidal concentrations (MBCs) from 100% to 3.2%. No growth from 100% to 25% shows death of *S. typhimurium*. Positive growth is seen from 12.5% to 3.2%

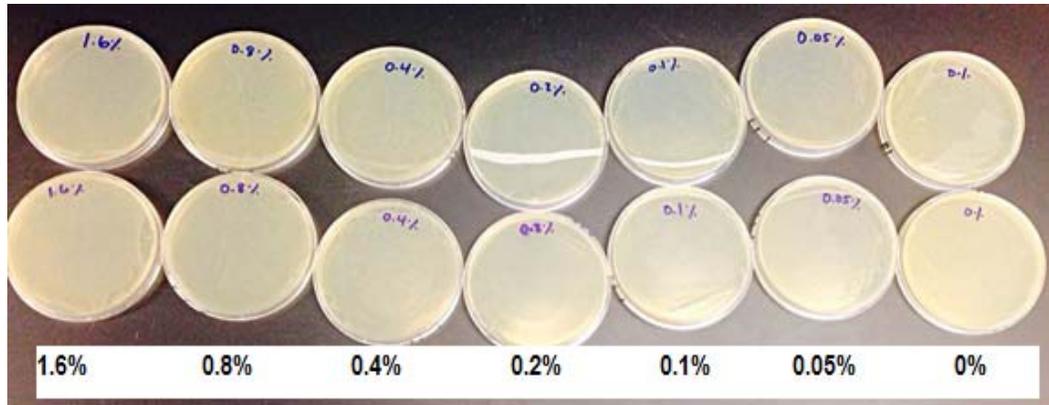
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Figure 8: Minimum bactericidal concentrations (MBCs) from 1.6% to 0%. Survival of *S. typhimurium* presented by positive growth on Nutrient agar plates

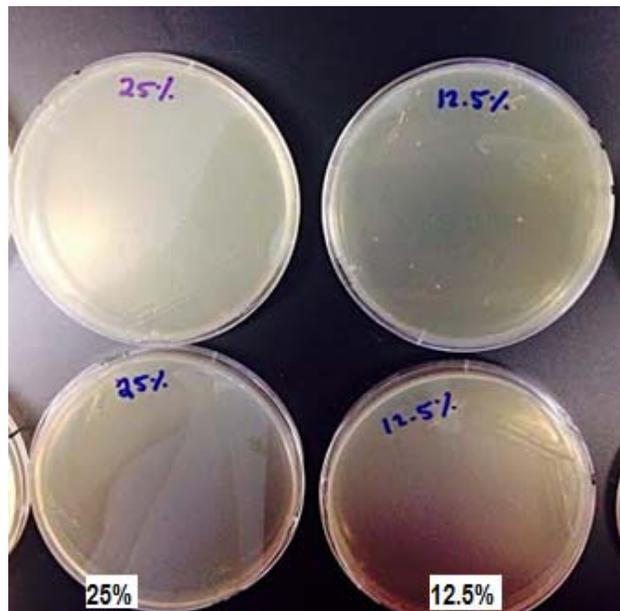


Figure 9: Minimum bactericidal concentration of 25% (MBC<sub>25</sub>) in both replicates

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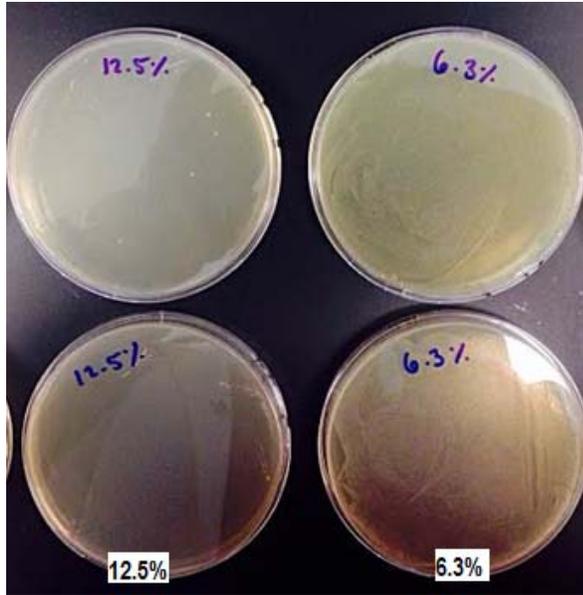


Figure 10: Formation of colonies in 12.5% in one plate (0 colonies in replicate). Lawn formation in 6.3% and lower

**RESISTANCE OF *S. TYPHIMURIUM*****III. Minimum inhibitory concentration (MIC), *Escherichia coli* KL25 strain:**

The minimum inhibitory concentration (MIC) of *E. coli* KL25 strain was determined. Figure 11 contains concentrations of 100%, 50%, 25%, 12.5%, 6.3%, 3.2%, 1.6%, and 0.8%, where 100% was one control. 100%, 25% and 12.5% revealed growth inhibition of *Escherichia coli* KL25. In figure 12, positive growth can be seen in concentrations 6.3%, 3.2%, 1.6%, 0.8%, 0.4%, 0.2% 0.1%, 0.05% and 0% (second control). Shown in figure 13 is the minimum inhibitory concentration for *E. coli* KL25 which is seen at 12.5% (MIC<sub>12.5</sub>).

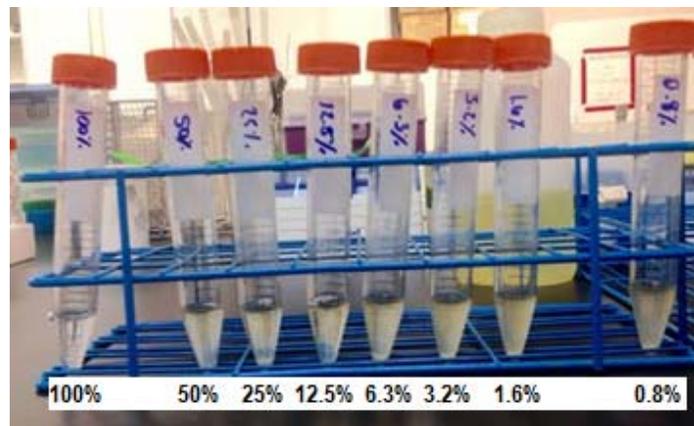


Figure 11: Minimum inhibitory concentrations (MIC) from 100% to 0.8% shows positive growth up to 12.5%.

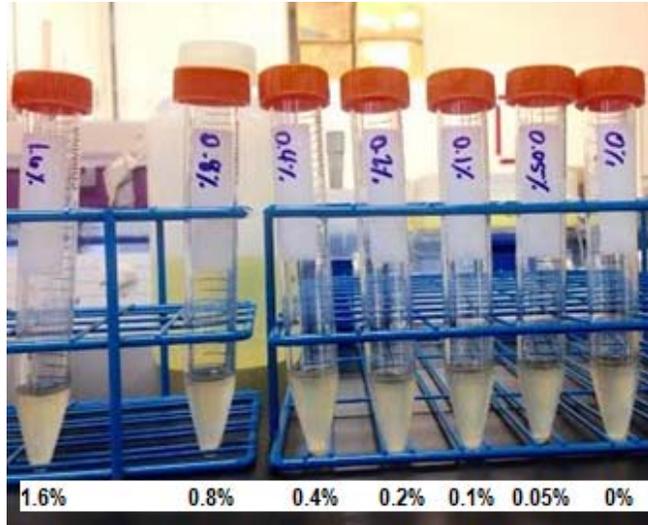
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Figure 12: Minimum inhibitory concentrations (MIC) from 1.6% to 0%. All tubes show positive growth.

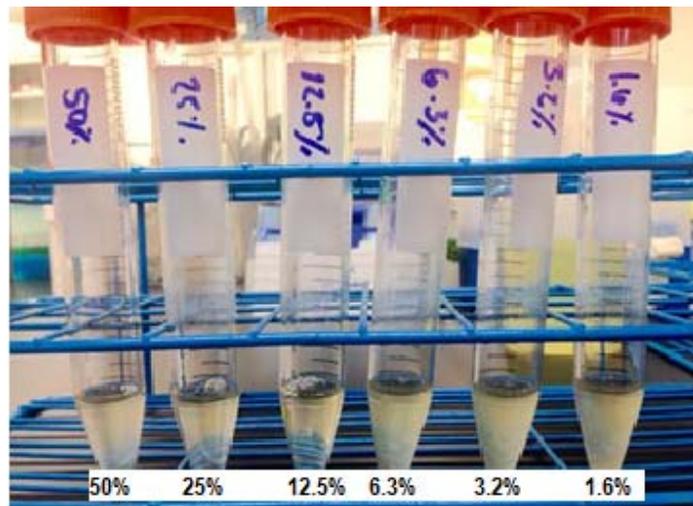


Figure 13: Minimum inhibitory concentration established at 12.5% (MIC<sub>12.5</sub>).

**RESISTANCE OF *S. TYPHIMURIUM*****IV. Minimum bactericidal concentration (MBC), *Escherichia coli* KL25 strain:**

Once grown on LB agar plates, the minimum bactericidal concentration (MBC) was determined for *Escherichia coli* KL25. Figure 14 shows the replicates starting at 100%, 50%, 25%, 12.5%, 6.3%, 3.2%, and 1.6%. No growth or death of *E.coli* KL25 was seen in plates of 100%, 50%, 25% and 12.5%. In figure 15, the remaining concentrations 1.6%, 0.8%, 0.4%, 0.2%, 0.1%, 0.05% and 0% (control) all showed to have positive growth in each replicate. The minimum bactericidal concentration (MBC) for *E. coli* can be seen at 12.5% (MBC<sub>12.5</sub>), in figure 16.

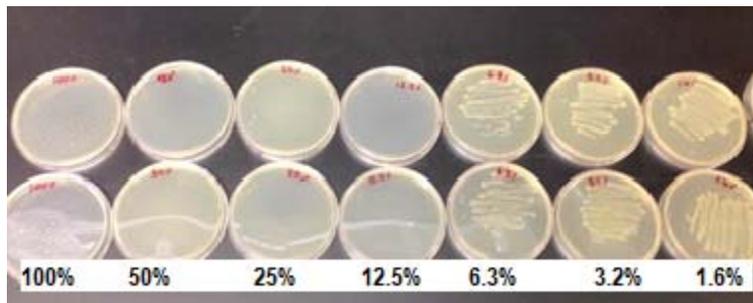


Figure 14: Minimum bactericidal concentrations (MBC) from 100% (control) to 1.6%.

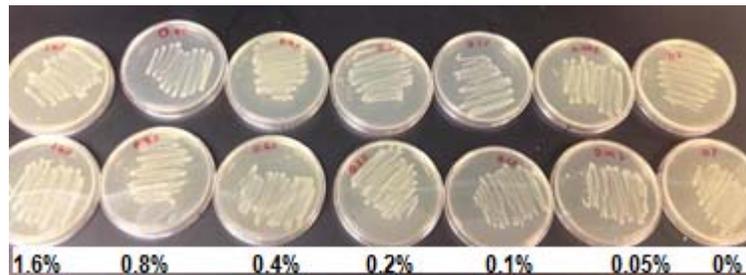


Figure 15: Minimum bactericidal concentrations (MBC) from 1.6% to 0% (control). All plates show positive growth.

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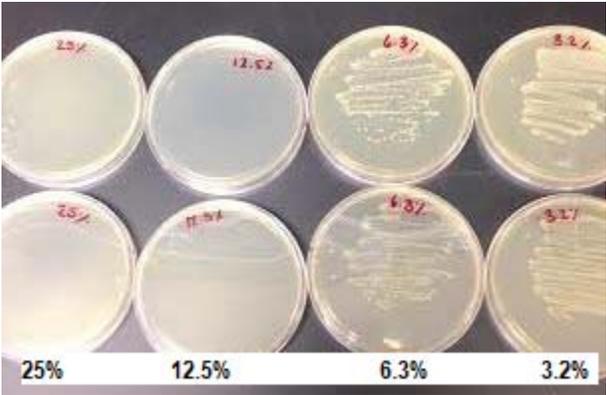


Figure 16: Minimum bactericidal concentration (MBC) of E. coli established at 12.5% (MBC<sub>12.5</sub>).

**RESISTANCE OF *S. TYPHIMURIUM*****V. Minimum inhibitory concentration (MIC), *S. typhimurium* Ames strain and *E. coli*****KL25 strain mixed culture:**

The of minimum inhibitory concentration (MIC) was observed for the mixed culture which contained *Salmonella typhimurium* Ames strain and *Escherichia coli* KL25 strain. In figure 17, concentrations starting at 100% (control), 50%, 25%, and 12.5% have inhibited the growth of both *S. typhimurium* Ames strain and *E.coli* KL25 strain. 6.3%, 3.2%, 1.6%, 0.8%, 0.4%, 0.2%, 0.1%, 0.05% and 0% (control) contain positive growth of the mixed culture (figures 17 and 18). The minimum inhibitory concentration can be seen in figure 19, at 12.5% (MIC<sub>12.5</sub>).

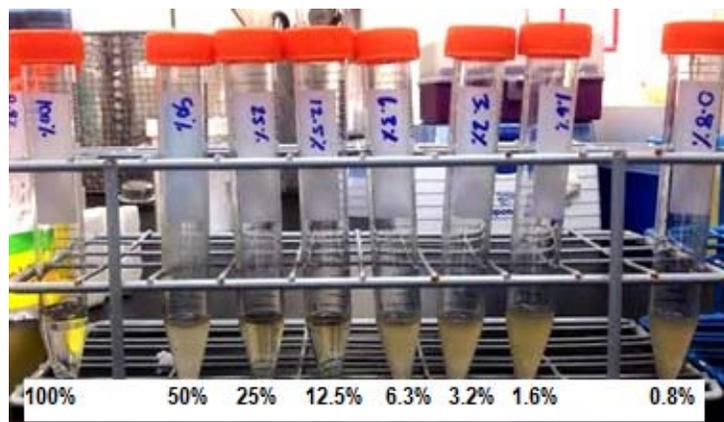


Figure 17: Test concentrations from 100% to 0.8% show negative and positive growth of mixed culture.

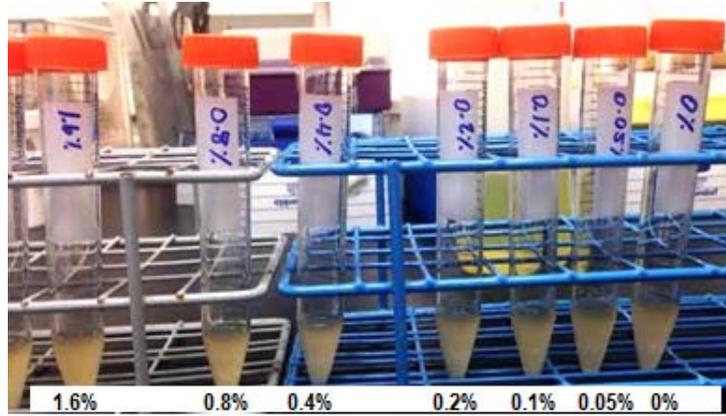
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Figure 18: Concentrations from 1.6% to 0% (control) show no inhibition of growth of mixed culture.

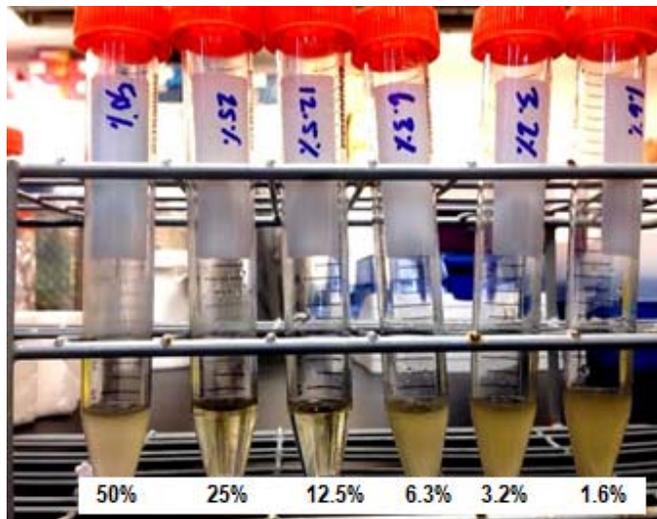


Figure 19: Minimum inhibitory concentration (MIC) established at 12.5% (MIC<sub>12.5</sub>).

**RESISTANCE OF *S. TYPHIMURIUM*****VI. Minimum bactericidal concentration (MBC), *S.typhimurium* Ames strain and *E.coli*****KL25 mixed culture:**

Minimum bactericidal concentration was determined for the mixed culture (*Salmonella typhimurium* Ames strain and *Escherichia coli* KL25 strain). Negative and positive growth is seen in figure 20. The minimum bactericidal concentration (MBC) is observed at 25% (MBC<sub>25</sub>) with it being the lowest concentration that contains no growth in both replicates. In figure 21, positive growth is seen in both replicates of concentrations 12.5%, 6.3%, 3.2%, 1.6%, 0.8%, 0.4%, 0.2%, 0.1% 0.05% and 0% (control).

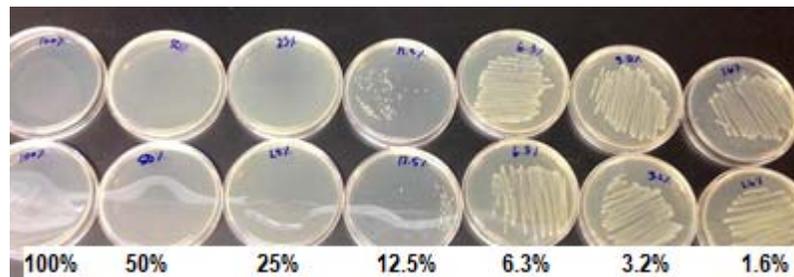


Figure 20: Minimum bactericidal concentrations (MBC) from 100% (control) to 1.6%. No growth is seen from 100% to 25%, positive growth from 12.5% to 1.6%. MBC is seen at 25% (MBC<sub>25</sub>).

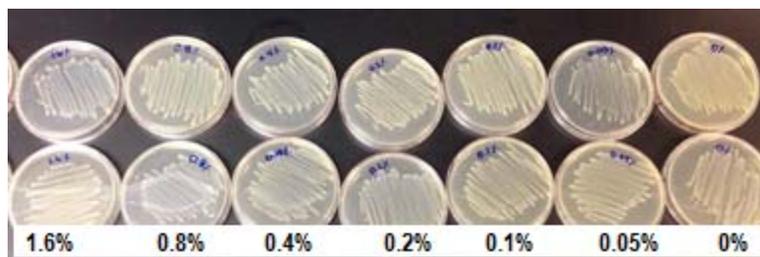


Figure 21: Concentration range from 1.6% to 0% (control) show positive growth in all replicates.

***RESISTANCE OF S. TYPHIMURIUM*****VII. Triple Sugar Iron (TSI) test, *S. typhimurium* Amest strain and *E. coli* KL25 mixed culture:**

The final test, Triple sugar iron (TSI) was necessary for establishing which colony survived and grew in 12.5% of the mixed culture. Figures 22 and 23 represent the controls for each *E. coli* KL25 and *S. typhimurium* Ames strain. A color change to yellow in the agar can be seen in figure 22. Figure 23 reveals a black/dark purple precipitate in the TSI agar which was caused from *Salmonella typhimurium* Ames strain. When testing the mixed culture, with replicates of 2 from both plates of the MBC test, each tube revealed a black/purple color change in the agar (figure 24).



Figure 22: TSI test with *E. coli* KL25 had no hydrogen sulfide (H<sub>2</sub>S) production.

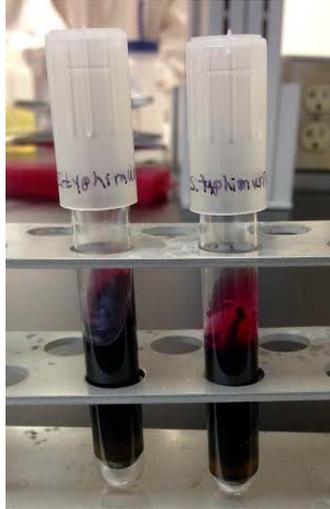
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Figure 23: TSI test with *S. typhimurium* Ames strain showed production of hydrogen sulfide (H<sub>2</sub>S).

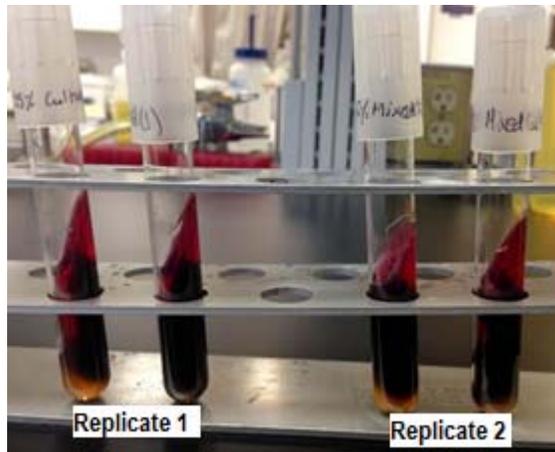


Figure 24: TSI test from mixed culture, replicates 1 and 2 of both plates produced hydrogen sulfide (H<sub>2</sub>S).

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**VIII. Comparison of minimal inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of test species, *Salmonella typhimurium* Ames strain and *Escherichia coli* KL25 strain:**

**Table 1:** Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) comparison of test species *S. typhimurium* and *E. coli* to mixed culture

Test Species	Minimum inhibitory concentration (MIC)	Minimum bactericidal concentration (MBC)
<i>Salmonella typhimurium</i> Ames strain	6.3%	25%
<i>Escherichia coli</i> KL25 strain	12.5%	12.5%
Mixed culture ( <i>S.typhimurium</i> + <i>E.coli</i> )	12.5%	25%

**RESISTANCE OF *S. TYPHIMURIUM******Discussion***

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Since the 1990's many *Salmonella* strains that are resistant to a range of antimicrobials have emerged and are now a serious concern to the public health. Over 2,500 different strains of *Salmonella* have been identified to date and are known to survive in extreme environments for several weeks to months (WHO 2013). *Salmonella typhimurium* and *Escherichia coli* contain similar characteristics in that they are both gram-negative, motile, typically located in the intestines of animals and humans and are well known to cause nosocomial (hospital-linked) infections which can be difficult to treat with antibiotics due to resistance.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) aided in determining the resistance of *S. typhimurium*. With close observations in the minimum inhibitory concentration and the minimum bactericidal concentration, the results produced evidence of some resistance for *Salmonella typhimurium* Ames strain. *S. typhimurium* produced a minimum inhibitory concentration of 6.3% (MIC<sub>6.3</sub>) and a minimum bactericidal concentration of 25% (MBC<sub>25</sub>). In other words, the lowest concentration of Advanced Gel Purell® that produced growth inhibition of *S. typhimurium* is 6.3% while 25% is the lowest concentration that resulted in cell death of the population. Further testing for MBC and MIC in *Escherichia coli* KL25 strain and a mixed culture of *S. typhimurium* Ames strain and *E. coli* KL25 were completed and used as comparisons for *S. typhimurium* Ames strain. *E. coli* KL25 presented a minimum inhibitory concentration of 12.5% (MIC<sub>12.5</sub>) and a minimum bactericidal concentration of 12.5% (MBC<sub>12.5</sub>). This would have been an expected result in the beginning, although the outcomes were much different for *S. typhimurium* and the mixed culture. It is interesting to see that the antibacterial agent inhibits growth and kills microbial population at the

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same concentration of 12.5%. The mixed culture containing *S. typhimurium* Ames strain and *E. coli* KL25 displayed the lowest concentration of growth inhibition at 12.5% (MIC<sub>12.5</sub>) and the lowest concentration that lead to microbial death of the entire population in the mixed culture occurred at 25% (MBC<sub>25</sub>). Table 1 displays the comparisons between each result for both minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) from every test. *E. coli* KL25 has a higher MIC than *S. typhimurium* Ames strain by 2-fold. However, *S. typhimurium*'s MBC was double of that of *E. coli*'s MBC. An inference could be made about *S. typhimurium*'s survival concentration in the mixed culture. Previous testing of this microbe presented evidence of it surviving until 25% (MBC<sub>25</sub>) however, the triple sugar iron (TSI) test was used to discriminate the species that formed colonies on both replicates of 12.5%. The formation of black/dark purple precipitate in the triple sugar iron agar indicates that *S. typhimurium* had produced hydrogen sulfide (H<sub>2</sub>S) (Sasahara, Heinzinger and Barrett 1997). *Escherichia coli* does not produce this black/dark purple precipitate due to its fermentation process of glucose and lactose producing acid and carbon dioxide, causing the phenol red indicator to turn yellow (American Society for Microbiology 2012).

Some strains of *Salmonella typhimurium* are known to be multidrug resistant with others being resistant to fewer drugs. Researchers have expanded this idea further, to test microbial resistance to antiseptics and disinfectants. One of the most common strains found in animals (bovine, swine, and poultry) and humans, *S. typhimurium* definitive type 104 (DT104) carries resistance to ampicillin, chloramphenicol, florfenicol, streptomycin, sulfonamides, and tetracycline (Khan et al. 2006). Many strains of *Salmonella typhimurium* including DT104 harbor a genomic island known as *Salmonella* genomic island 1 (SGI1). SGI1 is a 43-kb genomic island that contains a cluster of genes that may confer resistance to a variety of

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antimicrobials (Baucheron et al. 2004) (Carattoli et al. 2002). A partially deleted SGI1 may lack *flo<sub>st</sub>*, *tetR* and *tetA* genes which confer to chloramphenicol-florfenicol and tetracycline resistance (Carattoli et al. 2002).

Intrinsic mechanisms such as efflux pumps act as the primary evidence for mediating antimicrobial resistance. These pumps are transport proteins involved in the extrusion of foreign substrates (i.e. antibiotics, disinfectants and antiseptics) which may be specific to a substrate or a variety of compounds (Webber and Piddock 2003). Among the antimicrobial resistance genes, two code for efflux pumps, *floR* (florfenicol resistance gene) and *tet(G)* (tetracycline resistance gene) making this efflux system responsible for any characteristics of multidrug resistance (Baucheron et al. 2004).

The role of intrinsic (innate) resistance and acquired (developing overtime) resistance impact bacterial cells to change or mutate and activate specific defense mechanisms when exposed to an antimicrobial agent. Cells in various strains of *S. typhimurium* have shown higher mutability than others and the stable mutability factor has produced high gene mutability (Miyake 1960). The overexposure and long term environmental stress of antimicrobials play a primary role in the high mutability rate of *S. typhimurium* and gene transfer.

**RESISTANCE OF *S. TYPHIMURIUM******Conclusion:***

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*Salmonella typhimurium* is a common pathogen that has been identified as the major cause of salmonellosis. Salmonellosis is a prevailing food borne infection, targeting humans and animals in both North America and Europe (Carattoli et al. 2002). A gram-negative pathogenic bacterium like *Salmonella typhimurium* proves to have an increased survivability rate due to antimicrobial resistance. This resistance is due to the overexposure and long term effects of certain antimicrobial agents like the leading brand of Advanced Gel Purell®.

Since the production of hand sanitizer, antimicrobial resistance in bacteria has become an issue in healthcare. Some individuals are resorting to antiseptics as a substitution for hand washing. Production companies have made antiseptics safe enough to use on living tissue and claim to eliminate 99.9% of bacterial population. This percentage may seem impressive however, it may not be sufficient when considering the total amount of cells in a population. As a result, resistance may be acquired for cells that have survived as they begin to learn new resistance mechanisms, along with triggering their intrinsic mechanisms.

It is important to establish and follow a safe protocol to mitigate such resistance from bacteria like *S. typhimurium*. Hand hygiene protocol is one of the most important solutions to preventing the spread of infections. Hand sanitizers may eliminate the growth and bacterial populations; however hand washing establishes direct removal of bacteria and sebum from tissue surface. Food industry providers are recommended to practice proper hand hygiene before and after handling any type of food, especially those that are raw or undercooked foods (i.e. meat and eggs) to prevent cross contamination. Finally, healthcare professionals should practice hand

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hygiene at specific times to disrupt the transmission of microorganisms to patients. These key moments include before patient contact; after contact with blood, body fluids, or contaminated surfaces; before invasive procedures; and after removing gloves (CDC 2014).

**RESISTANCE OF S. TYPHIMURIUM****References**

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