**MULTI ANTIBIOGRAM-INTEGRON RELATIONSHIP IN SALMONELLASPECIES ISOLATED FROM LOCAL POULTRY CARCASSES**

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Abstract

This investigation was carried out to investigate the relationship between incidence of multidrug-resistant isolates and presence of class 1 integron in *Salmonella* recovered from poultry carcasses from local Egyptian markets. One hundred samples of raw chicken carcasses which obtained from ten poultry markets at Alexandria markets were examined for presence of *Salmonella* sp., via conventional microbiological methods, serotyping, real time PCR and lastly Conventional PCR for screening of *Salmonella* for sequencing and Class-1 Integrons presence. Detection rate of *Salmonella sp*. was (6%) with serotypes *S. kentucky, S. typhimurium, S. minnesota and S. arizonae* with positive results for *Salmonella* for conventional and quantitative PCR. Based on sequenced specific amplicons with 204 bp, *Salmonella* isolate no.1 was more identical to isolate no.2 which showed highest similarity to isolate no.4, while Isolate no.3 was more identical to isolate no.6, Isolate no.4 was more identical to isolate no.2, Isolate no.5 was more identical to isolate no.4. Lastly, isolate no.6 was more identical to isolate no.3. comparing with gene bank database, first, second, third, fourth, fifth and sixth isolates identified as S*. kentucky, S. minnesota, S. kentucky, S. minnesota and S. typhimurium and S. arizonae* respectively. Obtained results indicated that all six isolates considered multi-resistance isolates as (100 %) with varied resistance patterns. Class-1 Integron detection results reflected that first, second and fourth samples were Positive for class 1 integron (750 bp) which indicated relationship between antibiotic resistance and presence of integron in bacteria. Our obtaining findings cleared correlation between high antibiotics resistance rates exhibited by *Salmonella* isolates and presence of class 1 integron gene cassettes harboring resistance genes.

Keywords:

*Salmonella* – Phenotypic - Genotypic – Serotyping – quantitative PCR – multi-resistance isolates - Class-1 Integrons.

1. Introduction

*Salmonella* has been recognized as a major foodborne pathogen for human and animals causing illness as well as to medical and economical costs. Globally, *Salmonella* infection, food poisoning and *Salmonella* related deaths, constitute major public health problems (18). Moreover, *Salmonella* is one of the most important pathogens responsible for gastrointestinal infections in animals and poultry. Major source of *Salmonella* infections is contaminated poultry products (1).

More than 2500 serovars of *Salmonella enterica* that have been identified. The majority of human cases of non-typhoidal salmonellosis are caused by a limited number of serovars, which may vary from country to country and over time (2). Recently, a significant rise in *Salmonella* infections in animal and chicken meat has been recorded in Egypt (3). *S. enteritides* were isolated from broiler chicken, chicken meat and food poisoning patient (4). Masses of investigations of outbreaks and sporadic cases have indicated that food vehicles acting as the most common source of *Salmonella* infections in humans are poultry and poultry products (5). In poultry, severity of the infection depends on many factors, including the strain of *Salmonella*, the standard of hygiene, age of the bird, route of infection, and immune status of the bird (6).

Improper hygienic measures, unacceptable cooking, and abuse of temperature consider the main bacterial contamination factors for poultry. The dissemination of infection throughout plants during processing occurs in the evisceration, cooling, packaging, and transport stages (7). In addition, contamination can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing, handling, and preparation (8). Thus, under cooked poultry and poultry products should be avoided to prevent infection with such resistant *Salmonellae* (9). In human, *Salmonella* is responsible for approximately 93.8 million illnesses and 155000 deaths annually around the world (10). Furthermore, consumption of contaminated foodconsiders the main pattern fortransmission *Salmonella* to humans through. Most often contaminated food is of animal origin (such as eggs, beef, poultry, and milk) but can also include water and vegetables (11 and 12) which primarily cause of self-limiting acute enteritis (diarrhea, abdominal pain, and fever, with a typical duration of 4–7 days) (13).

Toimprove food safety*, Salmonella* surveillance and monitoring should be carried out according to reliable and efficient detection methods (14). Different methods were employed for *Salmonella* detection. Conventional microbiological methods followed by biochemical and serological confirmation tests serve as the basis for analysis in many food safety and public health laboratories due to the ease of use, reliability of results and lower cost compared to molecular-based technologies (15). On the other hand, requiring multiple subculture steps, time consuming and labor intensive and typically requires 5 to 7 days depending on the biochemical test and serological confirmation utilized considers mainly disadvantages for these methods (16 and 17). Analysis time of conventional and rapid methods depending on cell enrichment period to reach minimal cell concentration enough for *Salmonella* detection, cell enrichment process is typically lengthy in a conventional method whereas the rapid detection method generally requires at least 10⁴ cells ml⁻ᶥ of *Salmonella* concentration for detection (18). Antigenic analysis and agglutination reaction consider the base of serotyping methods to identify cultures serogroups. However, the same *Salmonella* serotype can vary with antigenicity due to change and loss of surface antigens, reducing the sensitivity of serological methods (19). Agglutination technique consider specific, uncomplicated and reliable *Salmonella* identification method due to employ latex particles coated with antibodies which react with antigens on the surface of *Salmonella* cells to form visible aggregates for identification of *Salmonella* positive samples. Thus, could be used as confirmatory analysis technique rather than screening test for *Salmonella* organisms (20 and 21). Recent traditional and Real-time PCR technologies, enable obtaining detection results from 5 to 24 hours with lot less time consuming comparing with other techniques (22).

Dramatic increase of antimicrobial agents in human and veterinary medicine, agriculture and aquaculture industries is promoting both the survival of resistant microorganisms and eliminate of susceptible ones in the antibiotic containing environments (23). Increasing occurrence of antibiotic-resistant microorganisms has augmented interest in the genetics and mechanisms of resistance evolved by bacteria to counteract the effect of antimicrobial agents. Several studies have reported the molecular bases of resistance in *Salmonella* isolated from animals and poultry worldwide (24). Integrons was identified as integrated genetic elements by site-specific recombination, gene cassettes and usually consult antibiotic resistance. Three classes of Integrons have been characterized in detail and are involved in antibiotic resistance (25).

Recently, occurrence of multidrug resistance (MDR) (to amoxicillin, chloramphenicol, sulfonamides, streptomycin, tetracycline, and trimethoprim) in Salmonella enterica serovar *Typhi* has been increasing (26), and MDR strains have been responsible for numerous outbreaks on the Asian continent. Due to founded Integrons in different serovars of S. enterica subsp. Enterica, studying integron consider the key role for evaluating contribution of Integrons to the antibiotic resistance of *salmonella* isolates (**27**). However, to date, no enough information has been available on the molecular bases of antimicrobial resistance in *Salmonella* isolated from poultry from local poultry carcasses in Egypt.

This investigation was aimed to detect the relationship between incidence of multidrug-resistant isolates and presence of class 1 integron in *Salmonella* recovered from poultry carcasses from local markets in Alexandria.

1. Methodology
   1. Samples collection

Total one hundred random samples of local poultry were collected after slaughtering from 10 different Alexandria local markets (ten samples from each market).

* 1. Samples Preparation

Immediately, collected samples were transferred in an insulated ice box to the laboratory. Then stored at – 18 °C till performing bacteriological examination. Under complete aseptic conditions, 25 grams of poultry flesh samples were homogenized with 225 ml of sterile buffered peptone water (BPW) (Oxoid, Basingstoke, United Kingdom) for 2 minutes using a stomacher and followed by incubation at 37°C for 22 h. Homogenates were used as initial samples for bacteriological analysis (28).

# Isolation and identification of Salmonella species

*Salmonella* detection was processed using conventional culture-based methods according to the [International Organization for Standardization [ISO] (2](https://www.frontiersin.org/articles/10.3389/fmicb.2017.02416/full#B18)9). After pre-enrichment incubated homogenates, 100 μl and 1 mL samples were taken and mixed with 10 ml of Rappaport Vassialidis soya (RVS) broth (Oxoid CM0866) and Muller Kauffmann tetrathionate-novobiocin (MKTTn) broth (Oxoid CM 1088), respectively. After incubated overnight at 37°C for MKTTn broth and at 41.5 °C for RSV broth, selective enrichment step was performed via streaked a loopful of each enriched on differential medium (Xylose Lysine Desoxyscholate (XLD) Difco 27732 ), S.S agar (Himedia Hi m081), MacConkey agar (Hi Media Him081) and Brilliant Green Agar (Difco 27533) and the plates were incubated at 37°C for 24 hours. Suspected colony subjected to biochemical examination for S*almonella* on TSI ( Himedia Hi m201). Lysine Iron Agar (Himedia M377), Urea agar (LAB MLab130), Simmons citrate (HimediaM099), Indole test, VogesProskaeur test and Methyl red was employed as biochemical tests. Then confirmed with ApiG-ve Kit and serologically as described below.

### Serological identification (ISO/TR 6579-3:2014)

Serotyping test was performed at the Animal Health Research institute, Dokki, Egypt. Briefly, slide agglutination tests with commercial predefined polyvalent and monovalent somatic and flagellar antisera was used. Somatic antigens were tested in pure cultures after 24 h of growth on nutrient agar; flagellar antigens were tested after a further 24 h of growth on semisolid medium according to Kauffmann–White serotyping scheme ([30](https://www.frontiersin.org/articles/10.3389/fmicb.2017.02416/full#B15)).

* 1. Confirmation of *Salmonella* isolates by real-time quantitative PCR (qPCR)

Quantitative PCR was performed to detect *Salmonella* species through SalSpp dtec-qPCR Test (genetic PCR Solution (GPS), GENETIC ANALYSIS STRATEGIES S.L, Spain) according to manufacture protocol by using MixStable qPCR.5x (5X mastermix solution containing a hot-start DNA polymerase, dNTPs, BSA and buffer. 400 µl, 100 rxn). StepOne™ Real time PCR programmed as follow, activation step 95 ºC for 15 min, 40 cycles 95 ºC for 15 Sec, hybridization at 60 ºC for 60 sec. finally, Fluorogenic signal should be collected during this step by using the FAM channel.

* 1. Antimicrobial susceptibility testing

Antimicrobial sensitivity phenotypes of *Salmonella* spp. were determined (31) for Amox-Clav, Ampicillin, Streptomycin, Gentamicin, Ciprofloxacin, Enrofloxacin, cefoxitin, sulphamethaxone/trimethoprim, Chloramphenicol, Tetracycline, Nalidixic Acid and Ceftriaxone by using the Kirby–Bauer disk diffusion method according to the standards and interpretive criteria described by the Clinical and Laboratory Standards Institute. Scoring results was recording based on zone-size interpretative chart supplied by the manufacturer.

* 1. Conventional PCR for screening *Salmonella* and Class-1 Integrons type

In this investigation, Conventional PCR was applied to identify *Salmonella* and Class-1 integron types through sequenced specific amplicons (32).

2.7.A. *Salmonella* screening:

To identify *Salmonella* through conventional PCR, total bacterial genomic DNA was purified through E.Z.N.A.® Bacterial DNA Kit (omega Bio- TEK, USA) according to manufacturer protocol. To amplify specific amplicon, DreamTaq PCR Master Mix (2X) (K1071, Thermo fisher. USA) was used as manufacturer protocol. As shown by Table (1), primers of OMPC gene encoding biosynthesis of outer membrane protein C of Salmonella was applied (33). for 10 minutes, followed by 35 cycles at 94ºC for 1 minute, annealing at 54ºC for 1 minute, and extension of DNA cassettes at 72ºC for 2 minutes, with final extension at 72ºC for 10 minutes (Silva *et al.*, 2006). After mixing each 7µL of PCR products with 3 µL of blue loading buffer (6X), stained 1% agarose gel with ethidium bromide solution (10mg/mL) was applied to migrate amplicon against 100 bp DNA ladder (G210A, 1500 bp ladder, Promega) via MultiSUB Mini Horizontal Electrophoresis System with PowerPro 300 Power supply (Cleaver Scientific, UK) and imaging with Gel documentation system (OmniDOC, Cleaver Scientific, UK). Data analysis was performed using Totallab analysis software (ww.totallab.com, Ver.1.0.1).

# 2.7.A.Class-1 Integrons type screening:

As shown by table (1), conserved Class-1 Integrons primers were used for the detection and identification class 1 Integrons as described (Ahmed et al., 2007). Resultant PCR products were purified with E.Z.N.A.®Gel Extraction Kit, (D2500-01, Omega BIO-TEK, USA). Samples were sequenced through Applied Biosystems® 3500 Genetic Analyzers. Aligned class 1 Integrons sequences were analyzed on NCBI website (http://www.ncbi.nlm.nih.gov/webcite) using BLAST to confirm their identity. MultiAlignments and identity matrix were creating via comparing the isolates of study with each other's using Clustal Omega software analysis (https://www.ebi.ac.uk/Tools/msa/clustalo/). The nucleotide sequences were also compared with highest homology sequences available in the GenBank.

Table (1): Primer used for detection in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primer | Sequence (5′ to 3′) | Target | Reference | Amplicon Size (bp) |
| Integron Class 1 5′-CS | GGCATCCAAGCAGCAAG | Class 1 integron | Ahmed et al. (2007) | Variable |
| Integron Class 1 3′-CS | AAGCAGACTTGACCTGA |
| OMPCF | ATCGCTGACTTATGCAATCG | *Salmonella* | Ahmed and Shimamoto, (2012). | 204 |
| OMPCR | CGGGTTGCGTTATAGGTCTG |

1. Results

Our present study was achieved to clear relationship between existence of multidrug-resistant isolates and presence of class 1 integron in *Salmonella* recovered from poultry from local markets in Alexandria. Thus, Serological and molecular biology techniques were employed.

* 1. Incidence of *Salmonella* in collected samples:

Varied detection level was recorded for isolated *Salmonella* from different Egyptian markets. As shown by Table (2) and Figure (1), among ten collected samples from each ten markets, one, two, one and two positive *Salmonella* isolates were detected from first, fifth, eight and nine local markets respectively.

Table (2) Incidence of *Salmonella* in this study

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Markets | First | Second | Third | Fourth | Fifth | Sixth | Seventh | Eighth | Ninth | Tenth |
| Total samples | 10 | | | | | | | | | |
| Positive samples | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 2 | 0 |
| Positivity Percentage | 10% | 0% | 0% | 0% | 20% | 0% | 0% | 10% | 20% | 0% |

Figure (1) positivity Percentage of *Salmonella*

3.2. Morphological and biochemical features of suspected *Salmonella* sp.

As shown by photograph (1), On XLD agar medium, suspected *Salmonella* grew as pinkish colonies with or without black center. on nutrient agar, isolates showed circular, raised, entire, colorless and translucent colonies, rod shaped gram negative and motile bacterium. According to biochemical tests, *Salmonella* sp. showed as negative indole production, negative Methyl Red production, negative Urease production, negative VP production, positive Citrate utilization and positive Nitrate and Catalase production.

Photo (1): Shows suspected *Salmonella* isolate grown on XLD agar producing pinkish colonies with or without black center.****

3.3. Serological confirmation of suspected salmonella sp.

For detection *Salmonella* serotyping, serological confirmation test was performed. As listed in table (3), different *Salmonella* serotypes were monitored. Interestingly, *Salmonella* serotype *kentucky* were isolated from first and fifth markets which confirm its spread comparing with other serotypes.

**Table (3): S**erological confirmation of six *Salmonella* isolates from different local markets.

|  |  |  |
| --- | --- | --- |
| Market | Sample number | Serotype |
| First | 1 | *S. kentucky* |
| Fifth | 1 | *S.minnesota* |
| 2 | *S. kentucky* |
| Eighth | 1 | *S. minnesota* |
| Ninth | 1 | *S. typhimurium* |
| 2 | *S.arizonae* |

3.4. Confirmation of *Salmonella species* by Real time PCR

Based on morphological, biochemical and serological data, all six Isolates were confirmed as *Salmonella* through Real time PCR using StepOne™ Real time PCR specific kit by Taqman probe for 18S target sequence. As shown by figure (2), all identified six isolates were confirmed as *Salmonella sp.*

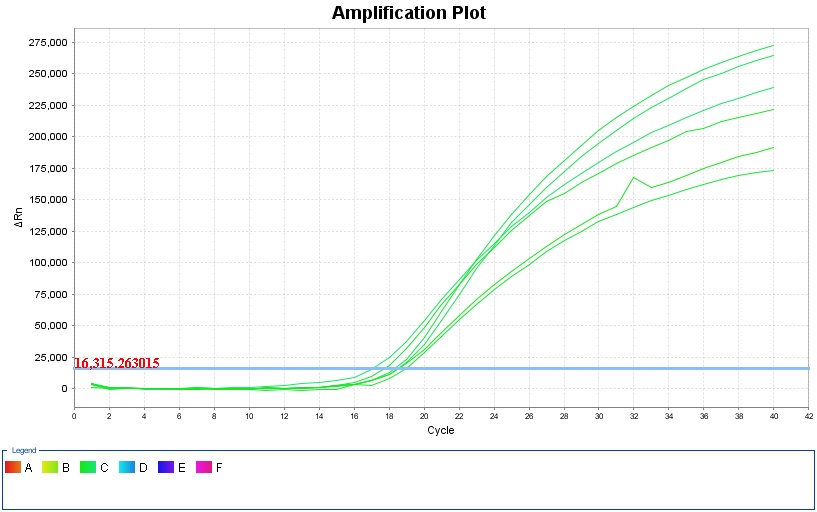
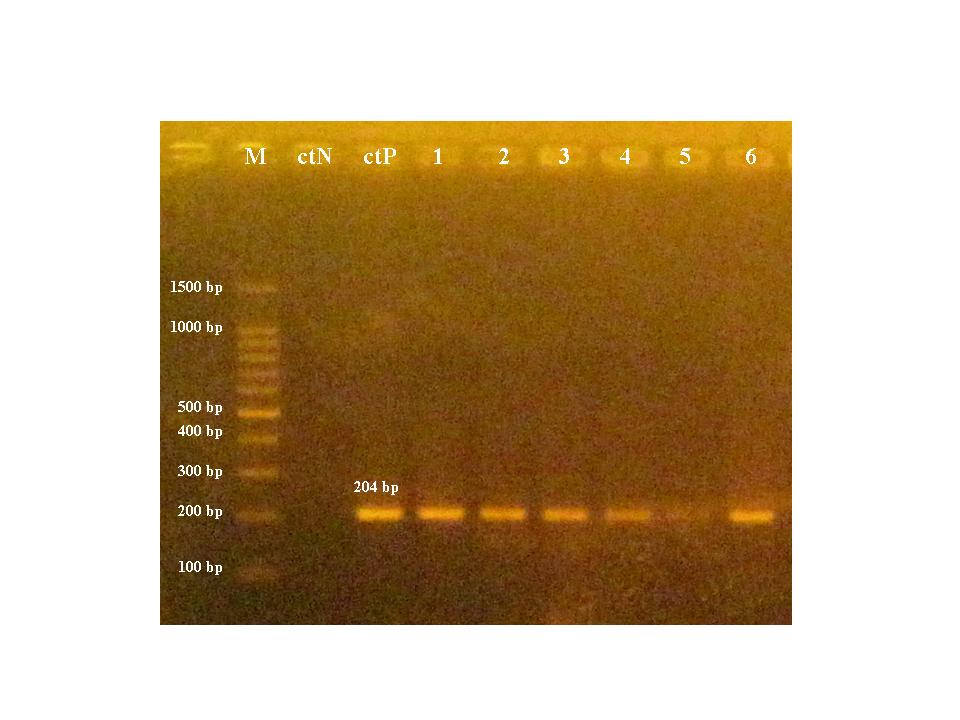


Figure (2). Amplification plot of 6 logs of linear dynamic range for a TaqMan® Assay of cDNA containing the 18S target sequence in tenfold serial dilutions.

3.5. Conventional PCR molecular marker technique for Salmonella Isolates identification

OMPC gene sequence which encoding biosynthesis of outer membrane protein C of Salmonella was applied as molecular biomarker for Salmonella identification. Furthermore, amplicons with specific fragment length (204 bp) were eluted, sequenced and alignments against highly similar Salmonella isolates in data base. As shown by Photograph (2), all six isolates reflected positive fragments (204 bp) which remarked Salmonella isolates. Table (4), cleared genetic similarity among all six Salmonella isolates. All OMPC gene sequences for all six Salmonella isolates were submitted to gene bank as MT221646, MT221647, MT221648, MT221649, MT221650 and MT221651 for first, second, third, fourth, fifth and sixth Salmonella isolates respectively. Table (), showed identified accession numbers and highly similar isolates for six Salmonella isolates under study. Table (5) and Figure (3) illustrate genetic similarity and Phylogenetic tree among six *Salmonella* isolates based on OMPC gene sequences. Highly genetic similarity was detected between sixth and third *Salmonella* isolates. Also, fourth and fifth reflected remarkable similarity. Interestingly, first *Salmonella* isolate was represented as separate cluster which indicated distinguishable dissimilarity.

**Photo (2).** Positive amplicons with (204 bp) of six *salmonella* isolates.

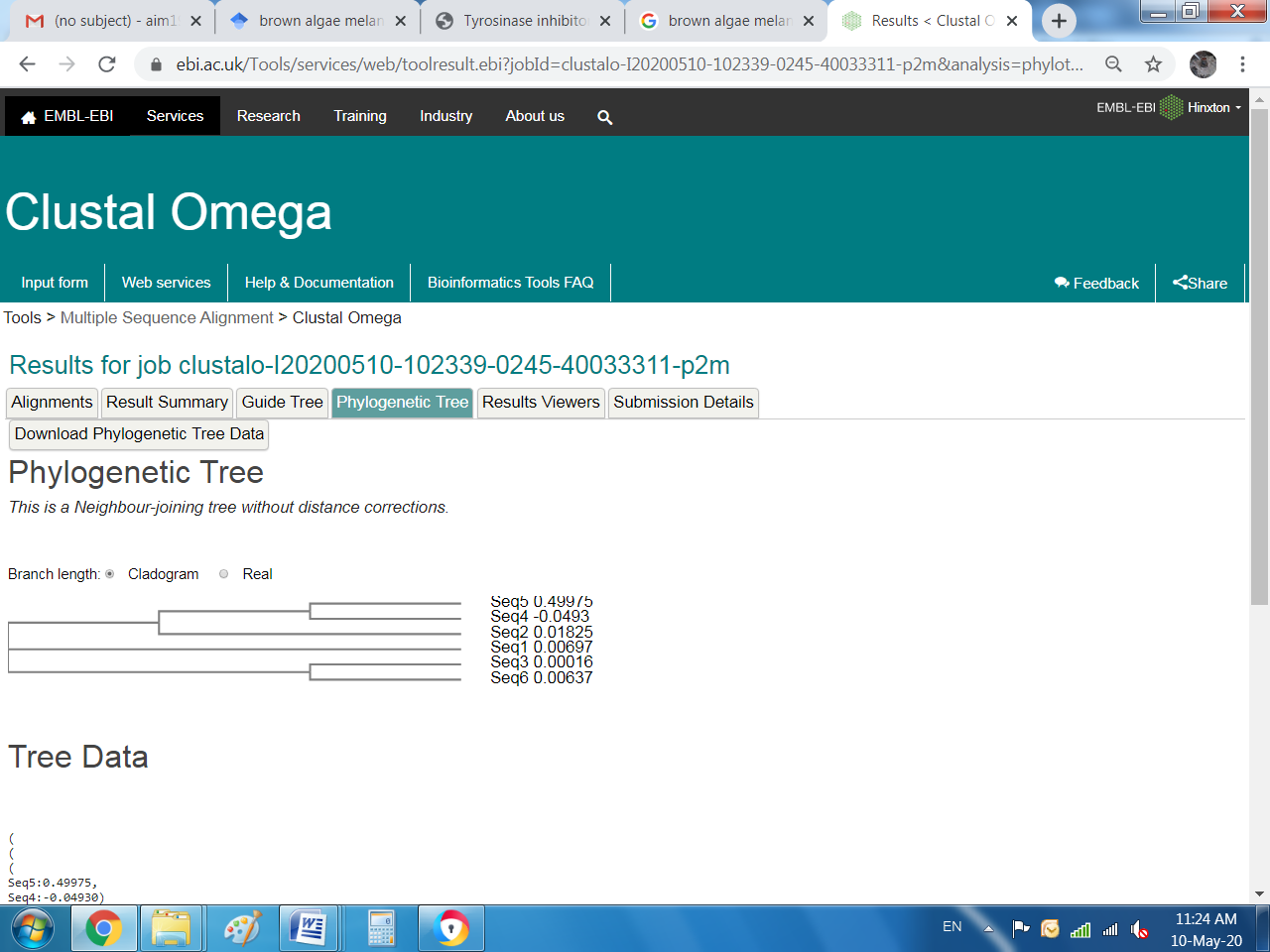
****

**Table (4):** Identity percentage among *Salmonella*isolates sequence and other related sequences from Gene Bank database (NCBI)**.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Isolates | Isolates  Accession number | Highly identity | Accession number | Percent Identity |
| Salm 1 | MT221646 | *S.enterica Sub spp. Enterica serovar Kentucky* | CP037917.1 | 97.81 % |
| Salm 2 | MT221647 | *Salmonella enterica subsp. Enterica serovar Gaminara* | CP030288.1 | 98.54% |
| Salm 3 | MT221648 | *S.enterica Sub spp. Enterica serovar Kentucky* | CP037917.1 | 99.35 % |
| Salm 4 | MT221649 | *Salmonella enterica subsp. enterica serovar Minnesota* | [CP019184.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP019184.1?report=genbank&log$=nucltop&blast_rank=24&RID=AGNT7GJ7014) | 97.52% |
| Salm 5 | MT221650 | *Salmonella enterica subsp. enterica serovar Typhimurium* | CP029029.1 | 98.54% |
| Salm 6 | MT221651 | *Salmonella enterica subsp. enterica serovar Typhimurium* | CP034230.1 | 98.82% |

**Table (5):** sequence identity matrix between different isolation sources of *Salmonella****.***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Identity** | Salm5 | Salm2 | Salm4 | Salm1 | Salm3 | Salm6 |
| Salm5 | 100 % | 38.10 % | 54.95% | 39.68% | 39.06 % | 35.44 % |
| Salm2 | 38.10 % | 100 % | 97.81% | 95.59% | 91.18 % | 91.91 % |
| Salm4 | 54.95 % | 97.81 % | 100 % | 93.38 % | 90.41 % | 90.30 % |
| Salm1 | 39.68% | 95.59 % | 93.38% | 100 % | 95.59 % | 94.85 % |
| Salm3 | 39.06 % | 91.18 % | 90.41 % | 95.59 % | 100 % | 99.35 |
| Salm6 | 35.44 % | 91.91 % | 90.30 % | 94.85 % | 99.35 % | 100 % |

****

**Figure (3):** Phylogenetic tree of *Salmonella* isolates based on OMPC gene sequences

**Table (6):** Identity percentage between ***Salmonella***isolates sequence and other related sequences from Gene Bank database (NCBI)**:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample 1** | | | | | |
| **97.08%**  **Salmonella enterica subsp. enterica serovar Goldcoast** | **97.08%**  **Salmonella enterica subsp. enterica serovar Karamoja** | **97.81%**  **Salmonella enterica subsp. enterica serovar Newport** | **97.08 %**  **Salmonella enterica subsp. enterica serovar Typhimurium** | **97.81 %**  **S.enterica**  **Sub spp.**  **Enterica**  **serovar Kentucky** | **Percent Identity** |
| **CP037960.1** | **CP034709.1** | **CP025230.1** | **CP034230.1** | **CP037917.1** | **Accession number** |
| **Stool** | **Hospital** | **Bovine hide** | **Chicken tissues** | **Chicken** | **Source of isolation** |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample 2** | | | | | |
| **96.35%**  **Salmonella enterica subsp. enterica serovar Goldcoast** | **96.35%**  **Salmonella enterica subsp. enterica serovar Karamoja** | **98.54%** **Salmonella enterica subsp. enterica serovar Minnesota** | **98.54%**  **Salmonella enterica subsp. enterica serovar Minnesota** | **98.54%**  **Salmonella enterica subsp. enterica serovar Gaminara** | **Percent Identity** |
| **CP037960.1** | **CP034709.1** | [**CP019184.1**](https://www.ncbi.nlm.nih.gov/nucleotide/CP019184.1?report=genbank&log$=nucltop&blast_rank=24&RID=AGNT7GJ7014) | [**CP017720.1**](https://www.ncbi.nlm.nih.gov/nucleotide/CP017720.1?report=genbank&log$=nucltop&blast_rank=21&RID=AGNT7GJ7014) | **CP030288.1** | **Accession number** |
| **Stool** | **Hospital** | **Collected by FDA** | **Mangoes** | **Blood** | **Source of isolation** |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample 3** | | | | | |
| **99.35%**  **Salmonella enterica subsp. enterica serovar Newport** | **98.69%**  **Salmonella enterica subsp. enterica serovar Goldcoast** | **98.69%**  **Salmonella enterica subsp. enterica serovar Karamoja** | **98.69 %**  **Salmonella enterica subsp. enterica serovar Typhimurium** | **99.35 %**  **S.enterica**  **Sub spp.**  **Enterica**  **serovar Kentucky** | **Percent Identity** |
| **CP025230.1** | **CP037960.1** | **CP034709.1** | **CP034230.1** | **CP037917.1** | **Accession number** |
| **Bovine hide** | **Stool** | **Hospital** | **Chicken tissues** | **Chicken** | **Source of isolation** |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample 4** | | | | | |
| **95.65%**  **Salmonella enterica subsp. enterica serovar Goldcoast** | **97.52%**  **Salmonella enterica subsp. enterica serovar Minnesota** | **97.52%** **Salmonella enterica subsp. enterica serovar Minnesota** | **97.52%**  **Salmonella enterica subsp. enterica serovar Montevideo** | **97.52%** **Salmonella enterica subsp. enterica serovar Oranienburg** | **Percent Identity** |
| **CP037960.1** | [**CP017720.1**](https://www.ncbi.nlm.nih.gov/nucleotide/CP017720.1?report=genbank&log$=nucltop&blast_rank=21&RID=AGNT7GJ7014) | [**CP019184.1**](https://www.ncbi.nlm.nih.gov/nucleotide/CP019184.1?report=genbank&log$=nucltop&blast_rank=24&RID=AGNT7GJ7014) | [**CP017976.1**](https://www.ncbi.nlm.nih.gov/nucleotide/CP017976.1?report=genbank&log$=nuclalign&blast_rank=10&RID=AGT5JHCH014) | [**CP033344.1**](https://www.ncbi.nlm.nih.gov/nucleotide/CP033344.1?report=genbank&log$=nucltop&blast_rank=5&RID=AGT5JHCH014) | **Accession number** |
| **Stool** | **Mangoes** | **Collected by FDA** | **Stool** | **Food** | **Source of isolation** |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample 5** | | | | | |
| **98.54%**  **Salmonella enterica subsp. enterica serovar Gaminara** | **98.54%**  **Salmonella enterica subsp. enterica serovar Montevideo str. CDC 07-0954** | **98.54%**  **Salmonella enterica subsp. enterica serovar Oranienburg** | **98.54%**  **Salmonella enterica subsp. enterica serovar Worthington** | **98.54%**  **Salmonella enterica subsp. enterica serovar Typhimurium** | **Percent Identity** |
| **CP030288.1** | **CP017974.1** | **CP033344.1** | **CP039509.1** | **CP029029.1** | **Accession number** |
| **blood** | **Stools** | **Raw Pecans** | **Stools** | **Human blood** | **Source of isolation** |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample 6** | | | | | |
| **98.82%**  **Salmonella enterica subsp. enterica serovar Typhi** | **98.82%**  **Salmonella enterica subsp. enterica serovar Typhi** | **98.82%**  **Salmonella enterica subsp. enterica serovar Typhimurium** | **98.82%**  **Salmonella enterica subsp. enterica serovar Goldcoast** | **98.82%**  **Salmonella enterica strain UFPRLABMOR1 chromosome** | **Percent Identity** |
| [**CP029942.1**](https://www.ncbi.nlm.nih.gov/nucleotide/CP029942.1?report=genbank&log$=nuclalign&blast_rank=84&RID=AGTY20E3014) | [**CP029956.1**](https://www.ncbi.nlm.nih.gov/nucleotide/CP029956.1?report=genbank&log$=nuclalign&blast_rank=85&RID=AGTY20E3014) | **CP034230.1** | **CP037960.1** | [**CP020101.1**](https://www.ncbi.nlm.nih.gov/nucleotide/CP020101.1?report=genbank&log$=nuclalign&blast_rank=1&RID=AGTY20E3014) | **Accession number** |
| **Missing** | **Missing** | **Chicken tissues** | **Stool** | **Broiler Carcass** | **Source of isolation** |

3.6. Antimicrobial susceptibility testing:

To evaluate antibiotics resistance for six *Salmonella* isolates under study, Antimicrobial susceptibility testing was performed. Tables (7 and 8) showed varied antibiotic resistance patterns. Among twelve tested antibiotics, Fourth *Salmonella* isolate was superior for antibiotic resistance with ten resistance patterns with 0.83 of MDR Index (a/b) comparing with other *Salmonella* isolates. By contrary, lowest antibiotic resistance was recorded for first *Salmonella* isolate which only resist four antibiotics with 0.25 of MDR Index (a/b)

**Table (7):** Shows results of antimicrobial susceptibility testing for the six *Salmonella* isolates under study.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| sample | 1-Amox-Clav | | | 2-Ampicillin | | | 3-Streptomycin | | | 4-Gentamicin | | | | 5-Ciprofloxacin | | | | | 6- Enrofloxacin | | | | 7- cefoxitin | | | 8-sulphamethaxone/  trimethoprim | | | 9-Chloramphenicol | | | 10-Tetracyclin | | | | 11- Nalidixic Acid | | | | 12- Ceftriaxone | | |
| Standard CLSI | R | I | S | R | I | S | R | I | S | | R | I | S | | R | I | S | R | | I | S | R | | I | S | R | I | S | R | I | S | R | I | S | R | | I | S | R | | I | S |
| ≤13 | 14-17 | ≥18 | ≤13 | 14-16 | ≥17 | ≤11 | 12-14 | ≥15 | | ≤12 | 13-14 | ≥15 | | ≤20 | 21-30 | ≥31 | ≤15 | | 16-20 | ≥21 | ≤14 | | 15-17 | ≥18 | ≤10 | 11-15 | ≥16 | ≤12 | 13-17 | ≥18 | ≤11 | 12-14 | ≥15 | ≤13 | | 14-18 | ≥19 | ≤19 | | 20-22 | ≥23 |
| 1 | 12 (R) | | | 0(R) | | | 14 | | | 20 | | | | 22 | | | | | 20 | | | | 0(R) | | | 10(R) | | | 24 | | | 14 | | | | 16 | | | | 20 | | |
| 2 | 0(R) | | | 0(R) | | | 10(R) | | | 16 | | | | 12(R) | | | | | 10(R) | | | | 0(R) | | | 10(R) | | | 18 | | | 0(R) | | | | 8(R) | | | | 0(R) | | |
| 3 | 0(R) | | | 0(R) | | | 10(R) | | | 20 | | | | 0(R) | | | | | 0(R) | | | | 0(R) | | | 18 | | | 20 | | | 0(R) | | | | 0(R) | | | | 16(R) | | |
| 4 | 0(R) | | | 0(R) | | | 12 | | | 0 + (R)growth | | | | 15(R) | | | | | 12(R) | | | | 0(R) | | | 0 + growth(R) | | | 20 | | | 0(R) | | | | 10(R) | | | | 0(R) | | |
| 5 | 0(R) | | | 0(R) | | | 9(R) | | | 18 | | | | 18(R) | | | | | 12(R) | | | | 0(R) | | | 16 | | | 16 | | | 0(R) | | | | 0(R) | | | | 0(R) | | |
| 6 | 14 | | | 0(R) | | | 12 | | | 18 | | | | 20 | | | | | 20 | | | | 0(R) | | | 16 | | | 22 | | | 12 | | | | 13(R) | | | | 18(R) | | |

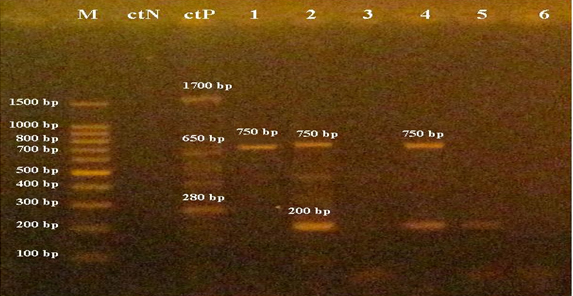
**Table (8):** Shows results of Multi Drug Resistance(MDR) for the six isolates under study.

|  |  |  |
| --- | --- | --- |
| **Isolate** | **No. of antibiotics to which the isolate was resistant (a)** | **MDR Index (a/b)** |
| **1**  Accession number: MT221646 | 4 out of 12 | 0.25 |
| **2**  Accession number: MT221647 | 10 out of 12 | 0.83 |
| **3**  Accession number: MT221648 | 9 out of 12 | 0.75 |
| **4**  Accession number: MT221649 | 10 out of 12 | 0.83 |
| **5**  Accession number: MT221650 | 9 out of 12 | 0.83 |
| **6**  Accession number: MT221651 | 4 out of 12 | 0.33 |

3.7. Conventional PCR for detection of class-1 Integron:

In this study, conventional PCR and sequencing technique were employed to detect class 1 integron type for all six *Salmonella* isolates. As shown by Photograph (3), only first, second and fourth *Salmonella* isolates amplified specific class 1 integron amplicons with 750 bp and confirmed as Positive for class 1 integron. After sequencing class 1 integron gene, gene cassette first, second and fourth *Salmonella* isolates were contained DHFR which is a bacterial enzyme that is uniquely associated with mobile gene cassettes within Integrons which confers resistance to the drug trimethoprim. Our obtaining results indicated the relationship between antibiotic resistance and presence of integron in bacteria as these isolates were resistant to Trimethoprim by using the Kirby–Bauer disk diffusion method according to the standards and interpretive criteria described by the Clinical and Laboratory Standards Institute (CLSI, 2011).

**Photo (3).** first, second and fourth *Salmonella* isolates positive amplicons for integron class 1.



1. Discussion

This study evaluated the incidence and prevalence of *Salmonella* sp. isolated from poultry samples from the local markets in Alexandria, Egypt. Biochemical features, colony morphology and microscopic features play a vital role in the characterization of enteric pathogens **(34** and **35).** Results also confirmed that all the isolates were identified using serological tests, PCR and sequencing.

The emergence of antimicrobial resistance within *Salmonella* species has been reported worldwide **(36).** In this study, only first *Salmonella* isolate was resistant to Nalidixic acid, which agrees with the studies in the Republic of Ireland. They reported resistance in only 2.6% of strains (**37 and** **38)**. Resistance sixth *Salmonella* isolate (*S. typhi*) to tetracycline and gentamycin was in accordance with findings which revealed the antibiotics resistance of *Salmonella* spp. from food sold in market place in Vietnam (39). All samples (except fourth *Salmonella* isolate) were resistance to class of aminoglycosides (Gentamicin). This is in agreement with the findings which stated the causes of resistance as an enzymic catalyzed inactivation of antibiotics as a result of mutation that affects the ribosomes and change in cellular permeability (40 and 41). Highest incidence of resistance to chloramphenicol was reported for *Salmonell*a strains isolated from chicken samples in China (42).

All of the surveys conducted in European countries reported rates of resistance to chloramphenicol ranging between 5.3% (chicken in Spain) and 37.5% (lamb in the UK). Frequency of resistance to this antibiotic among isolates from African countries was not higher than 4.8% (various meat products in Algeria), while no resistance to chloramphenicol was detected in *Salmonella* spp. from foods in Ethiopia and Tunisia (43).

Resistance nature of *Salmonella* isolates to commonly used antimicrobials. 77.8% of the *Salmonella* isolates tested for antimicrobial susceptibility were resistant to two or more antibiotics, which was similar to other results (44). On the other hand, all *Salmonella* isolates exhibit (100%) resistant to Cephalexin and Rifampicin while about 90% and 88% of the isolates were resistant to Ampicillin and tetracycline, respectively (45).

The results of PCR screening for Class-1 Integrons of this study agree with findings which indicated that isolated *Salmonell*a strains from poultry meat and humans were multidrug resistance and harboring class I Integrons that are chromosomally located including different profiles of class I Integrons with variable amplicon sizes ranged from 650– 3000 bp (46). On the other hand, lower detection rate (9 %) in *Salmonella* spp. isolated from poultry meat (47).

However, alarming multidrug resistance frequencies for *S. dysenteriae* and *S. typhi* had been attributed to the use of antibiotics in food producing animal and food preservatives (48).Antimicrobial resistance gene (class 1integron) transfer can be performed in vitro by concomitant culture of SE (receptor) and *E. coli* (donor) (49).

Multiple reports have suggested the dissemination of *Salmonella* resistance genes among *Vibrio* and *Escherichia* species. Such incidents are worrisome as it gives rise to new formidable variants of the once susceptible organisms(50). Furthermore, gene for resistance to antimicrobials (class 1 integron) was not observed in any of the 100 SE samples analyzed by PCR, unlike other studies (51).

**CONCLUSIONS**

High resistance rates exhibited by *Salmonella* isolates in this study to various types of antibiotics and revelation of class 1 integron gene cassettes harboring resistance genes. Thus, a wiser use of antibiotics in poultry farms is required.

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