VETERINÁRIA NOTÍCIAS

Short communication

Received: August, 2020; Accepted: June, 2021

# Nanocomposite of Ag-Doped ZnO and Ag2O nanocrystals in control of *Salmonella* Heidelberg biofilms formed in commercial eggs

*Pedro Lucas Figueiredo Nunes*[1], *Priscila de Castro Almeida*[2], *André Eduardo Schlemper*[3]*, *Thais Fernanda Martins Reis*[4], *Patrícia Giovana Hoepers*[5], *Gustavo Medina Schwerter*[6], *Noelio Oliveira Dantas*[7], *Anielle Christine Almeida Silva*[8]

**Abstract:** *Salmonella* spp. is an important causal agent of salmonellosis in humans. Controlling Salmonella spp. in eggs is important as the bacterium passes through the shell to an embryo and remains in the terrain. Disinfection is usually performed by using several sanitizers. However, novel, more efficient ways of controlling this agent have been studied with advances in nanotechnology, including nanoparticles. Preliminary studies of nanoparticles have shown they are successful in controlling such microorganisms. Standardizing the ideal concentration of this nanocomposite is fundamental for optimum efficiency in the control of *Salmonella* spp. In this study, eggs from commercial laying chickens were purchased from local trade and treated in laboratory with silver and zinc nanoparticles in different concentrations. Biofilm was formed 24 hours after that; then, the eggs were washed for the removal of free bacteria. Conventional microbiology was performed to isolate *Salmonella* spp., and PCR was performed to identify colonies. The effectiveness of using nanocomposite of silver oxide with silver-doped zinc oxide (ZnO:Ag-AgO) was evaluated in different concentrations to prevent the formation of eggshell biofilms.

[1] Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia, Brazil. https://orcid.org/0000-0003-4639-6429
[2] Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia, Brazil. https://orcid.org/0000-0002-1454-4944
[3] Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia, Brazil. Corresponding author: aeschlemper@gmail.com, Av. Ceará sem número, bloco 2D, Segundo andar, Campus Umuarama, Uberlândia-MG. https://orcid.org/0000-0002-0841-9485
[4] Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia, Brazil. https://orcid.org/0000-0002-9516-3640
[5] Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia, Brazil. https://orcid.org/0000-0002-0109-9810
[6] Departamento de Procesos Diagnósticos y Evaluación. Facultad de Ciencias de la Salud. Universidad Católica de Temuco, Chile. https://orcid.org/0000-0003-3889-1745
[7] Universidade Federal de Alagoas. https://orcid.org/0000-0001-6752-7382
[8] Universidade Federal de Alagoas. https://orcid.org/0000-0002-5274-156X

**Keywords:** Salmonella; silver nanoparticles (AgNps); biofilm; disinfection; Heidelberg.

## 1 Introduction

The control of infectious diseases such as *Salmonella* is important for public health because it affects a large part of the population. The European Union has reported ca. 100.000 cases in humans every year (Europe Food Safety Authority - EFSA., 2018). Salmonellosis is a disease caused by bacteria of the genus *Salmonella* that is often associated with outbreaks of foodborne illnesses. It is also classified as a zoonosis as it affects several species. S. Typhi and S. Paratyphique are human-specific serotypes, while S. Gallinarum and S. Pullorum are poultry specific (LOPES et al., 2013). *Salmonella* Heidelberg (SH) was isolated in Brazil in the 1960s but it has been recently found in the poultry production of various countries (BORSOI et al, 2011).

*Salmonella* affects several countries, as its control is hampered by both its high contamination rate in the population and cumbersome preventive measures (World Health Organization-WHO., 2018). In Brazil, about 2% of poultry carcasses are contaminated with this agent, causing serious financial damage to the poultry industry (MEDEIROS et al., 2011).

Eggs are constantly involved in contamination by *Salmonella* spp. in humans, with toxinfection caused inadequate handling for its consumption or use in food products (TAUXE., 1997). Much has been invested in measures for the sanitary control of Salmonella in eggs (JONES., 2011).

Bacteria of the genus *Salmonella* belongs to the family Enterobacteriaceae. They are bacilli shaped, gram-negative, facultative anaerobic, and non-spore forming. Due to oscillating ambient conditions, some organisms, including *Salmonella* spp., have developed the ability to adhere on a surface and agglomerate, forming a multicellular complex filled with a matrix of polysaccharides, in a process known as biofilm (LATASA et

al., 2012). Biofilm is beneficial to bacteria because it provides certain resistance to antibiotics, disinfectants, and the host's immune system. As some infections in hospitals and industries are associated with this process, it is important to create different compounds to eliminate and control biofilm (STEENACKERS et al., 2012).

Nanotechnology has no longer been restricted to the industry, with its use being increasingly common in our daily lives. This technology creates compounds with singular characteristics. Their small size increases contact surface and provides greater reactivity (GITIPOUR et al., 2017). Nanomaterials have different properties depending on the method used for their synthesis. Nanosilver particles (AgNPs) have antimicrobial activity and demonstrated ability to inhibit biofilm formation in different agents, including *Escherichia coli* and *Klebsiella* spp. (DAKHIL et al., 2017).

Zinc oxide (ZnO) nanocrystals are a biocompatible material generally recognized as a safe (GRAS) by the United States Food and Drug Administration. Silver nanoparticles have physical and chemical properties that account for their antimicrobial activities. Even though they aggregate in media with high concentrations of electrolytes, which reduces antibacterial activities (LOK, 2007), complexation with other composites stabilizes silver nanoparticles against aggregation, which retains their antibacterial activities.

In this article, we investigated this nanocrystal doped with silver (Ag) and silver oxide (Ag2O) nanocrystals (ZnO:Ag-AgO). The doping technique is used to increase the catalytic activity in ZnO nanocrystals and its ability to induce oxidative stress in bacteria, one of the major mechanisms of bactericidal action (PATI et al., 2014).

In this article, nanocomposites of silver oxide and silver-doped zinc oxide (ZnO:Ag-AgO) with different concentrations were synthetized to disinfect the surface of eggshell and thereby control *Salmonella* Heidelberg biofilm formation.

## 2. Material and methods

Nanoparticles were synthesized in the Laboratory of New Insulating and Semiconductor Materials (LNMIS) at the Institute of Physics. Biological study was carried out at the Laboratory of Molecular Epidemiology, and egg incubation was performed at the Incubation Laboratory. All laboratories integrate Universidade Federal de Uberlândia.

Several physical and chemical processes take place for nanoparticles synthesis. X-Ray Diffraction (XRD) was used to analyze the physical properties of nanoparticles. The method used for synthesis was coprecipitation (patent number BR 10 2018 0077147). The nanocomposite of ZnO:Ag-AgO nanocrystal was synthesized at room temperature via aqueous solutions of zinc chloride (ZnCl 2, 99.9%, 2M) and silver nitrate (99% AgNO 3, ranging from 0.1 to 11% Ag against Zn). The pH of the solution was adjusted to 11 using an aqueous solution of sodium hydroxide (98% NaOH). The nanoparticles formed were purified via centrifugation at 6000 rpm / 1 minute. All reagents were purchased from Sigma-Aldrich. The ZnO:Ag-AgO nanocomposite was doped with three different concentrations of silver.

Antibiograms were prepared to determine the level of silver doping used in the experiment, as described by Bauer et al. (1966), i.e., by inoculating a suspension of bacteria on Mueller Hinton agar plates and adding filter paper disks impregnated with the nanocomposite at three different dosages of silver doping (5, 9 and 11). Sulphonamide disks (300 µg) (LABORCLIN®) were used as a control. The plates were incubated for 20 hours at 37 °C, and the halos were measured to determine the inhibition spectrum.

The eggs used in this research came from a supermarket and stemmed from commercial laying hens from a farm in the State of Minas Gerais, Brazil. SH was isolated in our laboratory, where they were characterized and genetically typed.

Eggs were used to evaluate the antibacterial properties of nanocomposite of ZnO:Ag-AgO nanocrystal in control SH. A total of 51 eggs were divided into 7 groups, each group consisting of 8 eggs, except for the negative control, which was composed of 3 eggs. Each sample unit consisted of two eggs. The eggs were treated with different concentrations of nanoparticles as follows: (G1) Spraying approximately 1.0 mL of a solution containing 1.4 µg/mL of ZnO:Ag-AgO nanocomposite; (G2) Spraying approximately 1.0 mL of a solution containing 1.4 µg/mL of ZnO:Ag-AgO nanocomposite plus Tween 20 1% (surfactant substance); (G3) Spraying approximately 1.0 mL of a solution containing 14 µg/mL of nanosilver particles doped with zinc ZnO:Ag-AgO; (G4) Spraying approximately 1 .0 mL of a solution containing 1.4 mg/mL of ZnO:Ag-AgO Nanocomposite; (G5) Spraying approximately 1.0 mL of a solution containing peracetic acid (400 ppm) before contact with bacteria; (G6) (positive control) Spraying ultrapure water with bacteria; (G7) (positive control) Spraying ultrapure water.

Upon 24 hours, the eggs were separately submerged two by two in a suspension of 100 mL of TSB containing 105 CFU/mL of SH for a period of 24 hours at 25º C. In the seventh group, negative control, the eggs were also submerged for 24 hours, but in sterile TSB, without any bacteria. After this period of biofilm formation, the eggs were washed 3 times in ultrapure water to remove free bacteria. After drying the eggs, they were opened in laminar flow; their shell, albumen and yolk were separated and collected aseptically. The eggs were weighted in two samples of 10g for two different analyses carried out at the same time. 90mL of peptone water (Isofar®) were added to 10g of each sample, and the resulting solution was incubated for 24 hours at 37ºC. Later, 1mL aliquot was inoculated in the Rappaport (Oxoid™) culture medium, and 1mL was inoculated in the Tetrathionate (Merck™) culture medium. Both were depleted in XLD upon 24 hours of growth.

Colonies with typical characteristics of *Salmonella* were selected and submitted to a conventional PCR assay (ompC gene) reaction to conform

species. Genomic DNA was extracted by using the Wizard Genomic DNA Purification Kit (Promega, CITY, COUNTRY) according to the manufacturer's protocol. Purified DNA (10 ng) was used as template for all PCR assays. The primers used (ompC gene) in this analysis were (3'ATCGCTGACTTATGCAATCG 5' and 5'CGGGTTGCGTTATAGGTCTG 3') (Alvarez et al., 2004) producing a fragment of 204bp. GoTaq™ Green Master Mix kit (Promega, CITY, COUNTRY) was used following the manufacturer's instructions. The microtubes containing the PCR products were transferred to the thermocycler (Eppendorf®) for amplification, following these cycles: one initial denaturation cycle at 94°C for 5min, amplified in 35 cycles of denaturation at 94°C for 45s, annealing at 57°C for 1min; extension at 72 °C for 90s, with final extension at 72°C for 10 minutes. The strain of S. Enteritidis ATCC 13076 was used as a positive control of the reactions The amplified products were subjected to a 1.5% agarose gel electrophoresis and by a TBE 0.5x runner buffer (Invitrogen) with the standard value of 100pb marker (Invitrogen) as its molecular weight.

As the eggs were not sterilized, another microarray PCR assay was performed for serotype confirmation. The multiplex ligation detection reaction (LDR) generated DNA molecules collections. These DNA molecules were subsequently amplified by means of a single pair of amplimers over a PCR. The PCR products were then sorted by hybridization to a low-density DNA microarray. Positive hybridization was detected using a biotin label incorporated into one of the PCR primers. Later, tubes were inserted in the single-channel ATR03 array tube reader upon completion of the detection reaction, and images were acquired and interpreted with the manufacturer-supplied software (Check-Points, Wageningen, The Netherlands).

The analyses were performed using GraphPad Prism, version 7.0. Chi-square test and binomial between two proportions in relation to the positive control were used. The confidence level was set at 95% for all reports.

## 3. Results

The great dispersion in water is ideal for the good performance of nanoparticles. SH formed biofilm in all tested groups. The group (G4) that had the higher concentration of nanoparticle was the group that most controlled SH biofilm formation in relation to the positive control (G6). The results of PCR confirmed that the typical colonies were SH. Microarray PCR assay confirmed the SH serotype. The use of nanoparticles at the concentration of 1.4 was able to better control biofilm formation in the eggshell. Zinc-oxide (Zn-O) is an antimicrobial compound that acts through different mechanisms. This compound is also used to preserve food packages (ESPITIA et al., 2016). Some studies have noticed good efficacy when Zn is bounded with Ag to form nanoparticles against gram negative and gram-positive bacteria (PASQUET et al., 2014).

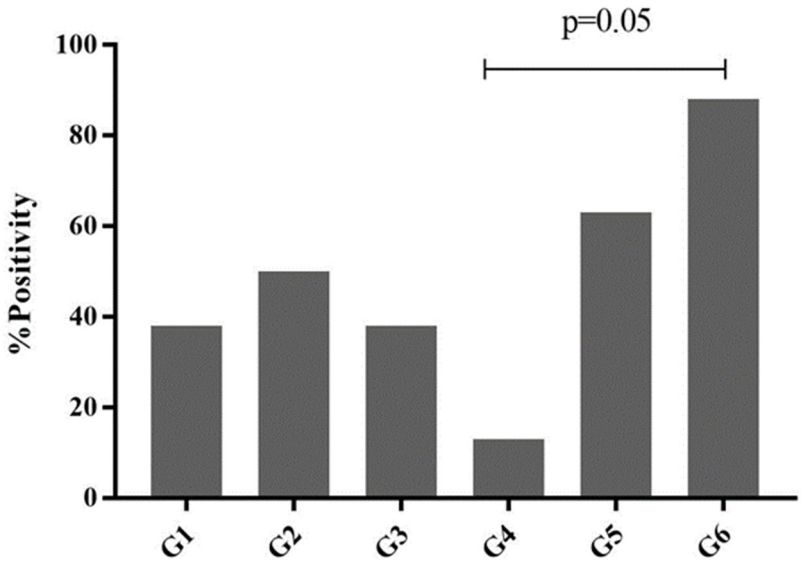


**Figure 1:** Percentage of positive samples after treatment with nanocomposite of Ag: ZnO+AgO nanocrystal in the eggshell for the following groups: G1: 1.4 µg / ml Ag: ZnO+AgO; G2: 1.4 µg / ml Ag: ZnO+AgO plus Tween 20 1%; G3: 14 µg / ml Ag: ZnO+AgO; G4: 1.4 mg / ml Ag: ZnO+AgO; G5: peracetic acid 400 ppm; G6: Positive Control. In G7: (Negative Control) the results were negative.

## 4. Discussion

The average size of the silver-doped ZnO nanocrystal varied from 15 to 28nm. The ZnO:Ag-AgO nanocomposites manufactured by us had favorable nanocrystal characteristics. The same nanoparticle can be manufactured through different methods which result in different properties. Methods of nanoparticle synthesis basically fall into two categories: physical methods (top-down), and chemical methods (bottom-up). This study used the physical methods, with physical processes, such as grinding, tempering, thermal decomposition, and irradiation, whereby the nanomaterial is formed from a larger sample. Other nanoparticles purchased both from Brazil and abroad have proved to be inefficient in our studies. In previous studies, we have noticed that our nanoparticles and nanocomposites are far superior compared to those acquired in the market (unpublished data).

The major risk associated with the use of nanoparticles is their ability to form free radicals in cells, including ROS (Reactive Oxygen Species), which can interfere with several intracellular processes (FU et al., 2013). However, ZnO:Ag-AgO nanoparticle is very stable. Silver loses reactive characteristics and its ability to bound with intracellular oxygen when it is bound with zinc oxide, ZnO:Ag-AgO has been shown to be safe to live in animals' cells. In previous analyses, we have found no toxicity of these nanocomposites in cell culture and chicken embryos (unpublished data).

In unfavorable ambient conditions, Salmonella can adhere to a surface, grow, and produce an extracellular matrix, in a process called biofilm. Biofilm provides bacteria with resistance to several external factors, which can be physical, chemical, and biological (PENG., 2016). Disinfectants are unable to remove the entire matrix of biofilm, they can only reduce bacterial load (SREY, et al., 2013). Thus, it is also associated with mechanical action to increase efficiency as this process can disorganize the external matrix and expose the microorganisms to the disinfectant (MAUKONEN et al., 2003).

The eggshell has several pores that are large enough to allow passage of microorganisms into the egg. Studies carried out by us (data unpublished) and Cardoso et al. (2001) show that SH can infect the egg interior upon 24-hour contact.

Because of the eggshell's irregular surface due its pores and roughness, the egg is a great experimental model to form biofilm. The structures developed in the process of biofilm formation allow the transport of nutrients and adapt well to this kind of surface. Besides, Salmonella control in eggs is very important to the poultry industry. Therefore, silver AgNP nanoparticles could be an excellent alternative for *Salmonella* control in eggs and in the environment (LOSASSO et al., 2014). Salmonellas cause a widespread zoonosis, salmonellosis. About 94 million cases are confirmed in humans worldwide every year (MAJOWICZ et al., 2010). The egg is a major animal source involved in most contamination by this agent. *Salmonella* Heidelberg sorovar has been responsible for an increased number of cases of salmonellosis in recent years (GIERALTOWSKI et al., 2016). Contamination is frequently linked to consumption of turkey and poultry products, which has generated several outbreaks in the USA (CENTERS OF DISEASE CONTROL AND PREVENTION – CDC., 2018).

SH is an important agent in poultry farms, because Salmonella, as shown in previously studies, can pass into the egg and infect embryos. Preventive treatment with nanoparticles is essential to avoid living forms of bacteria in the eggshell as it is difficult to eliminate the biofilm after its formation.

Our nanocomposites meet the minimum requirements to be classified as a safe and good nanoparticle. However, part of it still precipitates in water Because of our lack of ideal equipment for dispersion during manufacturing. This causes a loss of particles during the sprinkling process, leading to a waste of material. Even so, the nanocomposite of ZnO:Ag-AgO nanocrystal demonstrated more effectiveness than peracetic acid in high concentration to

control biofilm formation in eggshell. We are working to improve the dispersion of the nanoparticles and eventually decrease the concentration used in further studies.

## 5. Conclusion

New strategies are essential for the control of microorganisms in aviculture. The nanoparticles have shown excellent results in the control of the S. Heidelberg biofilm. Hence, the nanocomposite of ZnO:Ag-AgO nanocrystal is an alternative technology to control Salmonella in poultry.

## 6. Acknowledgment

This study was funded by Fundação de Apoio à Pesquisa de Minas Gerais (FAPEMIG).

***

## Nanocompostos de Ag-Doped ZnO e nanocristais de Ag2O no controle da formação de biofilmes de *Salmonella* Heidelberg em ovos comerciais

**Resumo:** As *Salmonellas* spp. são importantes agentes causadores de salmonelose em humanos. O controle da *Salmonella* spp. é importante, pois a bactéria ultrapassa a barreira da casca atingindo o embrião e infecta lotes de aves que podem levar a infecção ao ser humano. A desinfecção costuma ser feita por vários sanitizantes; porém, com os avanços da nanotecnologia, formas novas e mais eficientes de controle desse agente estão sendo estudadas, como as nanopartículas. Estudos preliminares dessas nanopartículas têm mostrado o sucesso de seu uso no controle de microrganismos. A padronização da concentração ideal de uso desse nanocomposto é fundamental para a máxima eficiência no controle de *Salmonella* spp. Ovos vermelhos oriundos de postura comercial foram comprados no comércio local e tratados em laboratório com as nanopartículas em diferentes concentrações; após 24 horas, formaram o biolfilme. Os ovos foram lavados para a retirada das bactérias livres. Realizaram-se exame microbiológico convencional, para isolamento de *Salmonella* spp., e PCR, para identificação das colônias. O objetivo deste

artigo foi avaliar a eficácia da utilização de nanocompostos de óxido de prata com óxido de zinco dopado com óxido de prata (ZnO: Ag-Ago) em diferentes concentrações na prevenção da formação de biofilmes na casca dos ovos.

***

## 7. References

Alvarez J, Sota M, Vivanco AB, et al. (2004). Development of a multiplex PCR technique for detection and epidemiological typing of Salmonella in human clinical samples. **Journal of Clinical Microbiology**. 42 (4), pp. 1734-1738. https://doi.org/10.1128/JCM.42.4.1734-1738.2004

Bauer, A. W. Kirby, W. M. Sherris, J. C. Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. **American Journal of Clinical Pathology**. 45 (4), pp. 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Borsoi, A. Santos, R. L. Rodrigues, B. L. Moraes, H. L. S. Salle, C. T. P. Nascimeto, V. P. (2011). Behavior of Salmonella heidelberg and *Salmonella* Enteritidis strains following broiler chick inoculation: evaluation of cecal morphometry, liver and cecum bacterial counts and fecal excretion patterns. **Brazilian Journal of Microbiology**. 42 (1), pp. 266-273. https://doi.org/10.1590/S1517-83822011000100034

Cardoso, A. L. S. P. Tessari, E. N. C. Castro, A. G. M. Kanashiro, A. M. I. Gama, N. M. S. Q. (2011). Pesquisa de coliformes totais e coliformes fecais analisados em ovos comerciais no laboratório de patologia avícola de descalvado. **Arquivos do Instituto Biológico**. 68 (1), pp. 19-22.

Centers of Disease Control and Prevention – CDC. (2018). Multistate Outbreak of Multidrug-Resistant *Salmonella* Heidelberg Infections Linked to Contact with Dairy Calves.

Dakhil, A. S. (2017). Biosynthesis of silver nanoparticle (AgNPs) using Lactobacillus and their effects on oxidative stress biomarkers in rats. **Journal of King Saud University**. 29 (4), pp. 462-467. https://doi.org/10.1016/j.jksus.2017.05.013

EFSA – EUROPE FOOD SAFETY AUTHORITY. (2018). Salmonella. https://www.efsa.europa.eu/en/topics/topic/salmonella

Espitia, P. J. P. Soares, N. F. F. Coimbra, J. S. R. Andrade, N. J. Cruz, R. S. Medeiros, E. A. A. (2012). Zinc Oxide Nanoparticles: Synthesis, Antimicrobial Activity and Food Packaging Applications. **Food and Bioprocess Technology**. 5 (1), pp. 1447-1464. https://doi.org/10.1007/s11947-012-0797-6

Fu, P. P. Qingsu, X. Huey-Min, H. Paresh, C. R. Yu, H. (2014). Mechanisms of nanotoxicity: Generation of reactive oxygen species. **Journal of Food and Drug Analysis**. 22 (1), pp. 64-75. https://doi.org/10.1016/j.jfda.2014.01.005

Gieraltowski, L. Higa, J. Peralta, V. et al. (2016) National Outbreak of Multidrug Resistant Salmonella Heidelberg Infections Linked National Outbreak of Multidrug Resistant Salmonella Heidelberg Infections Linked to a Single Poultry Company. **PLOS ONE**. 11 (9), pp. 1-13. https://doi.org/10.1371/journal.pone.0162369

Gitipour, A. Souhail, R. Al-abed, S. R. Scheckel, K. G. Tolaymat, T. (2017). Nanosilver as a disinfectant in dental unit waterlines: Assessment of the physicochemical transformations of the AgNPs. **Chemosphere**. 117 (1), pp. 245-252. https://doi.org/10.1016/j.chemosphere.2017.01.050

Jones, F. T. (2011). A review of practical Salmonella control measures in animal feed. **Journal of Applied Poultry Research**. 20 (1), pp.102-103. https://doi.org/10.3382/japr.2010-00281

Latasa, C. Garcia, B. Echeverz, M. et al. (2012). Salmonella Biofilm Development Depends on the Phosphorylation Status of RcsB. **Journal of Bacteriology**. 194(14), pp. 3708-3722. https://doi.org/10.1128/JB.00361-12

Lok, C. N. Ho, C. M. Chen, R. He, Q. Y. Yu, W. Y. Sun, H. Tam, P. K. H. Chiu, J. F. Che, C. M. (2007). Silver nanoparticles: partial oxidation and antibacterial activities. **Journal of Biological Inorganic Chemistry**. 12(1), pp. 527–534. https://doi.org/10.1007/s00775-007-0208-z

Lopes, F. L. Mendes, R. F. Santos, M. B. Andrade, M. A. Café, M. B. (2013). Salmonelose em sanidade avícola e saúde pública. **Revista eletrônica nutritime**. 5(10), pp. 2716-2751.

Losasso, C. Belluco, S. Cibin, V. et al. Antibacterial activity of silver nanoparticles: sensitivity of different Salmonella serovars. (2014). **Frontiers in Microbiology**. 5(1). p. 227. https://doi.org/10.3389/fmicb.2014.00227

Majowicz, S. E. Jennie, M. Elaine, S. Frederick, J. A. Martyn, K. Sarah, J. O. Timothy, F. J. Aamir, F. Robert, M. H. (2010.) For the International Collaboration on Enteric Disease "Burden of Illness" Studies.The Global Burden of Nontyphoidal Salmonella Gastroenteritis. **Clinical Infectious Diseases**. 50 (6). pp. 882-889. https://doi.org/10.1086/650733

Maukonen, J. Mättö, J. Wirtanen, G. Raaska, L. Mattila-Sandholm, T. Saarela, M. (2003). Methodologies for the characterization of microbes in industrial environments: a review. **Journal Industrial Microbiology and Biotechnology**. 30(6). pp. 327-356. https://doi.org/10.1007/s10295-003-0056-y

Medeiros, M. Oliveira, D. Rodrigues, D. Freitas, D. (2011). Prevalence and antimicrobial resistance of Salmonella in chicken carcasses at retail in 15 Brazilian cities. **Revista Panamericana de Salud Pública**. 30 (1). pp. 550-560. https://doi.org/10.1590/S1020-49892011001200010

Pasquet, J. Yves, C. Jocelyne, P. Emmanuelle, C. Dominique, B. Marie-Alexandrine, B. (2014). The contribution of zinc ions to the antimicrobial activity of zinc oxide.

**Colloids and Surfaces A: Physicochemical and Engineering Aspects**. 457(5). pp. 263-274. https://doi.org/10.1016/j.colsurfa.2014.05.057

Pati, R. Mehta, R. K. Mohanty, S. et al. (2014) Topical application of zinc oxide nanoparticles reduces bacterial skin infection in mice and exhibits antibacterial activity by inducing oxidative stress response and cell membrane disintegration in macrophages. **Nanomedicine**. 10(6). pp. 1195-1208. https://doi.org/10.1016/j.nano.2014.02.012

Peng, D. (2016). Biofilm Formation of Salmonella. **Microbial Biofilms Dharumadurai Dhanasekaran**. 1(1). https://doi.org/10.5772/62905

Srey, S. Jahid, K. I. Ha, S. (2013). Biofilm formation in food industries: A food safety concern. **Food Control**. 31(2). pp. 572-585. https://doi.org/10.1016/j.foodcont.2012.12.001

Steenackers, H. Hermans, K. Vanderleyden, J. Keersmaecker, S. C. J. (2012). Salmonella biofilms: An overview on occurrence, structure, regulation and eradication. **Food Research International**. 45(2). pp. 502-531. https://doi.org/10.1016/j.foodres.2011.01.038

Tauxe, R. V. (1997). Emerging foodborne diseases: an evolving public health challenge. **Emerging Infectious Diseases**. 3(4). pp. 425-434. 10.3201/eid0304.970403

WHO – WORLD HEALTH ORGANIZATION. (2018). Salmonella (non-typhoidal). http://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal).