## Comparative Studies of Food Poisoning Salmonella among Slaughtered Animals.

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#### **Abstract:**

Salmonella is one of the major zoonotic foodborne pathogens worldwide. The presented study find out that the level of Salmonella contamination on cattle skin excision, fresh carcasses, liver, kidney and swabs after transportation and display at abattoir and butcher shops at Sharkia Governorate, Egypt. A total of 444 fresh Cow (50), Buffalo (48) and Camel (13) carcasses represented by Liver, Kidney, Swab and Skin excision samples The collected samples were subjected to isolation and identification of salmonella. Rate of resistant of the streptomycin was 100%, 96.2% for erythromycin, cefotaxim (80.8%), nalidixic acid (69.2%), sulphamethoxazol (65.4%), chloramphenicol (53.8%) and amikacin (50%). On contrary the sensitivity was 96.2% for gentamicin, (84.6%) ciprofloxacin, (73.1%) ampicillin and 76.9% for kanamycin. The high level of resistance may be due to many bacteria come in a close contact with many types of antibiotics miss used in animal medication, these processes contribute to resistance by overexposing cultures to these bactericidal or bacteriostatic chemicals. The data showed that 71.4 % of S. Typhimurium isolates displayed five or more antimicrobial resistant profiles . 80 % of S. Enteritidis isolates displayed five or more antimicrobial resistant profiles, 75 % of S. Infantis isolates displayed five or more antimicrobial resistant profiles. Furthermore, multiple antibiotic resistance (MAR) index of isolated Salmonella species ranged from 0.143 to 1 with an average of 0.483.

The MAR index results higher than 0.2 could be due to a contamination from high-risk sources, such as farm animals frequently exposed to antibiotics, resulting in potential risk to consumers. The reduction percent of the used disinfectants increased by the time to reach 100% or nearly approach it in a case of most used disinfectants. Besides alkadox followed by aldekol were the most superior disinfectant while, swift was the least one.

### 1.Introduction

Meat and edible organs are considered as an excellent source of high quality animal protein, vitamins especially B complex, especially iron (**Gracy**, **1986**). Foodborne diseases pose a major Public Health problem because of the increasing number of episodes, the emergence of new forms of transmission, the appearance of vulnerable population groups, the increasing pathogen resistance to antimicrobial compounds, and their socioeconomic impact (**Prado et al.**, **2002**; **Ruttler et al.**, **2002**).

Salmonella is one of the major zoonotic foodborne pathogens worldwide. It can cause avariety of clinical manifestations from mild gastroenteritis to bacteremia and typhoid fever. the global burden of nontyphoidal salmonella gastroenteritis has been estimated to be 93.8 million cases of gastroenteritis each year, with 155000 deaths (Majowics SE et al., 2010) Salmonella species are aleading cause of acute gastroenteritis in several countries, and salmonellosis remains an important public health problem worldwide, particularly in the developing countries (AddisZ et al., 2011). The presence of salmonella in food animals at slaughter and the consequent cross-contamination of edible carcass present asignificant food-safety hazard (Kikuvi et al., 2010). Foodborne bacterial contamination of meat is responsible for several thousand illnesses per year in Egypt. The primary means of reducing or

preventing this type of disease is to identify the sources of contamination and minimize or remove the sources from the production process. skin excision, fresh carcasses have been consistently implicated as the main source of the foodborne pathogens that contaminate carcasses Barkocy-Gallagher et al. (2003), Barkocy-Gallagher et al. (2001), Bosilevac et al. (2004) and Nou et al. (2003). In response to this finding, various forms of antimicrobial interventions targeting cattle skin excision, fresh carcasses have been developed. These interventions have been implemented in commercial beef processing facilities and have been effective for reducing carcass contamination Arthur et al. (2007) and Bosilevac et al. (2005). However, even after the implementation of these interventions, pathogenic bacteria are still found in ground beef, possibly indicating that other sources of these bacteria may be present. Possibility of contamination of meat products with food poisoning bacteria especially Salmonella organism has been extensively reported (Reham, 2004; Erdem et al., 2005). Salmonella has been associated with a number of food-producing animals, which makes animals and their products important sources of human infections (Acha & Szyfres 2001; Davies, Dalziel & Gibbens 2004). The risk of Salmonella contamination may be present at any stage of food animal production ranging from the live animal to environmental factors (Alexander, Warnick & Wiedmann 2009; Troutt & Osburn, 1997). At the farm level, cattle hides may become exposed to Salmonella through contact with contaminated faeces, feed, or the environment, which poses a risk to food safety if these organisms are transferred on the carcass

Antimicrobial-resistant Salmonella is increasing due to the use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated meat (Forough et al., 2013). Antimicrobial compounds have been used to treat bacterial infections since the middle of the twentieth century. These compounds were highly successful in treating various diseases and were 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). Control of antibiotic resistant Salmonella is most efficiently through the reduction of consumption antibiotic. Control of animal feed, husbandry, hygiene in abattoir routinely, sanitation at all stages and food services are ways to minimize the need for antibiotic treatment (Leila, 2012). Good manufacturing practices in processing plants may reduce final product contamination, and the control of critical points is essential along the entire production chain (Bailey et al., 2001). One of the mechanisms adopted in the control of critical points to control and to reduce the presence of Salmonella spp during carcass processing and in the finalproduct is the use of disinfectants. A wide range of active principles, such as chlorine, quaternary ammonium, glutaraldehyde, and iodine, is available for the use in meat production, bothduring rearing and processing. The expected efficacy of disinfectants depends on their correct application, considering recommended dilutions, time of contact withthe surface to be sanitized, previous removal of organic matter, the quality of the water used for cleaning, and general characteristics of the action of disinfectants (Martinez et al., 1999), in addition to product concentration, and environment temperature and pH (Merianos,

### 2 -MATERIALS AND METHODS

### 2.1. Collection of samples:

A total of 444 samples were collected (50 liver, 50 Kidney, 50 Swab and 50 skin excision) from cattle, (48 liver, 48 Kidney, 48 Swab and 48 skin excision) from camel and (13 liver, 13 Kidney, 13 Swab and 13 skin excision) from buffalo animals carcasses including of cattle, buffalo and camel. All samples collected from different butchers shops and abattoir of different sanitation levels at Sharkia government well identified, packed in sterile plastic bags then labeled and immediately transferred under sanitary precaution to the laboratory respectively.

## 2.2.Antibiotic Resistance of *Salmonella* species (Antibiogramme)

Antimicrobial susceptibility was tested by the single diffusion method according to **Srivani** (2011) for Salmonella species. Sensitivity discs with variable concentrations were used to determine the susceptibility of the isolated bacterial strains (Oxoid Limited, Basingstoke, Hampshire, UK). plate method was applied by using of nutrient agar as a substrate for growth of the tested bacterium for its antibiotic sensitivity. The bacterial culture was uniformly spread on the surface of nutrient agar. Then the antibiotic discs were placed over the surface of inoculated plate. Moreover, the plate was then incubated at suitable temperature (25°C) for 2-7 days and checked for the growth of the bacterium around the antibiotic discs. The maximal inhibition zone for the growth of microbe is said to that antibiotic had maximum effect on the microbe growth. Therefore, the antimicrobial susceptibility testing was applied according to the guidelines stipulated by National Committee for Clinical Laboratory Standards "NCCLS" (2001). Accordingly, the antimicrobial discs and their concentrations as well as the diameters of the zones of inhibition for the tested strains are demonstrated in the following table:

Supplementary table (1): Antimicrobial discs, concentration and interpretation of their action on the isolated *Salmonella* species.

| Antimicrobial agent        | Sensitivity disc | <b>Resistant</b> | <b>Intermediate</b> | <b>Susceptible</b> |
|----------------------------|------------------|------------------|---------------------|--------------------|
|                            | content (ug)     | (mm)             | (mm)                | (mm)               |
| Ampicillin (AM)            | <u>10</u>        | 13 or less       | <mark>14-17</mark>  | 18 or more         |
| Nalidixic acid (NA)        | <mark>30</mark>  | 13 or less       | <mark>14-18</mark>  | 19 or more         |
| <b>Tetracycline (T)</b>    | <mark>30</mark>  | 14 or less       | <mark>15-18</mark>  | 19 or more         |
| Gentamicin (G)             | <mark>10</mark>  | 12 or less       | <mark>13-14</mark>  | 15 or more         |
| Kanamycin (K)              | 30               | 13 or less       | <del>14-17</del>    | 18 or more         |
| Ciprofloxacin (CP)         | <u>5</u>         | 15 or less       | <mark>15-19</mark>  | 20 or more         |
| Amikacin (AK)              | <mark>30</mark>  | 12 or less       | <b>13-15</b>        | 16 or more         |
| Streptomycin (S)           | 10               | 11 or less       | 12-14               | 15 or more         |
| Cefotaxim (CF)             | <mark>30</mark>  | 17 or less       | 18-22               | 23 or more         |
| Neomycin (N)               | <mark>30</mark>  | 12 or less       | <del>13-16</del>    | 17 or more         |
| Cephalothin (CN)           | <mark>30</mark>  | 14 or less       | <mark>15-17</mark>  | 18 or more         |
| Erythromycin (E)           | <mark>15</mark>  | 13 or less       | 14-22               | 23 or more         |
| <b>Chloramphenicol (C)</b> | <mark>30</mark>  | 12 or less       | <b>13-17</b>        | 18 or more         |
| Sulphamethoxazol (SXT)     | <mark>25</mark>  | 10 or less       | <b>11-15</b>        | 16 or more         |

The tested strains were evaluated as susceptible, intermediate and resistant. Multiple Antibiotic Resistance (MAR) index for each strain was determined according to the formula stipulated by **Singh et al. (2010)** as follow: **MAR index**= No. of resistance (Isolates classified as intermediate were considered sensitive for MAR index) / Total No. of tested antibiotics.

## 2.3. In vitro Disinfectant efficacy against Salmonella Typhimurium:

- 2.3.1. Preparation of test strains (Sutton et al., 2002)
- 2.3.2. Preparation of Disinfectant Agent (Linton et al., 1987)
- 2.3.3. Disinfectant agents:

Alkadox®, Aldekol®, Virkon-S®, Iodoline®, Phenodex® and Swift® were prepared as per production procedure and/or supplier guideline so that the test solution is made to its final dilution using USP purified water with pH 5.0-7.0 from the facility distribution water system.

- 2.3.4. Antimicrobial Effectiveness Test
- 2.3.4.1.In vitro Surface Challenge Test (Clontz, 2008)

# Supplementary table (2): sources and active ingredient of disinfectant:

| Source                   | Active ingredient  |
|--------------------------|--|
| Antec International LTD  | Potassium peroxymonosulfate and Sodium   |
| UK                       | chloride   |
| EWABO Chemkalien,        | Glutaraldehyde, Quaternary ammonium  |
| Gmbh                     | compound, Formalin   |
| Chemi-care, A.R.E        | Phenol, sodium sulphate salt, anionic surfactants  |
| Chemi-care, A.R.E        | Sodium hypochlorite, Sodium carbonate  |
| Tristel, Cambridgeshire, | Chlorine dioxide   |
| CB8 7NY, UK              |  |
| Jordan Resource for      | Iodine, phosphoric acid, sulphoric acid  |
|                          | Antec International LTD UK EWABO Chemkalien, Gmbh Chemi-care, A.R.E Chemi-care, A.R.E Tristel, Cambridgeshire, CB8 7NY, UK |

### 2.4. Statistical analysis:

The obtained data were statistically analyzed using analysis of variance (ANOVA) test and comparative of means were performed according to Duncan Multiple Range test for comparison of Means according to **Snedecor**, (1969) using **SPSS14(2006)**.

### 3- Results and Discussion

Antibiotic resistance can be spread via residual antibiotics in food products, through the transfer of resistant foodborne pathogens, or through the ingestion of resistant strains among original food microflora and the transfer of resistance to pathogenic microorganisms (Pesavento et al., 2007).

In the current study the rate of resistant to the streptomycin was 100%, 96.2% for erythromycin, cefotaxim (80.8%), nalidixic acid (69.2%), sulphamethoxazol (65.4%), chloramphenicol (53.8%) and amikacin (50%). On contrary the sensitivity was 96.2% for gentamicin, (84.6%) ciprofloxacin, (73.1%) ampicillin and 76.9% for kanamycin (table 2). The findings to some extent comparable with **Esaki et al., (2004)** who found that Salmonella were isolated from food-producing animals samples and tested for antimicrobial susceptibility.

Table (3) Antimicrobial susceptibility of Salmonella species (n=26).

| Antimicrobial agent    |    | S    |    | I    |    | R    |
|------------------------|----|------|----|------|----|------|
|                        | NO | %    | NO | %    | NO | %    |
| Streptomycin (S)       | -  | -    | -  | -    | 26 | 100  |
| Erythromycin (E)       | 1  | -    | 1  | 3.8  | 25 | 96.2 |
| Cefotaxim (CF)         | 1  | 3.8  | 4  | 15.4 | 21 | 80.8 |
| Nalidixic acid (NA)    | 2  | 7.7  | 6  | 23.1 | 18 | 69.2 |
| Sulphamethoxazol (SXT) | 6  | 23.1 | 3  | 11.5 | 17 | 65.4 |
| Chloramphenicol (C)    | 8  | 30.8 | 4  | 15.4 | 14 | 53.8 |
| Amikacin (AK)          | 10 | 38.5 | 3  | 11.5 | 13 | 50.0 |
| Cephalothin (CN)       | 11 | 42.3 | 5  | 19.2 | 10 | 38.5 |
| Tetracycline (T)       | 15 | 57.8 | 1  | 3.8  | 10 | 38.5 |
| Neomycin (N)           | 15 | 57.8 | 2  | 7.7  | 9  | 34.6 |
| Kanamycin (K)          | 20 | 76.9 | -  | -    | 6  | 23.1 |
| Ampicillin (AM)        | 19 | 73.1 | 3  | 11.5 | 4  | 15.4 |
| Ciprofloxacin (CP)     | 22 | 84.6 | 2  | 7.7  | 2  | 7.7  |
| Gentamicin (G)         | 25 | 96.2 | -  | -    | 1  | 3.8  |

S:Sensitive. I: Intermediate. R:Resistant

ampicillin, isolates were resistant to dihydrostreptomycin, kanamycin, The oxytetracycline, chloramphenicol, bicozamycin, nalidixic acid, oxolinic acid and trimethoprim during Japanese veterinary antimicrobial resistance monitoring program mean while Molla and Zewdu (2004) who reported that isolates of Sal-monella from food items and personnel from Addis Ababa were resistant to commonly used antibiotics including streptomycin, ampicillin and tetracycline and susceptible to gentamicin more over **Tesfaw et** al., (2013) found that 83.3, 50, 16.7, and 16.7% of Salmonella isolates were sensitive to tetracycline, ampicillin, amoxicillin, and chloramphenicol, respectively. However, all the isolates were susceptible to gentamycin, ceftriaxone, ciprofloxacin, and sulfamethoxazole. Nearly similar findings for Salmonella species isolated from abattoir in Adama town, Oromia, Ethiopia Abunna et al., (2018) who found that all isolates were 100%, 81.8% and 81.8% sensitive to gentamycin, kana-mycin, sulphamethazole respectively. On the other hand the isolates were 72.7%, 63.6%, and 54.5% resistant to streptomycin, cefoxitin and ampicillin respectively. The high level of resistance may be due to many bacteria come in close contact with many types of antibiotics miss used in animal medication, these processes contribute to resistance by overexposing cultures to these bactericidal or bacteriostatic chemicals (miss use of antibiotics in farm animals ) (Everage et al., 2014). This may lead to a development of antibiotic-resistant bacteria.

The data illustrated in table(3) showed that 71.4 % of S. Typhimurium isolates displayed five or more antimicrobial resistant profiles while 80 % of S. Enteritidis isolates displayed five or more antimicrobial resistant profiles, 75 % of S. Infantis isolates displayed five or more antimicrobial resistant profiles. Furthermore, multiple antibiotic resistance (MAR) index of isolated Salmonella species ranged from 0.143 to 1 with an average of 0.483. Salmonella Typhimurium and S. Infantis are serotypes frequently isolated from food of animal origin and food poisoning cases in Japan. With the antibiotic resistance genes integrated in the chromosome, most isolates show multi antibiotic resistant to five drugs (Threlfall et al., 1994). Moreover, 40.7% of S. Typhimurium isolates and was more often multi-drug resistant during Japanese veterinary antimicrobial resistance monitoring program (Esaki et al., 2004).

Table (3): Antimicrobial resistance profile of Salmonella species

| NO    | Strains        | Antimicrobial resistance profile                 | MAR   |
|-------|----------------|--|-------|
|       | G 75 1 1       |  | index |
| 1     | S. Typhimurium | S, E, CF, NA, SXT, C, AK, CN, T, N, K, AM, CP, G | 1     |
| 2     | S. Typhimurium | S, E, CF, NA, SXT, C, AK, CN, T, N, K, AM        | 0.857 |
| 3     | S. Typhimurium | S, E, CF, NA, SXT, C, AK, CN, T                  | 0.643 |
| 4     | S. Typhimurium | S, E, CF, NA, SXT, C, AK                         | 0.500 |
| 5     | S. Typhimurium | S, E, CF, NA, SXT                                | 0.357 |
| 6     | S. Typhimurium | S, E, CF, NA                                     | 0.286 |
| 7     | S. Typhimurium | S, E   | 0.143 |
| 8     | S. Enteritidis | S, E, CF, NA, SXT, C, AK, CN, T, N, K, AM        | 0.857 |
| 9     | S. Enteritidis | S, E, CF, NA, SXT, C, AK, CN, T, N               | 0.714 |
| 10    | S. Enteritidis | S, E, CF, NA, SXT, C                             | 0.428 |
| 11    | S. Enteritidis | S, E, CF, NA, SXT                                | 0.357 |
| 12    | S. Enteritidis | S, E, CF   | 0.214 |
| 13    | S. Infantis    | S, E, CF, NA, SXT, C, AK, CN, T, N, K            | 0.786 |
| 14    | S. Infantis    | S, E, CF, NA, SXT, C, AK                         | 0.500 |
| 15    | S. Infantis    | S, E, CF, NA, SXT                                | 0.357 |
| 16    | S. Infantis    | S, E   | 0.143 |
| 17    | S. Virchow     | S, E, CF, NA, SXT, C, AK, CN, T, N, K            | 0.786 |
| 18    | S. Virchow     | S, E, CF, NA, SXT, C, AK, CN, T, N               | 0.714 |
| 19    | S. Virchow     | S, E   | 0.143 |
| 20    | S. Montevideo  | S, E, CF, NA, SXT, C, AK                         | 0.500 |
| 21    | S. Montevideo  | S, E, CF   | 0.214 |
| 22    | S. Montevideo  | S  | 0.071 |
| 23    | S. Heidelberg  | S, E, CF, NA, SXT, C, AK, CN, T, N               | 0.714 |
| 24    | S. Heidelberg  | S, E, CF   | 0.214 |
| 25    | S. Paratyphi A | S, E, CF, NA, SXT, C, AK, CN, T, N, K, AM, CP    | 0.928 |
| 26    | S. Haifa       | S, E   | 0.143 |
| Avera | age 0.483      |  |       |

S: Streptomycin
SXT: Sulphamethoxazol
CN: Cephalothin
CP: Ciprofloxacin

E: Erythromycin
CF: Cefotaxim
CF: Cefotax

Furthermore the results were comparable to **Molla and Zewdu**, (2004) who reported that 25% anti¬microbial resistant Salmonella isolates (**Tesfaw et al.**, 2013) found that 50% of Salmonella isolates were multiple antimicrobial resistant and **Abunna et al.** (2018) revealed that 54.5% were multiple antimi¬crobial resistant.

The variation in the MAR index could be attributed to differences in the sources of samples geographic distribution, which has differential selective pressures for the antibiotic resistance levels (Lesley et al., 2011); and test methodologies (Robert-Pillot et al., 2014).

The MAR index results higher than 0.2 could be due to contamination from high-risk sources, such as farm animals frequently exposed to antibiotics, resulting in potential risk to consumers. The high MAR in the current study indicated that the isolates originated from high-risk source samples; therefore, monitoring of antimicrobial resistance is essential to identify the effectiveness of new generations of antibiotics and to ensure the safety of meat.

One way to limit the occurrence and spread of *Salmonella* within the abattoir environment is through appropriate cleaning and disinfection programmes. Several approaches have been

investigated however, difficulties in eliminating *Salmonella* remain. Reasons for this include production of biofilms, or developed resistance to the cleaning agents and/or disinfectants, or harboring sites (i.e., cracks and holes in the lairage pens, drains) that are not easily cleaned or disinfected, all of which allow *Salmonella* to be survive ( **Stewart et al., 2001**). The reduction percent of *S.Typhmurium* isolated from abattoir after exposure to some chemical disinfectant at 0.5 % concentration of Alkadox ,Aldekol, Virkon-S, Iodoline, Phenodex, Swift, respectively and contact time of 30, 60, 90 and 120 minutes was recorded in table (4), The table results revealed that the initial count of *S.Typhmurium* (zero time) was 4 x 10<sup>6</sup> /cm<sup>2</sup> surface after 30 mintes This count was reduced by 42.5, 37.5, 27.5, 25,15 and 12.5% respectively. Moreover, the reduction percent increased gradually after 60 minutes became 57.5, 47.5, 40, 35,27.5 and 22.5%, respectively. While the reduction percentages after contact time 90 minutes became 83, 67.5, 55, 50, 40 and 32.5%, respectively. Finally, after 120 minutes the reduction % was 97.6, 90.1, 79.5, 75.2, 67.5 and 55 %, respectively.

Table (4): Effect of different disinfectants (0.5%) against Salmonella Typhimurium  $(4.0 \times 106/\text{ cm}^2)$  at various contact times.

| Disinfectant      | Contact time |         |        |         |        |         |        |            |        |  |
|-------------------|--------------|---------|--------|---------|--------|---------|--------|------------|--------|--|
| (0.5%)            | Zero<br>time | 30 min  | R<br>% | 60 min  | R<br>% | 90 min  | R<br>% | 120<br>min | R<br>% |  |
| Alkadox®          | 4.0×106      | 2.3×106 | 42.5   | 1.7×106 | 57.5   | 6.8×105 | 83.0   | 9.5×104    | 97.6   |  |
| Aldekol®          | 4.0×106      | 2.5×106 | 37.5   | 2.1×106 | 47.5   | 1.3×106 | 67.5   | 3.7×105    | 90.1   |  |
| Virkon-S®         | 4.0×106      | 2.9×106 | 27.5   | 2.4×106 | 40.0   | 1.8×106 | 55.0   | 8.2×105    | 79.5   |  |
| <b>Iodoline</b> ® | 4.0×106      | 3.0×106 | 25.0   | 2.6×106 | 35.0   | 2.0×106 | 50.0   | 9.9×105    | 75.2   |  |
| <b>Phenodex</b> ® | 4.0×106      | 3.4×106 | 15.0   | 2.9×106 | 27.5   | 2.4×106 | 40.0   | 1.3×106    | 67.5   |  |
| <b>Swift®</b>     | 4.0×106      | 3.5×106 | 12.5   | 3.1×106 | 22.5   | 2.7×106 | 32.5   | 1.8×106    | 55.0   |  |

The reduction percent of S.Typhmurium isolated from abattoir after exposure to some chemical disinfectant at 1 % concentration of alkadox, aldekol, virkon-S, iodoline, phenodex and swift, respectively and contact time of 30, 60, 90 and 120 minutes was recorded in table (5), they also noticed that the initial count of S.Typhmurium (zero time) was 4 x  $10^6$  /cm² surface after 30 minutes . This count was reduced by 60, 52.5.5, 42.5, 37.5,30 and 25%, respectively. Moreover, the reduction percent increased gradually after 60 minutes became 80, 72.5, 65, 55,42.5 and 40%, respectively.

Table (5): Effect of different disinfectants (1%) against Salmonella Typhimurium  $(4.0 \times 106/\text{ cm}^2)$  at various contact times.

|                   | Contact time |         |        |         |        |         |        |            |        |  |
|-------------------|--------------|---------|--------|---------|--------|---------|--------|------------|--------|--|
| Disinfectant (1%) | Zero<br>time | 30 min  | R<br>% | 60 min  | R<br>% | 90 min  | R<br>% | 120<br>min | R<br>% |  |
| Alkadox®          | 4.0×106      | 1.6×106 | 60.0   | 7.7×105 | 80.7   | 9.2×103 | 99.7   | -          | 100    |  |
| Aldekol®          | 4.0×106      | 1.9×106 | 52.5   | 1.1×106 | 72.5   | 8.3×104 | 97.9   | 5.1×103    | 99.8   |  |
| Virkon-S®         | 4.0×106      | 2.3×106 | 42.5   | 1.4×106 | 65.0   | 2.9×105 | 92.8   | 2.4×104    | 99.4   |  |
| <b>Iodoline</b> ® | 4.0×106      | 2.5×106 | 37.5   | 1.8×106 | 55.0   | 7.4×105 | 81.5   | 8.7×104    | 97.8   |  |
| <b>Phenodex</b> ® | 4.0×106      | 2.8×106 | 30.0   | 2.3×106 | 42.5   | 1.1×106 | 72.5   | 3.9×105    | 90.2   |  |
| Swift®            | 4.0×106      | 3.0×106 | 25.0   | 2.4×106 | 40.0   | 1.6×106 | 60.0   | 7.2×105    | 82.0   |  |

The reduction percentages after contact time 90 minutes became 99.7, 97.9, 92.8, 81.5, 72.5 and 60%, respectively. Finally, after 120 minutes the reduction % were 100, 99.8, 99.4, 97.8, 90.2 and 82 %, respectively. The reduction percent of *S.Typhmurium* isolated from abattoir after exposure to some chemical disinfectant at 1.5 % concentration of alkadox, aldekol, virkon-S, iodoline, phenodex and swift, respectively and contact time of 30, 60, 90 and 120 minutes was recorded in table (6). also noticed that the initial count of *S.Typhmurium* (zero time) was 4 x 10<sup>6</sup> /cm<sup>2</sup> surface after 30 minutes . This count was reduced by 75, 70, 55, 50,42.5 and 37.5 %, respectively. Moreover, the reduction percent increased gradually after 60 minutes became 99.1, 98, 72.5, 70, 60 and 52.5%, respectively. The reduction percentages after contact time 90 minutes became 100, 99.8, 97.9, 94.5, 85 and 70%, respectively. Finally, after 120 minutes the reduction % were 100, 100, 99.7, 99.6, 97.8 and 91 %, respectively.

Table (6): Effect of different disinfectants (1.5%) against Salmonella Typhimurium  $(4.0 \times 106/\text{ cm}^2)$  at various contact times.

| Disinfectant      | Contact time |         |        |         |        |         |        |            |        |
|-------------------|--------------|---------|--------|---------|--------|---------|--------|------------|--------|
| (1.5%)            | Zero<br>time | 30 min  | R<br>% | 60 min  | R<br>% | 90 min  | R<br>% | 120<br>min | R<br>% |
| Alkadox®          | 4.0×106      | 1.0×106 | 75.0   | 3.5×104 | 99.1   | -       | 100    | -          | 100    |
| Aldekol®          | 4.0×106      | 1.2×106 | 70.0   | 7.9×104 | 98.0   | 4.6×103 | 99.8   | -          | 100    |
| Virkon-S®         | 4.0×106      | 1.8×106 | 55.0   | 1.1×106 | 72.5   | 8.1×104 | 97.9   | 9.8×103    | 99.7   |
| <b>Iodoline</b> ® | 4.0×106      | 2.0×106 | 50.0   | 1.2×106 | 70.0   | 2.2×105 | 94.5   | 1.5×104    | 99.6   |
| <b>Phenodex</b> ® | 4.0×106      | 2.3×106 | 42.5   | 1.6×106 | 60.0   | 6.0×105 | 85.0   | 8.7×104    | 97.8   |
| Swift®            | 4.0×106      | 2.5×106 | 37.5   | 1.9×106 | 52.5   | 1.2×106 | 70.0   | 3.6×105    | 91.0   |

The reduction percent of S.Typhmurium isolated from abattoir after exposure to some chemical disinfectant at 2 % concentration of alkadox, aldekol, virkon-S, iodoline, phenodex and swift, respectively and contact time of 30, 60, 90 and 120 minutes was recorded in table (7).

Table (7): Effect of different disinfectants (2%) against Salmonella Typhimurium  $(4.0 \times 106/\text{ cm}^2)$  at various contact times.

|                   |              | Contact time |      |         |      |         |      |         |      |
|-------------------|--------------|--------------|------|---------|------|---------|------|---------|------|
| Disinfectant (2%) | Zero<br>time | 30 min       | R %  | 60 min  | R %  | 90 min  | R %  | 120 min | R %  |
| Alkadox®          | 4.0×106      | 5.3×104      | 98.6 | -       | 100  | -       | 100  | -       | 100  |
| Aldekol®          | 4.0×106      | 1.1×105      | 97.2 | 9.0×103 | 99.8 | 1       | 100  |         | 100  |
| Virkon-S®         | 4.0×106      | 4.7×105      | 88.3 | 2.6×104 | 99.3 | 3.4×103 | 99.9 | •       | 100  |
| <b>Iodoline</b> ® | 4.0×106      | 8.0×105      | 80.0 | 5.5×104 | 98.6 | 9.1×103 | 99.7 | -       | 100  |
| <b>Phenodex</b> ® | 4.0×106      | 1.0×106      | 75.0 | 3.9×105 | 90.2 | 7.5×104 | 98.1 | 9.0×102 | 99.9 |
| Swift®            | 4.0×106      | 1.5×106      | 72.5 | 8.3×105 | 79.3 | 1.0×105 | 97.5 | 4.8×104 | 98.8 |

It is noticed that the initial count of S.Typhmurium (zero time) was  $4 \times 106$  /cm2 surface after 30 minutes in. This count was reduced by 98.6, 97.2, 88.3, 80,75 and 72.5 %, respectively. Moreover, the reduction percent increased gradually after 60 minutes became 100, 99.8, 99.3, 98.6, 90.2 and 79.3%, respectively. The reduction percentages after contact

time 90 minutes became 100, 100, 99.9, 99.7, 98.1 and 97.5%, respectively. Finally, after 120 minutes the reduction % were 100, 100, 100, 100, 99.9 and 98.8 %, respectively.

It is clear from tables (4, 5, 6 and 7) that the reduction percent of the used disinfectants increased by the time to reach 100% or nearly approach as in case of most disinfectants used. The first disinfectant was alkadox followed by aldekol while considered as the most superior disinfectant while, swift was the least one. Regarding to the effect of quaternary ammonium compound active ingredient in aldekol nearly similar effects which lead to declines in the probability of detecting Salmonella 17/72 of swabs positive for Salmonella. A greater reduction in the number of Salmonella positive swabs 1/72 was detected following the combined use of quaternary ammonium compound and the chlorocresol based disinfectant, resulting in a reduction in the probability of detecting Salmonella (Walia et al., 2017). Moreover, Salmonella Typhimurium strain was selected and decontaminated with glutaraldehyde, QAC and formaldehyde-based products (Aldekol) on abattoir surfaces and showed great reduction (Gosling et al.2017). Several studies have revealed the efficacy of chlorine dioxide (swift) as a sanitizer for reducing Salmonella from fruit surfaces and food processing plants (Du et al., 2003). A recent study in Republic of Korea by Ahmed et al. ( 2017) found that Salmonella was reduced in all measuring period; with the highest disinfection rate occurring at 6 hours under the effect of chlorine dioxide the active ingridient of swift. Nearly similar reduction % was obtained by examined Sodium hypochlorite 5%, 8% and 10% achieved 78%, 94% and 100% killing efficacy after 4 h contact, respectively. Morover, Carbolic acid 6.5% achieved a highly significant 100% killing efficacy after 4 hours exposure (Soliman, et al., 2016).

### 4-Conclusion

Higher rate of resistance in Salmonella to the common used antibiotics such as streptomycin, erythromycin, sulphamethoxazol. Meanwhile, sensitivity was detected for gentamicin, ciprofloxacin, ampicillin and for kanamycin.

The most effective disinfectant for reducing the viability of Salmonella Typhimurium is the disinfectant which contains quaternary ammonium compound, potassium peroxymonosulphate, sodium chloride and Glutraladyde in its components.

#### 5- References

- Acha, P.N. & Szyfres, B., 2001, 'Salmonellosis', in Zoonoses and communicable diseases common to man and animals, pp. 233–246, Scientific and Technical Publication No. 580, Pan American Health Organization, Washington, DC.
- **Abunna F, Bedashu A, Beyene T, Ayana D, Feyisa A, Duguma R (2018):** Occurrence of Salmonella and antimicrobial sensitivity test in Abattoir and Dairy farms in Adama town, Oromia, Ethiopia. J Vet Med Res 5(3): 1127.
- Addis Z, Kebede N, Sisay Z, Alemayehu H, Wubetie A, Kassa T 2011. Prevalence and antimicrobial resistance of Salmonella isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross sectional study. BMC Infect Dis 2011; 11:222

- Ahmed, S. T., Bostami, A. B. M., Mun, H. S., & Yang, C. J. (2017): Efficacy of chlorine dioxide gas in reducing Escherichia coli and Salmonella from broiler house environments. *The Journal of Applied Poultry Research*, 26(1), 84-88.
- **Alanis, A. J. 2005:** Resistance to antibiotics: are we in the post-antibiotic .Arch Med Res.; 36: 697-705.
- **Alexander, K.A., Warnick, L.D. & Wiedmann, M., 2009**, 'Antimicrobial resistant *Salmonella* in dairy cattle in the United States', *Veterinary Research Communication* 33, 191–209.
- Arthur, T.M., Bosilevac, J.M., Brichta-Harhay, D.M., Kalchayanand, N., Shackelford, S.D., Wheeler, T.L. & Koohmaraie, M., 2007, 'Effects of a minimal hide wash cabinet on the levels and prevalence of *Escherichia coli* O157:H7 and *Salmonella* on the hides of beef cattle at slaughter', *Journal of Food Protection* 70, 1076–1079.
- **Bailey J.S, Cox NA, Stern N.J. 2001:** HACCP Symposium. Risk Management factors associated with implementation of HACCP in poultry industry .Available from: <a href="http://www.cfsan.fda.gov">http://www.cfsan.fda.gov</a>.
- Barkocy-Gallagher, G. A., T. M. Arthur, G. R. Siragusa, J. E. Keen, R. O. Elder, W. W. Laegreid, and M. Koohmaraie. 2001. Genotypicanalyses of *Escherichia coli* O157:H7 and O157 nonmotile isolatesrecovered from beef cattle and carcasses at processing plants in the
- midwestern states of the United States. Appl. Environ. Microbiol. 67:3810–3818.
- Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X.Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial peef processing plants. *J. Food Prot.* 66:1978–1986.
- **Bosilevac, J. M., T. M. Arthur, T. L. Wheeler, S. D. Shackelford, M.Rossman, J. O. Reagan, and M. Koohmaraie. 2004.** Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and *Enterobacteriaceae* are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. *J. Food Prot.* 67:646–650.
- Bosilevac, J. M., X. Nou, M. S. Osborn, D. M. Allen, and M. Koohmaraie.2005. Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing
- plant. J. Food Prot. 68:265-272.
- Bosilevac, J. M., M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2009. Prevalence and characterization of *Salmonellae* in commercial ground beef in the United States. *Appl. Environ. Microbiol.* 75:1892-1900.
- **Clontz, L. (2008):** Microbial Limit and Bioburden Tests: Validation Approaches and Global Requirements, second edition, CRC Press NewYork, USA.

- **Davies, J., and D. Davies. 2010**. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 74(3):417-433.
- **Du, J., Y. Han, and R. H. Linton. 2003:** Efficacy of chlorine dioxide gas in reducing *Escherichia coli* O157:H7 on apple surfaces. Food Microbiol. 20:583–591.
- **Erdem B,Ercis S, Hascelik G, Gur D and Aysev AD. 2005.** Antimicrobial resistance of salmonella enterica group c isolated from human in Turkey , 2000-2002.int.J.Antimicrob.agents,26:33-37.
- Esaki H, Morioka A, Ishihara K, Kojima A, Shiroki S, Tamura Y, et al. 2004: Antimicrobial susceptibility of Salmonella isolated from cattle, swine and poultry (2001-2002): report from the Japanese veterinary antimicrobial resistance monitoring program. J Antimicrob Chemother.; 53: 266-270.
- Everage, T. J., Boopathy, R., Nathaniel, R., Lafleur, G., and Doucet, J. q 2014 plant in Thibodaux, Louisiana, USA. Int. Biodeterior. Biodegradation 95, 2–10.
- **Forough T, Elahe T, Ebrahim R, and Manochehr M. 2013:** Determination of antibiotic resistance in Salmonella species isolated from raw cow, sheep and goat's in Chaharmahal VaBakhtiyari Provience, Iran. Global Veterinaria.; 10: 681-685.
- **Gracey JF.(1986).**Meat Hygiene , 8<sup>th</sup> Ed. The English Book Sic. And Baillier ,Tindall,London.
- Gosling, R. J., Mawhinney, I., Vaughan, K., Davies, R. H., & Smith, R. P. 2017: Efficacy of disinfectants and detergents intended for a pig farm environment where Salmonella is present. *Veterinary microbiology*, 204, 46-53
- **Kikuvi GM, Ombui JN and Mitema ES. 2010:** Serotypes and antimicrobialresistance profiles of Salmonella isolates from pigs at slaughter in Kenya. J Infect Dev Ctries; 4: 243-248.
- **Leila Y. 2012:** Antibiotic resistance in *Salmonella enteric* and the role of animal and animal food control. A literature review of Europe and USA:; 12-13.
- Lesley, M.B., Velnetti, L., Cheah, Y.K., Son, R., Kasing, A., Samuel, L., Micky, V., Nishibuchi, M., 2011: Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from cockles (Anadara granosa) at Tanjung Karang, Kuala Selangor. Int. Food Res. J. 18, 1183–1188.
- **Linton, Y.; Hugo, W. B. and Russel, A. D. 1987:** Disinfection: In Veterinary and Farm Animal Practice. 2nd Ed. Oxford, London, Edinburgh, Blackwell Scientific Publ.ication, UK
- Martinez, F., Berchieri, J. A., Paulillo, A.C. 1999: Ação de desinfetantes sobre Salmonella na presença de matéria orgânica. Revista Brasileirade Ciência Avícola; 1:17-35.
- Majowicz SE ,MMusto J , Scallan E ,Angulo FJ, Kirk M,O Brien SJ,Jon es TF,Fazil A,Hoekstra RM .The Global Burden of Nontyphoidal Salmonella Gastroenteritis.Clin Infect Dis.2010;50:882-889

- **Merianos, J.J. 1991:** Quaternary ammonium antimicrobial compounds. In: Block SS, editor. Disinfection, sterilization and preservation. 4thed. Philadelphia: Lea & Febiger; p. 225-252
- **Molla B, Zewdu E. 2004:** Prevalence distribution and antimicrobial resistance profile of Salmonella isolated from food items and personnel in Addis Ababa, Ethiopia. Tropical Veterinary Medicine.
- National Committee for Clinical Laboratory Standards "NCCLS" 2001: Performance standards for antimicrobial susceptibility testing. Supplement M100-S11. Villanova, PA, USA.
- Nou, X., M. Rivera-Betancourt, J. M. Bosilevac, T. L. Wheeler, S.D. Shackelford, B. L. Gwartney, J. O. Reagan, and M. Koohmaraie.2003. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses in a commercial beef processing plant. *J. Food Prot.* 66:2005–2009.
- Prado V, Solari V, Alvarez IM, Arellano C, Vidal R, Carreno M, Mamani N, Fuentes D, O'Ryan M and Munoz V. 2002. Epide-miological situation of foodborne diseases in Santiago, Chile in 1999-2000. Rev Med Chil. 130:495-501
- **Pesavento, G., Ducci, B., Comodo, N. & Lo Nostro, 2007:** A. Antimicrobial resistance profile of Staphylococcus aureus isolated from raw meat, a research fo methicillin resistant Staphylococcus aureus (MRSA). J Food Control. 18, 196–201.
- **Reham A. A. 2004**.Microbial evaluation of some meat products. M.V.Sc. Thesis(Meat hygiene),Fac.Vet.Med.,Moshtohor, Zagazig University. (Benha Branch)
- **Robert-Pillot, A., Copin, S., Himber, C., Gay, M., Quilici, M.L., 2014:** Occurrence of the three major Vibrio species pathogenic for human in seafood products consumed in France using real-time PCR. Int. J. Food Microbiol. 189, 75–81.
- Ruttler ME, Renna NF, Balbi L, Garcia B, Guidone L, FernandezR, Puig O and Ortiz A. 2002. Characterization of enteroaggregative Escherichia colistrains isolated from childrenwith acute diarrhea, in Mendoza, Argentina. Rev Argent Microbiol. 34:167-70.
- **Singh, A., Yadav, S., Singh, S. and Bharti, P. 2010:** Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Res. Inter., 43: 2027-2030.
- **Sndecor, G.W. and Cochran, W.G. 1969:** Statistical methods 6th. The Iowa state Univ. Press, Iowa, USA.
- **Soliman, E.S., Moawed, S.A. and Ziaan, A.M. 2016:** Assessing cleaning and disinfection regime in a slaughterhouse against carcasses contamination. Adv. Anim. Vet. Sci. 4(9): 449-45.
- **SPSS. 14 2006:** Statistical Package for Social Science, SPSS for windows Release 14.0.0, 12 June, 2006. Standard Version, Copyright SPSS Inc., 1989-2006, All Rights Reserved, Copyright SPSS Inc.

- **Srivani, R. 2011:** Studies on antimicrobial susceptibility pattern of Salmonella isolates from Chennai, India. Inter. J. Pharma and Bio Sciences, 2: 435-442.
- **Stewart, P.S., Rayner, J., Roe, F., Rees,W.M., 2001:** Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. J. Appl. Microbiol. 91, 525–532.
- Sutton, S.V.; Proud, D.W.; Rachui, S. and Brannan, D.K. 2002: Validation of Microbial Recovery from Disinfectants. PDA J. Pharmaceutical Sci. and Technol., 56 (5): 255-266.
- **Tesfaw, L., Taye, B., Alemu, S., Alemayehu, H., Sisay, Z., & Negussie, H. 2013:** Prevalence and antimicrobial resistance profile of Salmonella isolates from dairy products in Addis Ababa, Ethiopia. *African Journal of Microbiology Research*, 7(43), 5046-5050.
- **Threlfall, E. J., Frost, J. A., Ward, L. R. et al. 1994:** Epidemic in cattle and humans of Salmonella typhimurium DT 104 with chromosomally integrated multiple drug resistance. Veterinary Record *134*, 577.
- Walia, K., Argüello, H., Lynch, H., Grant, J., Leonard, F. C., Lawlor, P. G., ... & Duffy, G. 2017: The efficacy of different cleaning and disinfection procedures to reduce Salmonella and Enterobacteriaceae in the lairage environment of a pig abattoir. *International journal of food microbiology*, 246, 64-71.

## الملخص العربي

# دراسات مقارنة عن مدى تواجد سالمونيلا التسمم الغذائي في ذبائح الحيوانات

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أختبار حساسية السالمونيلا للمضادات الحيوية المختلفة: وجد أن عزلات السالمونيلا لها القدرة على مقاومة الأستريبتومايسين بنسبة 09.2% والسيفوتاكسيم 80.8% و النالديكسيك أسيد 69.2% والسلفاميسين بنسبة 69.2% والأماسيكين 50% بينما كانت عزلات السالمونيلا حساسة للجنتاميسين بنسبة 65.4% والأماسيكين 50% بينما كانت عزلات السالمونيلا حساسة للجنتاميسين بنسبة 73.1% والأمبيسلين بنسبة 76.9% وقد عزيت هذه المقاومة أن المجتريا قد سبق أن تعرضت لهذه المضادات الحيوية أثناء علاج الحيوانات خلال فترة التربية أو أستخدام المضادات الحيوية كمحفز ات للنمو.

بحساب معامل مقاومة المضادات الحيوية وجد أن 71.4 % من عزلات السالمونيلا تيفيميوريم لها القدرة على مقاومة سبع مضادت حيوية أو أكثر و 80 من السالمونيلا أنترتيدس لها القدرة على مقاومة خمس مضادات حيوية أو أكثر و 75% من السالمونيلا أنفانتس لها القدرة على مقاومة خمس مضادات حيوية أو أكثر.

بلغ متوسط معامل مقاومة المضادات الحيوية 0.483 وكان ينحصر بين 0.143 و 1 ويعتبر المؤشر دليلا على مقاومة المضادات الحيوية عندما يزيد عن 0.2 أو يكون للميكروب القدرة على مقاومة ثلاثة أنواع من المضادات الحيوية من مجموعات مختلفة.

تأثير بعض المطهرات على السالمونيلا تيفيميوريم: أوضحت النتائج المعملية أن معدل الخفض في ميكروبات السالمونيلا يزداد تدريجيا حتى يصل إلى 100% مع زيادة الوقت وزيادة التركيز في المطهرات المستخدمة وكانت أفضل النتائج باستخدام الألكودكس متبوعا بالأديكول ينما كان السويفت أقل النتائج.

خلصت الدراسة الى أن درجة تلوث الذبائح وأعضائها بميكروب السالمونيلا في مجازر الشرقية عالية كما أن ميكروبات السالمونيلا التي تم عزلها عالية الضرواة ولها القدرة على مقاومة العديد من المضادات الحيوية.