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Multidrug-Resistant *Salmonella* spp. Isolated from Local Food Markets: Molecular Factors

E. A. Magthab[1] and Asmaa M. S. Al-Bayati[2]

[1]Department of Medical Laboratory Techniques, College of Health and Medical Techniques, Northern Technical University, Kirkuk, IRAQ.

[2]Department of Medical Laboratory Techniques, College of Health and Medical Techniques, Northern Technical University, Kirkuk, IRAQ.

[1]Corresponding Author: ebtsam@ntu.edu.iq

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ABSTRACT

Antibiotic-resistant *Salmonella* spp. linked with food remains a primary concern globally as it is associated between animals and humans, causing foodborne illness and zoonotic diseases. One hundred *Salmonella* isolates out of 241 swab specimens taken from slaughtered chicken were identified and investigated for their antimicrobial susceptibility pattern. Ampicillin (62%), tetracycline (59%), amoxicillin-clavulanic acid (46%), trimethoprim-sulfamethoxazole (35%), and ceftriaxone (24%) were the drugs with the highest prevalence of impedance. At 12%, 11%, and 8%, respectively, resistance to ciprofloxacin, chloramphenicol, and azithromycin was very negligible. In order to assess the frequency of resistance genes, six different kinds of genes were examined in this research. The findings revealed that the proportion of these genes included in the survey were tetA (10%), blaCMY-2 (32%), dfrA7 (17%), tetC (20%), sul2 (25%) , and blaTEM-1 (47%).

Keywords- Antibiotic-resistance, *Salmonella,* foodborne, prevalence.

I. INTRODUCTION

Salmonellosis is a zoonosis that is transmitted via food and is responsible for 155,000 annual fatalities and 93.8 million annual episodes of gastroenteritis in the United States alone. Inappropriate use of antibiotics, in particular within the cattle and poultry sectors, has been associated to this condition. This is especially true in the United States. Salmonella outbreaks may almost always be traced back to this factor. Because of this, the spread of MDR non-typhoidal Salmonella (NTS) from chicken is a significant risk to the health of the general public. NTS is a subtype of Salmonella. The development of antibiotic resistance as a consequence of Salmonella is a huge challenge for the medical community to address. The development of Salmonella strains that are resistant to several drugs is facilitated in large part by the irresponsible use of antibiotics in both human and animal patients. The development of bacterial resistance, including resistance to Salmonella and other types of bacterial resistance, is due to a group of genes known as proto-impedance genes. These genes are responsible for the development of bacterial resistance. The extensive use of antibiotics in animal feed, either as a food additive or as a growth stimulant, is a likely source of the multidrug-resistant condition that Salmonella strains have attained, according to the evidence that has been gathered. These antibiotics are used in one of two ways: either as a food supplement or as a growth stimulant. These antibiotics were used in either of two distinct capacities: first, as a growth stimulant; second, as a dietary supplement. The widespread use of antibiotics for the purpose of curing ailments in both human beings and animals ultimately results in a flourishing transmission of antibiotic resistance genes across different bacterial species. This is a consequence of the extensive use of antibiotics in modern medicine. Due of this problem, doctors are increasingly urging patients to

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adopt treatments that do not use antibiotics in an attempt to lessen the extent to which antibiotic resistance is a widespread problem.

Characteristics of S. enterica that make it resistant to antibiotics in the host through a variety of mechanisms, such as the inhibition and degradation of some antimicrobial agents by enzymes, the activation of antimicrobial efflux pumps, a change in the site where antibiotics are most effective, and the prevention of cell permeability to antibiotics. PCR has been used to locate numerous genes in the genome of microbes that are resistant to antibiotics. These genes have caused the microbes to be resistant to a variety of antibiotics. Polymerase chain reaction, often known as PCR, is a technique that, when combined with the right primers, may be used to multiply genes for antibiotic resistance as well as mutant genes. It was possible to carry out DNA amplification in a thermocycler by using the processes of denaturation, annealing, and extension since the conditions that were necessary were satisfied. The quality of the amplified segments was determined by the use of an agarose gel electrophoresis.

Researchers are able to ascertain whether or not a treatment is successful by analyzing bacterial strains for the presence of antibiotic resistance genes and locating these genes via the use of genetic tools. Because of this, they are able to assess whether or not a certain drug is successful in treating a given setting. The researchers are able to establish whether or not a certain pharmaceutical will be beneficial in treating a specific ailment by using this approach. With this method, the researchers are able to determine whether or not a certain medicine will be useful in the treatment of a particular illness. As a result, it is much simpler to write the right prescription for patients who have been diagnosed with a specific bacterial infection. This research intended to assess antimicrobial susceptibility and identify causes of Salmonella spp. resistance isolated from chickens that had been killed and sold in local markets. The study's participants were people who had purchased chickens from local markets. This course of action is preferable to the administration of antibiotics, which might result in the development of germs that are resistant to the medication. Both of these goals were supposed to be accomplished with the help of this study.

II. MATERIALS AND METHODS

Specimens collection and screening

From January 2021 to April 2022, 241 swabs were taken from killed chickens at local markets. Bacterial cells in the swabs were preserved in tetrathionate broth before culture. Bacteria were grown on SS agar. After overnight incubation, the suspected *Salmonella* cells were picked for biochemical tests including gram staining, triple sugar iron agar, MacConkey agar, oxidase, urease and catalase. Confirmation for isolates took place by Vitek 2 compact system, and preserved in the infusion broth of brain heart that supplemented with 20% glycerol at -20°C.

Antimicrobial susceptibility test

Salmonella spp. isolates. They were exposed to tryptone soy broth containing 10 milliliters of volume at a temperature of 37 degrees Celsius for a period of 18 hours. In line with the recommendations presented by CLSI, the antimicrobial susceptibility profiles of the isolates were examined. Antibiotic Tablets ceftriaxone (30 mcg), ampicillin (10 mcg), chloramphenicol (30 mcg), amoxicillin clavulanate (20 mcg), sulfamethoxazole (25 mcg), ciprofloxacin (5 mcg), tetracycline (30 mcg), and trimethoprim (15 mcg) micrograms) were inoculated on Muller-Hinton agar plates. A positive control of Escherichia coli strain of ATCC 25922 based on CLSI guidelines was applied to determine the efficacy of the antibiotics used in the experiment. To test antibiotic resistance, cultures were incubated at 37°C for 16 hours. Estimating the diameter of the bacterial-free region around the discs gave the clearing area (mm). CLSI classifications were sensitive, impedance, or intermediate.

DNA purification

ESBL-producing In Luria-Bertani broth, Salmonella strains were allowed to grow at 37 degrees Celsius for 18 to 24 hours after being isolated. In order to get genomic DNA in its purest form from the bacterial culture, a commercially available DNA extraction kit was used. In order to determine the purity of the DNA, a Qubit fluorometer (version 3.0) from Thermo Fisher Scientific in New Hampshire, United States was used.

Amplification by PCR amplification of antibiotic resistance genes

The use of PCR-linked techniques with already registered primers was necessary for the search for genes in Salmonella isolates that are responsible for antibiotic resistance. The results of this search are shown in Table 1. The quantity of 24 microliters that was present in the tube throughout the reaction was made up of 12 microliters of the master mix and 0.5 microliters of the master mix. In order to make any kind of primer, you will need 6 microliters of PCR-grade water in addition to 5 microliters of template DNA (forward and backward). The only difference between the positive control and the negative control is that the positive control has the DNA template present, whereas the negative control just has PCR-grade water present. DNA that had been collected in the past from an organism that was already known to be Salmonella was used so that a positive control could be created. This was done in line with the methodology that was supplied by the provider. The Qiagen store in Germany was visited in order to get the QIAquick PCR Purification Kit. The conclusions about the sequences of amino acids were arrived at with the help of the translation program ExPASy (Expert Protein Analysis System). In a thermocycler (BioRad, Singapore), PCR was done with three minutes of denaturation at 95 degrees Celsius, thirty amplification cycles of thirty

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seconds each, and thirty seconds of annealing at a given temperature. The primers were heated, then final extended for eight minutes at the same temperature after being extended for one minute at 72 degrees Celsius.

Table 1: The research used primers with annealing temperatures for gene amplification.

The sequence of primers	Gene	Temperature of annealing (°C)	Reference
R: 5′-GTG TGC GGATGA AGT CAG-3 F: 5′-CGG CAT CGT CAA CATAACC-3′	sul2	60	Poppe et al., 2006
R: 5′-GTGAACAGTAGACAAATGAAT-3′ F: 5′-AAATGGCGTAATCGGTAATG-3′	dfrA7	52	Frank et al., 2007
R: 5′-CATAGATCGCCGTGAAGAGG-3′ F: 5′-GCTACATCCTGCTTGCCTTC-3′	tetA	52	Fonseca et al., 2006
R: 5′-CAGTAGCGAGACTGCGCA-3′ F: 5′-ATAACCACCCAGTCACGC-3′	blaCMY-2	50	Poppe et al., 2005
R: 5′-ATGGTCGTCATCTACCTGCC-3′ F: 5′-CTTGAGAGCCTTCAACCCAG-3′	tetC	42	Fonseca et al., 2006
R: 5′-TAATTGTTGCCGGGAAGC-3′ F: 5′-TTGGGTGCACGAGTGGGT-3′	blaTEM-1	56	Guerra et al., 2001

Statistical analysis

With the assistance of the SPSS application, the statistical analysis was carried out (version 20.0; SPSS, Chicago, IL, USA). In order to determine whether or not the differences in ratios are significant, a chi-square test was carried out. In order to be considered statistically significant, a result needed to have a P value that was lower than 0.05.

III. RESULTS AND DISCUSSION

Of the 241 samples, 170 (71%) showed positive growth on SS agar, and this was observed in pale colonies centered with black, as would be expected for *Salmonella* species on SS agar. Out of the 170 putative salmonella, 100 were confirmed to be positive by biochemical tests and the Vitek 2 system. The characteristics of the isolates are shown in table 2.

Table 2: Morphological characteristics of suspected *Salmonella* Spp (Percival and Williams, 2014).

Test	Observation
Grams stain	Gram (-) rods
Urease	-
Oxidase	-
Catalase	+
MacConkey agar	Lactose fermenter
Triple sugar iron agar	A/A + ±*

±: some isolates produce H2S, others are not.

This survey's salmonella isolates were resistant to all medications, according to Kirby-Power disc publication. Amoxicillin-clavulanic acid (46%), trimethoprim-sulfamethoxazole (35%), ampicillin (62%), and tetracycline (59%). Isolates varied greatly (graph 1). Azithromycin, ciprofloxacin, and chloramphenicol showed modest resistance at 12%, 11%, and 8%. The study found moderate ciprofloxacin resistance in 37% of isolates. Figure (2) shows agarose and nucleic acid in the gel.

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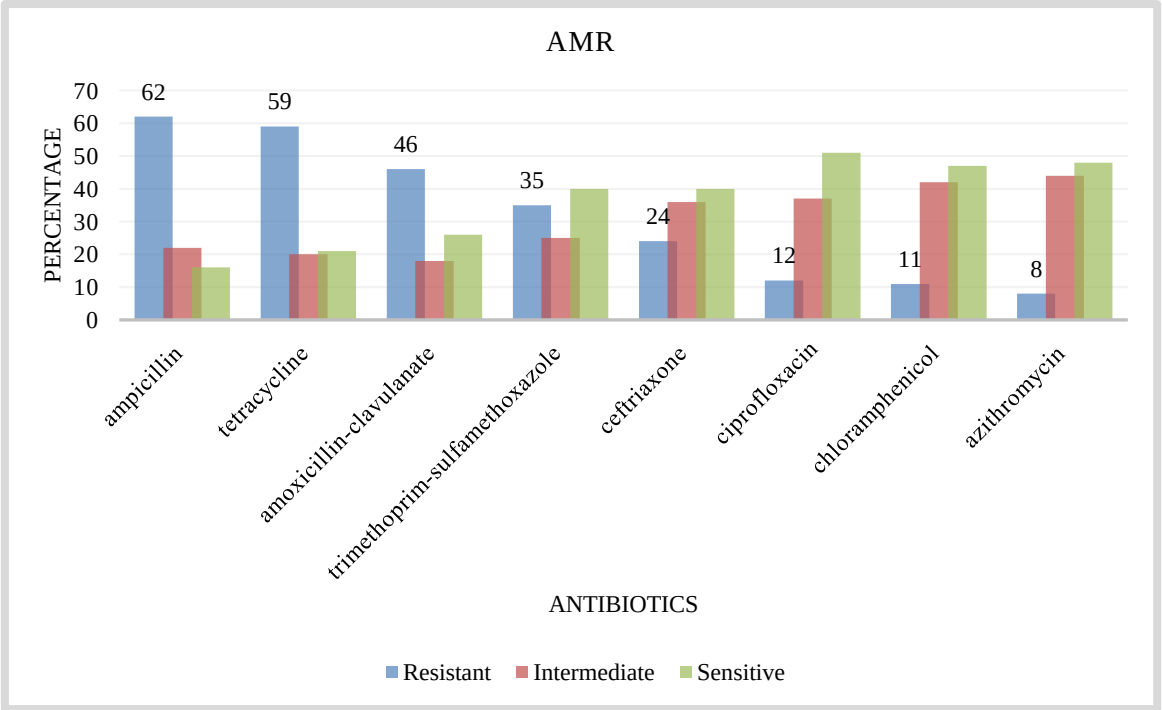

Figure 1: Antimicrobial Susceptibility in *Salmonella* isolates.

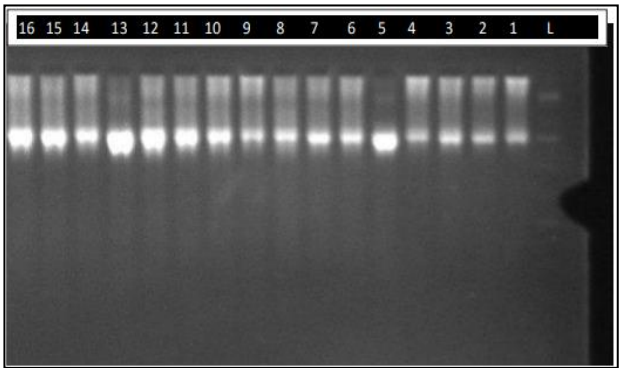

Figure 2: DNA that has been purified as shown by agarose gel electrophoresis. DNA Ladder is L.

The commonness of the genes was as follows: *tetA* (10%), bla_{TEM-1} (47%), *tetC* (20%), *dfrA7* (17%), *sul2* (25%), and bla_{CMY-2} (32%), (Figure 3).

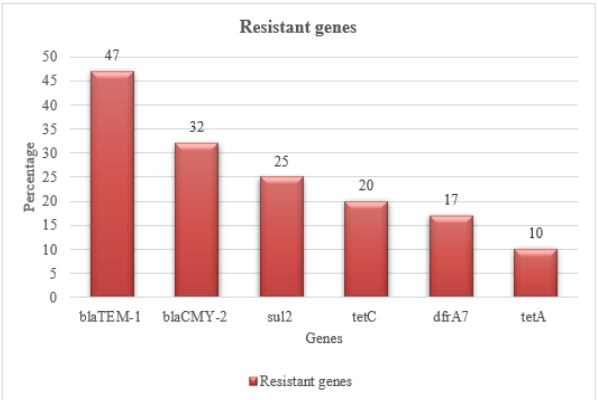

Figure 3: Prevalence of resistant genes in the survey.

The results in the present survey were consistent with other researches that recorded high rates of impedance to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole. Azithromycin (16.9%) and ceftriaxone (24.07%) had higher resistance rates than the present research. With median sensitivities of 67% and 33%, chloramphenicol and ciprofloxacin had decreased efficacy.

The resistance to antibiotic is mediated by genetic elements that encode the various mechanisms bacteria use to evade the impacts of antibiotics. In the present survey, blaTEM-1, blaCMY-2, dfrA7, tetA, tetC, and sul2 were found. Enzymes in the blaTEM-1 and blaCMY-2 genes hydrolyze β-lactam antibiotic rings. To avoid trimethoprim-sulfamethoxazole, the sul2 and dfrA7 genes synthesize dihydrobutyrate and dihydrofolate reductase-insensitive versions. TetC and tetA keep tetracyclines out.

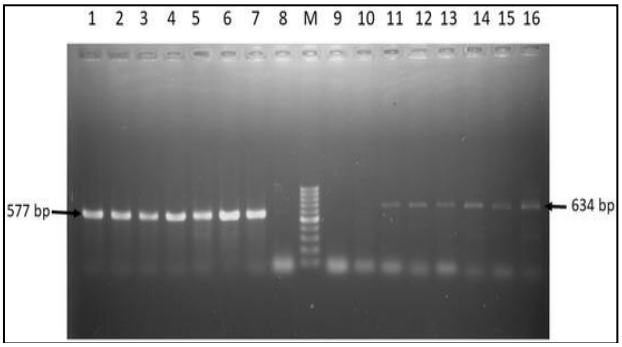

Figure 4: Salmonella sppblaCMY-2 .'s and blaTEM-1 genes were amplified using PCR. 100 bp DNA marker for path M.

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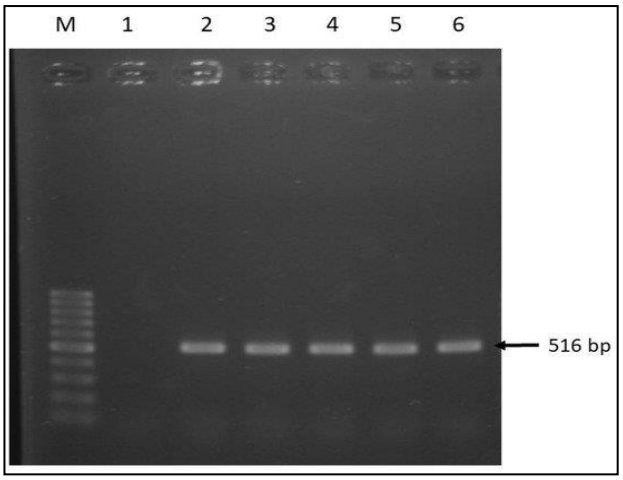

Figure 5: Salmonella spp. tetA and tetC genes PCR amplification Lane M: 100 bp an DNA marker

The prevalence of blaCMY-2 in the current investigation was almost identical to the rate of occurrence that was documented in an earlier test, which was 38.88%. Since extended-release beta-lactams like ceftriaxone are the antibiotic of choice for the treatment of salmonellosis, it is essential that researchers continue to investigate the higher prevalence of beta-lactam-degrading components found in current study.

These genes are responsible for the bacteria's ability to replicate. There is a correlation between the blaCMY-2 gene and the tetC gene and their engagement in the production of cell wall and transmembrane movement activities, respectively. It is possible that their connection may be explained by the fact that both the blaTEM-1 gene and the blaCMY-2 gene have the same precise -lactam degradation and extended -lactam activity. In addition, the transposable plasmids include the blaCMY-2 and blaTEM-1 genes. It is possible that the locations of the sul2-producing genes and the -lactamase genes (blaCMY-2 and blaTEM-1) in Salmonella will establish a connection between the two.

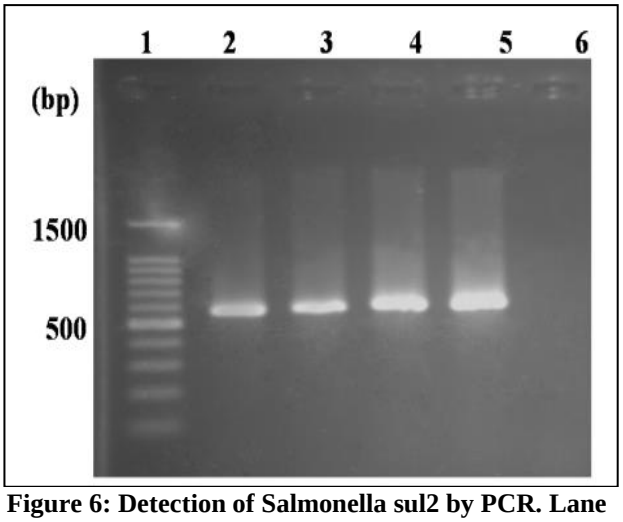

Figure 6: Detection of Salmonella sul2 by PCR. Lane 1: 100 bp DNA ladder.

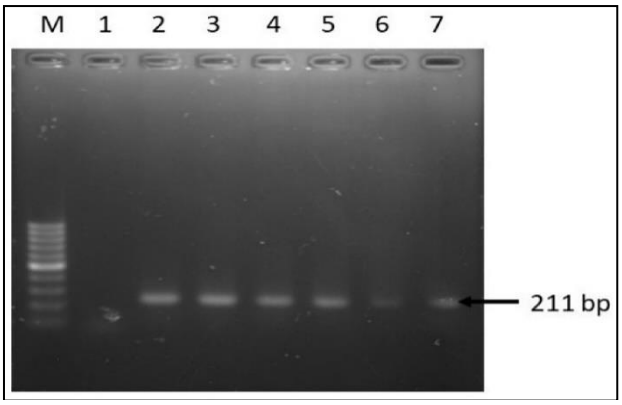

Figure 7. PCR amplification of genes from Salmonella dfrA7 Pathway M: 100 bp for a DNA tag,

In a previous study, it was discovered that class 1 integrins were associated with transposons and elements of input sequence (IS) that carried antimicrobial impedance genes. The linkage between resistance genes found in the current survey is consistent with the findings that were reported in that earlier study. The impedance genes are generally transferred by integrins as gene strands. The present research revealed the association between β-lactamase genes (blaCMY-2 and blaTEM-1) in IS elements related with class 1 manufacturing.

Higher grades of antimicrobial impedance were noted in comparison with resistance genetic determinants. In the current survey, the risk of human exposure to antibiotic-resistant *Salmonella* bacteria is highlighted. The high grade of moderate susceptibility to ciprofloxacin raises inquiry about the efficacy of this antibiotic in the future. Furthermore, *Salmonella* resistant to fluoroquinolone was detected. Included in the WHO's high majority list is alarming.

Salmonellosis in children is treated with β-lactams due to infection risk. Another issue to be noted is that these classes are not only utilized to medicate salmonellosis, but also contagions caused by other bacterial species.

Sequencing the whole genome of the chosen specimens will provide a clearance genomic characterization of the resistance of the isolates. Continuing surveillance will provide an early caution system for antibiotic-impedance *Salmonella*, helping us to reduce the public health burden.

IV. CONCLUSIONS

Despite the availability of antibiotics, the global burden of illness, death, and economic losses caused by human and animal enteric pathogenic bacteria, such as Salmonella spp., is enormous. This is due to the fact that Salmonella spp.. The large-scale study of antibiotic resistance in the previous few decades has clarified the current risks of antibiotic impedance in many bacterial species. The development and commonness of antibiotic

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impedance will continue to be demonstrated by the study of the pathogenesis and epidemiology of Salmonella. Resistance of bacterial pathogens consists of several determinants, i.e., MDR pumping of inactivating enzymes to antibiotics and plasmid-delivered antibiotics elements.

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