

CAN ENVIRONMENT CONTROL MEASURE AND LABORATORY DIAGNOSTIC TECHNIQUE BE USED TO CONTROL THE SPREAD OF SALMONELLA INFECTION

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INTRODUCTION

Salmonella infection is the one of the most common foodborne diseases in the world and is caused by a bacterial infection. There are many serotypes of Salmonella bacteria which may cause salmonellosis in animals and human. The two most commonly found types of Salmonella are *S. typhimurium* and *S. enteritidis*, however, the frequency of Salmonella serotypes varies between countries. ¹ Approximately 40,000 cases of salmonellosis are reported annually in the U.S., 400 of these end in death, often as a result of dehydration from chronic diarrhea. ¹ It is also estimated that the actual number may be at least 30 times the number reported by the CDC since milder cases of salmonella are not diagnosed or reported. ¹ Any person can contract salmonellosis, however, it is most often found in children below the age of ten years old. Those with impaired immune systems such as young children and old adults, as well as people with illnesses are most likely to have severe infections.

Additionally, people with AIDS are especially vulnerable, not only suffering from the most severe infections but also from episodes that are likely to recur. In the last decade salmonella infections have been shown to be decreasing in the United States, however, some types are still increasing. Although this disease can be found across the world, cases occurring in North America and Europe are most often reported by health experts. ² A number of Salmonella serotypes have developed resistance to several antibiotics most often used in salmonella treatment, this presents a potentially serious public health threat. ¹

The purpose of this review is to examine the effectiveness of environmental control measures and laboratory diagnostic techniques in controlling the spread of salmonella worldwide. This is an important question to address because of the high frequency of infections of salmonella globally as well as the nature and vulnerability of the populations most at risk. This literature review format is appropriate in answering this question because it deals with the comparative evidence of antimicrobial resistance from different points of transmission and geographical locations and will address factors that promote progress in salmonella treatment and may provide insight into preventive actions that can be employed.

TRANSMISSION

Food products such as eggs, meat, and poultry and sometimes unwashed fruit are common places where the Salmonella bacteria can be found. Surfaces used to prepare food can become cross-contaminated with Salmonella bacteria when they come in contact raw meat and poultry products. The Centers for the Disease Control and Prevention (CDC) has also recently reported cases of salmonella infection occurring after consumption of raw alfalfa sprouts grown in contaminated soil.⁴ Salmonella can also be contracted from pets, particularly reptiles.² Salmonella can become a chronic infection even if the patient does not have visible symptoms². In addition, though no symptoms may be present, the disease can still be spread by failure to wash hands properly before preparing food for others. Health experts recommend that persons infected with Salmonella should not handle or prepare food or water for others until laboratory tests² confirm that the Salmonella bacteria is no longer present.²

SYMPTOMS

Salmonella may include a range of symptoms including diarrhea, fever, abdominal cramps, and headache. Sometimes there are additional symptoms associated with Salmonella which may last up to 7-9 days these may also include, dramatic appetite loss, vomiting, and possible nausea. More severe symptoms will occur in individuals with compromised or weak immune symptoms such as the elderly, young children, and individuals with chronic conditions such as diabetes or HIV.⁵

REITER'S SYNDROME

Persons infected with salmonella will normally recover however in a few individuals a chronic condition known as Reiter's syndrome may develop. Reiter's Syndrome can last for years and may lead to the individual developing arthritis. Symptoms include joint pains irritable eyes and painful urination. Inadequate treatment of salmonella can result in the organism spreading to other organ of the body and may eventually lead to death.

TYPHOID FEVER

Salmonella typhi bacteria is the causative agent for typhoid fever. This can be fatal if left untreated and it is of particular importance in developing countries. Approximately 21.5 million persons are affected by typhoid each year. This is mainly due to the consumption of contaminated water. The Centers for Disease Control and Prevention (CDC) has indicated that approximately 400 cases occur annually in the United States, of this 75 percent are acquired through international travel.

DIAGNOSIS

Healthcare provider use lab tests that identify Salmonella in human body fluids and stool. Salmonella can also be isolated from food and water sources. A medical history and a physical examination performed by a physician are required to make a diagnosis for Salmonellosis. The physician does this by first asking questions about the patient's symptoms, recently consumed foods, as well as work and home environments. The diagnosis is confirmed using laboratory tests on human and food samples.

TREATMENT

In most cases salmonella leaves the body on its own as a result of the work of the immune system within five to seven days and further treatment will not be necessary, however, if the patient has symptoms which include severe diarrhea then intravenous fluids are required. People who have been treated with oral antibiotics and younger people tend to carry the bacteria longer than others which may last up to several months. If the disease spreads from the intestines of the patient into the bloodstream, then the healthcare provider should be able to treat it with antibiotics. Many Salmonella serotypes have developed resistance to several antibiotics normally used to treat people with salmonella disease, this may become a potential public health threat.¹

OVERVIEW OF RESEARCH STUDIES

METHODOLOGY

A total of ten journal articles, two books and 4 web based articles were reviewed with regards to the issue of Laboratory diagnosis, antimicrobial susceptibility, environmental control and prevention of Salmonella that causes food borne diseases and typhoid fever. The research studies chosen for this literature review focused on the impact of salmonella bacteria in both food-borne disease and typhoid and also investigations of antimicrobial resistance of salmonella. Textbooks covering the subject area as well as journals from National Centre for Biotechnology Information (Pubmed database), Pubmed Central, JSTOR, Proquest, Highwire and EBSCO were the main sources of electronic literature research. In addition web based articles from the Center for Disease Control (CDC) and New South Wales (NSW) proved useful for the review. The literature selected for review were based on current publications no older than 10 years, with the majority chosen from between and including 2005 to 2013.

LABORATORY DIAGNOSIS AND ANTIMICROBIAL RESISTANCE TESTING

Salmonella can be isolated from several sources or sites. The salmonella organism can be found in food such as eggs poultry and meats and also from human specimen blood, feces and urine Salmonella is a gram negative rod bacteria and is divided into three subgenera and consists

of several hundred different serotypes. These serotypes are differentiated based on their antigenic make-up. A limited number of sera can be used to serotype the organism.¹⁵

In order to isolate salmonella blood is taken from persons with a febrile disease. Approximately 10 mls of blood is placed in a 90-100 ml of bile broth and incubated at 35°C. The incubation period last up to 14 days before a negative sample is reported. For persons who are being investigated for Salmonella typhoid the samples should be taken during the first or second week of onset. If the samples are negative then repeat samples should be taken in the second week of onset. Blood cultures from the same patient will show positive results before the organism is picked up in the stool. Antimicrobial testing can be done using several different techniques

1. Tube dilution method – The organisms susceptibility (ability to grow in varying concentrations of antibiotic prepared using serial dilutions is tested
2. Agar plate dilution method – The antibiotic is incorporated into solid agar in varying concentrations and the organisms susceptibility is examined. The point at which no growth occurs is determined as the minimum inhibitory concentration level
3. Standardized Disc – Agar Diffusion method – Filter discs are impregnated with antimicrobial agents and placed on agar plates that have been inoculated with bacterium. The size of the diameter of the zone of no growth around the disc will determine if the organism is susceptible¹⁵

A comprehensive look at 5 articles reviewed for Antimicrobial Resistance of Salmonella illustrates a trend of increasing antimicrobial resistance exhibited by various strains of salmonella over the last decade. These increased levels of resistance come from salmonella isolated from various points of transmission which include, animal-human, food-human and human-human. One study specifically identifies a recent increase in the level of resistance in salmonella to the drug ciprofloxacin.

A similar trend was observed for animal isolates, in regards to resistance to ceftriaxone particularly from salmonella in cattle which had increased from 0% in 1997 to 21.6% in 2003¹⁴ and then leveled-off according to the study. The results of one study shows that there is a variation among resistance patterns of organisms isolated from humans and animals. In organisms isolated from humans the average resistance to tetracycline was 16.9% and from animals, 34.9%, and in particular the average resistance for turkey and swine was greater than 50%.¹⁰ The resistance patterns for Sulfamethoxazole/sulfisoxazole isolated from humans was 15.3% and for animals, 19.9% Streptomycin resistance was 14.4% in humans and 26.3% in animals. Ampicillin and resistance chloramphenicol was 14.3% and 8.8% and 16.0% and 7.3% for animals respectively.¹⁰ The resistance for gentamicin was 2.1% in humans and 6.5% in animals however, this drug is not usually used to treat human infections in the U.S. Levels of resistance to gentamicin was detected at 2.1% in isolates from humans and 6.5% from animals.¹⁰

In another study conducted from a farm in Kentucky a stratified random collection of 1,888 samples was gathered between 2009 to 2011 from cattle, camels, poultry, fish, vegetables, and humans.⁴ The Salmonella isolates obtained were then serotyped and tested for antimicrobial susceptibility by MIC determinations.⁴ 149 isolates representing 17 serotypes were isolated with

(7.9% prevalence). The overall antimicrobial resistance was low, however *S. Enteritidis* and *S. Eko* revealed much variability in its antimicrobial resistance patterns as well as 7 out of the 17 tested antimicrobials from *S. Kentucky* for ciprofloxacin and nalidixic acid susceptibility. There were 3 isolates that showed decreased resistance. The *S. Hadar* isolates revealed reduced susceptibility to ciprofloxacin and susceptibility to nalidixic acid and harbored the plasmid-mediated quinolone resistance gene *qnrS1*.⁴

In a study done from 2000-2011 in France 1% of all *Salmonella* strains were identified as *S. Kentucky*, the same study conducted in Morocco had 30 of 226 of isolates from soil samples belonging to *S. Kentucky*. Results reveal an increase in the resistance to the antimicrobial drug ciprofloxacin in France and 40% of isolates from *S. Kentucky* obtained had a resistance in 2000-2008, which later rose to (83%) or 376 of 489 had a resistance in 2009-2011.

There is also evidence of lateral gene transfer among *Salmonella* strains and other bacteria species which also plays a major role in the increase of antimicrobial resistance in *Salmonella* serotypes such as *S. Kentucky* to antimicrobial drugs and medicine. The history and originations obtained from the study suggested that *S. Kentucky* infections originated predominantly in east Africa, North Africa, West Africa, the Middle East, and India. β -lactamase (CTX-M-1, CTX-M-15), plasmid-encoded cephalosporinase (CMY-2), or carbapenemase (OXA-48, VIM-2) genes by ciprofloxacin-resistant isolates of *S. Kentucky* ST198-X1 from the Mediterranean area since 2009.³

In another study done in Japan a total of 82 *Salmonella* isolations from food animals were tested for antimicrobial susceptibility. Resistance was shown to a variety of antimicrobial drugs used to combat various strains of *Salmonella* these include: ampicillin, dihydrostreptomycin, kanamycin, oxytetracycline, chloramphenicol, bicozamycin, nalidixic acid, oxolinic acid and trimethoprim. *Salmonella* Dublin demonstrated high levels of resistance particularly to older quinolones. There was also one strain, *Salmonella Choleraesuis*, isolated from pigs which showed resistance to Fluoroquinolone, this was the first ever incidence of Fluoroquinolone resistant salmonella reported Japanese which implies that there is a recently developed strain of *Salmonella*.

Most *Salmonella* Typhimurium isolates showed resistance to ampicillin, chloramphenicol, dihydrostreptomycin and oxytetracycline. *S. Typhimurium* DT104 accounted for 40.7% of *S. Typhimurium* isolates and was more often multi-drug resistant. There were also *Salmonella* isolated from poultry such as *S. Infantis* which was dihydrostreptomycin, oxytetracycline, trimethoprim or kanamycin resistant. There was also one strain of salmonella, *Salmonella* Enteritidis, which is the most common cause food-poisoning in Japan, that show resistance only resistance to dihydrostreptomycin.⁵

DISCUSSION

From the mid-20th century to present Antimicrobial compounds have been an important part of the treatment used for bacterial infections. Due to the highly success rate in treating various diseases these were then widely used in both human and veterinary medicine. However,

resistance to these compounds was detected in target pathogens only a few years after initiation of therapeutic use in humans (Alanis, 2005). The selective pressure created by the use of antimicrobials was identified as a driving force behind the emergence of resistance which was genetically encoded, inherited by subsequent progeny of the resistant pathogens, and in some cases could be transferred horizontally even to distantly related bacteria [as reviewed by Linton

These AR antimicrobial drugs were also widely used in the food animal industries to promote growth of livestock in order produce yield of meat. However these drugs were often given below the required dosages to adequately destroy all the bacteria and as a result surviving bacteria proliferated and AR resistance quickly began to emerge. The number of human infections as a result of AR strains also began to dramatically increase.

Salmonella, a common pathogen was among the long list bacteria species that developed antimicrobial resistances. Goldberg and Rubin et al. explains that some strains of Salmonella for example serovars of *S. enterica* can survive in a wide variety of hosts and cause different diseases such as Salmonella Typhimurium, which causes no symptoms in adult poultry, can cause gastroenteritis in humans, or cause highly invasive systemic enteric fever in mice.¹⁰ The type of hosts utilize can also vary among food animals to companion animals to humans. D'Aoust, 1997; Lavigne and Blanc-Potard, 2008). Further studies also indicate that certain servora stains can shift the frequency in which type of hosts they are found in over time. Servora's have also become host specific such for example Salmonella typhi.¹⁰

In the U.S., Salmonella is estimated to cause over one million human infections each year (Scallan et al., 2011). Most of the infections are resolved after a few days and may simply result in gastroenteritis however in more severe cases where those with compromised immune systems are concerned this requires the use of Antimicrobial drug treatment in order to prevent further morbidity or mortality (Alcaine et al., 2007). In order to successfully treatment salmonella in the U.S. drugs have been categorized in first line, second line and third line drugs based on the level resistance that salmonella virus displays. First line resistance drugs typically included typically a fluoroquinolone-like ciprofloxacin or a third generation cephalosporin β -lactam such as ceftriaxone (Mandal, 1990; Guerrant et al., 2001; Hohmann, 2001; Habib, 2004; Parry and Threlfall, 2008), if the salmonella strain proved resistant they move to the second line of drugs which were also usually reserved for more chronic infections.

CONTROL AND PREVENTION

Salmonella infections occur after eating contaminated food or sometimes after contact with another person with the infection.¹⁴ The principal vehicle is water, milk, or food contaminated by feces. The most dangerous link in a chain of infection is the food handler who is a carrier

A comprehensive list of strategies to prevent the spread of salmonella may be enumerated under four main categories: Cooking, Food Handling, Temperature Control and Food Contamination. Food infection is caused by the multiplication of bacteria. This multiplication usually takes place in the intestine. Food that is prepared in large quantities is more often the source of infection than when smaller quantities of food is prepared. This as the food prepared in

large quantity is sometimes not penetrated with enough heat to destroy the bacteria. If the meat of infected animal is not cooked properly it may convey the disease to man but it is usually conveyed from outside sources. These sources are the intestinal contents of the slaughtered animal, the intestinal contents of animals that have contaminated the food or human carriers who have prepared or handled the prepared food.

When cooking meats for example: poultry, beef or fish one needs to make sure all foods are thoroughly cooked before consumption. Eating foods that are not well done or those that have a pink or red center increases the chances of salmonella infection. Additionally, consumption of raw meats should be avoided. Eggs should also be cooked thoroughly. It is recommended that poultry products are cooked to an internal temperature of 170°F for breast meat and 180°F for thigh meat.⁶

It is recommended that human carriers of *Salmonella* should not work in the food industry and if possible should undergo gallbladder removal and antibiotic therapy for an attempt to cure the carrier state. Public-health authorities will recall products that have been contaminated with salmonella or other pathogenic organisms.⁶ Recalls for contaminated beef in July and August 2009 contained *Salmonella* with multiple drug resistance.⁶ It is recommended that people should wash their hands after contact with animal feces. Reptiles should not be as pets.⁷ This as reptiles are particularly likely to have *Salmonella*, and it can contaminate their skin, everyone should immediately wash their hands after handling reptiles.⁷ Children can be exposed to the bacteria by simply holding, cuddling, or kissing the birds. Children should not handle baby chicks or other young birds. Everyone should immediately wash their hands after touching birds, including baby chicks and ducklings, or their environment.⁷

In October 2010 to February 2011, there was an outbreak of 91 Salmonella Enteritidis infections in Alberta, Canada. An investigation conducted by local public health department revealed that the 91 cases were all associated with the consumption of food that was purchased from mobile food-vending vehicles that operated from worksites in Alberta. A catering company which provided lunch to the trucks and vendors was also implicated. In 85 (93%), of the cases it was reported that patients consumed food prepared by the catering company 7 days before getting sick. Six of the patients were employed to the catering company. It was found that two of the food samples collected from the catering company tested positive for Salmonella Enteritidis. From investigation it was deduced that the source of the contamination was from food obtained from illegal source. The contamination was deemed to have happened directly or indirectly from eggs contaminated with Salmonella Enteritidis and by employees who were infected with Salmonella Enteritidis. The intervention that was made by Public Health personnel to control the spread of the disease included the screening for Salmonella, removal from duty employees involved in food-handling and training.¹⁶

The use of liquid soap and chlorine for the cleaning and disinfection of contaminated surfaces on a frequent basis is an effective measure to control the transmission of salmonella in food preparation facilities.

In a study on residual viral and bacterial contamination of surfaces after cleaning and disinfection, data on infectious doses and efficiencies of transfer was used to estimate a target level to which the residual contamination should be reduced. It was found that a single wipe with liquid soap then with 250-ppm free chlorine solution was sufficient to reduce the contamination to below the target level for most of the pathogens tested.⁹ “ The sterility of surfaces is monitored in hospitals by determining the levels of reduction of bacteria like *Staphylococcus* and also *Salmonella Enterica*, Serovar and *Enteritidis* in food preparation facilities.”⁹

Temperature control is also important in preventing the spread of salmonella. Eggs products must be kept refrigerated and cracked or dirty eggs should always be discarded. Raw or unpasteurized milk and dairy products should also be avoided. Pasteurized milk products should be kept refrigerated. The expiry date should also be inspected before consumption and preferably before purchase at the local food store.

Food contamination also plays an important role in the spread of salmonellosis. This occurs when the virus does not originate from within the food but instead is brought to it by an external agent. Hands are one of the main culprits, which is why the proper washing of hands are emphasized. Utensils and surfaces must also be kept clean. Contamination may also happen before the product reaches to the store therefore it is important to wash foods immediately after they are purchased.

A study in China concluded that water and food sanitation and environmental awareness is very effective in reducing food and water borne diseases. This study was conducted between 2006-2010 and aimed to “identify the sources, transmission processes, and determinants of typhoid and paratyphoid fever in the Hongta District.”¹⁷

In this retrospective case-control study, (80 cases and controls) geographical detectors and epidemiological surveys were used to identify transmission sources and population that had been exposed. The results showed that there was an increased risk of salmonella infection (typhoid and paratyphoid fever) from adding fresh mint (OR = 2.17, 95% CL: 1.04–4.54) to breakfast, eating uncooked vegetables (OR = 2.29, 95%CL: 1.24–4.24) at restaurants or roadside food sites, and eating flavoring that contained fresh caraway and mint (OR = 2.38, 95%CL: 1.00–5.69).¹⁷

A study conducted in Taiwan in January 2009 to October 2010 revealed that salmonella was the most common isolate in children suffering from gastroenteritis. A matched case control study (360 cases 930 controls)was conducted in children less than 5 years a Multivariate analysis which identified household contacts with symptoms of diarrhea was conducted using conditional logistic regression, the matched Odds ratio was 17.9; 95% confidence interval [CI]: 8.82-36.34; P < 0.0001), Milk powder consumption (mOR, 2.04; 95% CI: 1.05-3.94; P = 0.0344), Heath center visits (mOR, 1.66; 95% CI: 1.12-2.48; P = 0.0126) ground water (mOR, 1.50; 95% CI: 1.06-2.11; P = 0.0214).All factors were associated with an increased risk of salmonella infection. Factors such as hand washing, breastfeeding, chicken consumption and preparation of food by

caregivers showed a decreased risk of salmonella infection. The study concluded that major mode of Salmonella infection in Taiwanese Children was found to be person-to-person, waterborne and environmental contacts. The study could not eliminate the possibility of powdered milk and groundwater contamination as routes of Salmonella infection as well.¹¹

The underlying consensus from all the articles agreed that salmonella infections occur after eating contaminated food or sometimes after contact with another person with the infection.

VACCINATION

Salmonella vaccines are available for poultry and animals, typhoid vaccines are available for humans. Research is ongoing for the development of vaccines against other types of salmonella. Animal vaccines are currently being used in Europe to reduce the likelihood of hens transmitting diseases to their eggs and to other hens. Egg producers using the vaccine on poultry should continue to maintain sanitary conditions since the vaccine is not 100%⁶ protective.⁸

CONCLUSION

The control of Salmonella remains a worldwide challenge it contributes to millions of human infections and deaths per year. It is also a real threat in children particularly so in developing countries as one of the organisms associated with diarrheal disease that result in 1.4 million deaths annually.

Given the high level of resistance in salmonella it is important that antimicrobial susceptibility tests be conducted on organisms isolated from food and human samples to identify effective treatment regimes. These tests should be done before the drug administration. There is a need to ensure that treatment guidelines are revised to include relevant information on antibiotic treatment. There is evidence to support that plasmids that encode multidrug resistance may contribute to the spread of drug resistance. It is therefore important to control this as it can pose a significant problem in emerging and re-emerging infections.

Prevention is the most effective strategy to control salmonella infection. Prevention strategy should include approaches on a personal and community basis. On a personal basis measures must be put in place to prevent the transmission of disease for the ill person and approaches from the community basis should include implementation of effective mechanisms to provide and monitor pasteurized milk, water supplies, sewage disposal, public food production and eating facilities, Diagnosis and effective treatment of infected persons and carriers, control of flies and administration of vaccines where required. It is very important to provide information to the public during outbreaks. This information should include information about the outbreak, implicated food products and advice on personal hygiene.

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