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Salmonella Enterica Diarozoniae Strain's Pattern of Increased Antibiotic Resistance

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ABSTRACT
Salmonella enterica subsp. diarizonae is a bacterial strain that has been of amassed alarm due to its influence on community health. This bacteria is frequently isolated from cold-blooded reptiles, sheep and monkeys. Lately, it has been identified from birds migrating transversely the continent of Asia and Europe. This study is the first of its kind in Iraq to diagnose this serotype of Salmonella from chicken meat and study the level of its resistance to antibiotics. Ten types of antibiotics were used, and this bacteria was extremely unaffected to seven antibiotics out of a total of ten antibiotics and sensitive to only three, with an average diameter of inhibition of Tetracycline 10 mm and Chloramphenicol 7mm. Salmonella were used as a comparison sample, and this bacteria was sensitive to a large number of antibiotics notwithstanding its high resistance to antibiotics, according to a large number of research. The occurrence of antibiotic resistance in Salmonella enterica diarizonae additional confuses the controlling of infections and increases distresses about the efficiency of antibiotic treatment.

1. Introduction

Salmonella enterica subsp. *diarizonae* is a Gram-negative rod subspecies of the Salmonella enterica bacterium that is commonly found in the gastrointestinal tracts of birds, including chickens. Most frequently, it infects domesticated cold-blooded animals, such as reptiles (Andino & Hanning, 2015). Furthermore, *Salmonella enterica* subsp. *diarizonae* was found in sheep in Greece, Switzerland and Norway. (Pławińska-Czarnak,. et al. 2022). This subspecies has been a significant concern in poultry farming due to its potential to cause foodborne illness in humans through contaminated poultry products (Haile, 2023). This particular strain has been isolated from chicken meat, posing a potential risk for foodborne illness. The emergence of antibiotic resistance in *Salmonella Enterica diarizonae* further complicates the management of infections and raises concerns about the effectiveness of antibiotic treatment (Teklemariam et al., 2023). The rise of antibiotic resistance in *Salmonella enterica diarizonae* poses a significant threat to public health. When bacteria become resistant to antibiotics, infections become more difficult to treat and can lead to prolonged illness, increased healthcare costs, and higher mortality rates. This presents a major challenge for healthcare providers, as they must seek alternative treatment options that may not be as effective or readily available. (Nair et al., 2018)

Furthermore, the potential for the spread of antibiotic-resistant strains of *Salmonella enterica diarizonae* within the community and food supply chain is a source of concern. This strain can be transmitted through contaminated food or water, putting the general population at risk of exposure (Russo et al., 2022). It is imperative for public health officials and researchers to closely monitor the prevalence of antibiotic-resistant *Salmonella enterica diarizonae* and to develop strategies to combat its spread. This may include enhanced surveillance, education on safe food handling practices, and the promotion of responsible antibiotic use in both humans and animals (Mkangara, 2023) Understanding the impact of antibiotic resistance in *Salmonella enterica diarizonae* is crucial for developing effective public health interventions and minimizing the potential negative consequences for the population. Therefore, in this research, we aim to explore the antibiotic resistance patterns of *Salmonella enterica diarizonae* isolated from chicken meat and evaluate its implications for public health. Understanding the resistance profiles of this pathogen is crucial for effective disease control and the development of appropriate public health interventions.

2. Methodology

Cultivation of *Salmonella*

Based on the previous identification achieved by Al-Essawi et al. (2023). Two strains of *salmonella enterica* subsp *diarozoniae* were cultivated and purified using Two selective enrichment media, xylose lysine deoxycholate agar (XLD; GRASO) and Salmonella Chromagar agar (CHROMagar Salmonella PLUS; GRASO), were used as described in Al-Esawi et al., (2023). These media were chosen for their ability to selectively isolate and differentiate *Salmonella* species from other bacteria. XLD agar contains xylose, lysine, and deoxycholate, which inhibits the growth of most Gram-positive bacteria and allows for the detection of *Salmonella* based on its ability to ferment xylose. On the other hand, CHROMagar Salmonella PLUS agar utilizes chromogenic substrates that produce distinctive color colonies for easy identification of *Salmonella*.

Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility Testing is commonly performed on *Salmonella* isolates to determine their sensitivity to various antibiotics. The MIC patterns of *S. enterica* subsp. *diarizonae* were analyzed using the Merlin MICRONAUT system. The MICs were interpreted based on the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) and FDA breakpoints (Kowalska-Krochmal & Dudek-Wicher, 2021)- The antimicrobial susceptibility test was conducted to determine the effectiveness of these agents against the target pathogens. Antibiotic agents from Himedia were used, which consist of a circular ring containing antibiotics and their concentrations shown next to each (Table 1). The ring of antibiotic tablets was placed using sterile forceps in the middle of the dish (Figure 1). The dish was placed in the incubator upside down at a temperature of 37°C for 24 hours, then the results were recorded the next day.

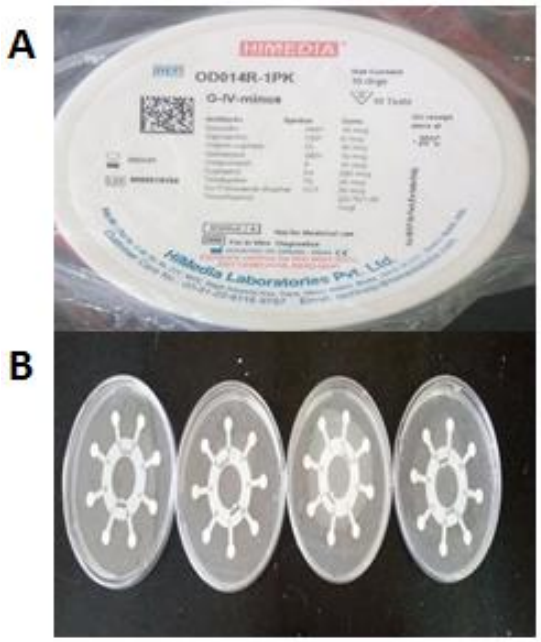

Figure1: Antibiotic agents used against *salmonella enterica* subsp *diarozoniae* and *Salmonella*. A: list of antibiotics used. B: Applying antibiotics ring on *Salmonella* strain.

3. Results and Discussion

Salmonella enterica Diarizonae is a strain of Salmonella that is known for causing gastrointestinal illness in humans. This particular strain has become a growing concern due to its increasing antibiotic resistance(Pławińska-Czarnak,. et al. 2022).. In Iraq, where the consumption of chicken meat is common, it is essential to assess the prevalence and antibiotic resistance of Salmonella spp in chicken meat to evaluate the potential risks to public health. By examining the prevalence and antibiotic resistance patterns of Salmonella spp, we can gain insights into the effectiveness of current control measures and identify areas for improvement in food safety practices. This study aims to provide a comprehensive understanding of the prevalence and antibiotic resistance of Salmonella spp in chicken meat in Iraq, with the ultimate goal of enhancing food safety and protecting public health.

The results shown in Table (1) showed the measurement of the diameters of inhibition for the used isolates, where the results showed that the isolate of *Salmonella enterica* subsp *dirarizonae* is a bacterium that is resistant to most of the antibiotics that were used for sensitivity testing except for its sensitivity to the antibiotic Tetracycline, where the average diameter of inhibition was 10 mm, followed by the antibiotic Chloramphenicol with a diameter of Inhibition reached 7 mm, while other antibiotics did not show any inhibitory activity (Fig. 2).

Table 1 : Antimicrobial resistance of the *Salmonella enterica* subsp. *diarizonae*

Antibiotics	symbol	Concentration mcg /disc	Diameter of Inhibition zone
Ampicillin	Amp	10mcg	0
Cephalothin	Cep	5	0
Colistin Sulphate	Cl	25	0
Gentamycin	Gen	10	0
Streptomycin	S	10	0
Sulphatrid	S3	200	0
Tetracycline	Te	25	10
Co-Trimoxazole	Cot	25	0
Chloramphenicol	Chl	25	7

Figure2 : Antimicrobial resistance of the *Salmonella enterica* subsp. *diarizonae*

As for the second isolate, which was used as a comparison isolate, Salmonella, it was clearly sensitive to four antibiotics: Tetracycline, with an average inhibitory diameter of 32 mm, Cephalothin, 19 mm, while the inhibitory diameter of the antibiotic Chloramphenicol was 20 mm, and for the antibiotic Gentamycine, the average diameter of inhibition was 12 mm Table 2, Figure 4**.**

Table (2) shows the average diameter of inhibition zone of Salmonella in millimeters, which were used as a negative control

Antibiotics	Symbol	Concentration mcg /disc	Diameter of Inhibition zone (mm)
Ampicillin	Amp	10	0
Cephalothin	Cep	5	19
Colistin Sulphate	Cl	25	0
Gentamycin	Gen	10	12
Streptomycin	S	10	0
Sulphatrid	S3	200	0
Tetracycline	Te	25	32
Co-Trimoxazole	Cot	25	0
Chloramphenicol	Chl	25	20

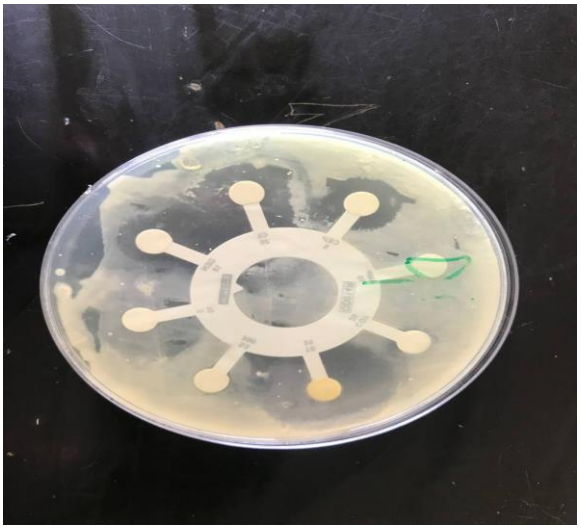

Figure 3: The sensitivity of *salmonella* to antibiotics

To the best of my knowledge, there haven't been many studies on *Salmonella. enterica* subsp. *diarizonae* bacteria in poultry meat, with the exception of one conducted in Poland, where the bacteria were isolated from migrating swans and their antibiotic resistance was investigated. The study's findings revealed the presence of two subspecies of *S. enterica* subsp. *diarizonae*, which were highly resistant to 14 of 33 antibiotics. The researchers concluded that the resistance of these isolates could be caused by the presence of resistance genes (blaTEM, aadA, and strA/strB) (Pławińska-Czarnak,. et al. 2022). The study examined the prevalence, serotype distribution, and antimicrobial susceptibility of nontyphoidal Salmonella in poultry farms in Adama and Modjo towns. Isolates were collected from various sources, including fecal droppings, feed, floor swabs, and stools from in-contact humans. Most were positive for Salmonella. Salmonella isolates, 19 (76%) and 20 (80%) were resistant to tetracycline and streptomycin, respectively. Nineteen isolates, or 76% of the total, had resistance to two or more antibiotics (Dagnew et al., 2020; Pławińska-Czarnak, et al. 2022). Out of the 17 antimicrobial tested, every isolate of *Salmonella* isolated from different Slaughtered Cattle in Addis Ababa that were either resistant or somewhat resistant to at least four of them. There was a high rate of resistance to ampicillin 19 (67.9%), cephalothin 20 (71.4%), amoxicillin+clavulanic acid 19 (67.9%), and streptomycin 25 (89.3%). Twenty (71.5%) of the isolates had antimicrobial resistance to five or more drugs (Ketema et al., 2018). These results underline the necessity for producers, government agencies, and researchers studying poultry to identify strategies to lessen the effects of antibiotic use in poultry, with a particular emphasis on constant monitoring and the development of antibiotic substitutes (Wessels et al., 2021). 46 eligible articles were chosen for the final analysis from the initial 13,186 studies that were retrieved, based on the inclusion and exclusion criteria. In clinical specimens from Iran, the pooled prevalence of quinolone-resistant Salmonella serotypes was 2.9% to ciprofloxacin and 48.1% to nalidixic acid. The following information on antibiotic resistance was additional: 54.3% went towards tetracycline, 50.6% towards ceftizoxime, 50.2% towards streptomycin, 37.9% towards ampicillin, 36.5% towards kanamycin, 33.5% towards trimethoprim-sulfamethoxazole, 27.2% towards chloramphenicol, 19.1% towards cephalothin, 8.8% towards ceftriaxone, 7.6% towards cefotaxime, 7.4% towards aztreonam, 7.2% towards gentamicin, 7% towards cefepime, 6.8% towards ceftazidime, 5.8% towards cefixime, 2.7% towards imipenem, and 2.2% towards meropenem (Khademi et al., 2020). Some researchers reported that the focus on antibiotic resistance may overshadow other important factors contributing to the spread of this pathogen. They emphasize the need for comprehensive public health strategies that address not only antibiotic use but also food safety, animal husbandry practices, and environmental contamination. Additionally, there are concerns about the overuse of antibiotics in agriculture and its potential contribution to resistance in *Salmonella enterica diarizonae* (Jahantigh et al., 2015; 2018 ; Giner-Lamia et al., 2019).

Furthermore, some experts suggest that antibiotic resistance is a natural phenomenon that occurs as bacteria evolve and adapt to their environments. They argue that while antibiotic resistance should be managed and monitored, it may not always pose an immediate and direct threat to public health. Instead of solely focusing on the development of new antibiotics, there are calls for more investment in alternative treatment approaches and preventive measures to combat *Salmonella enterica diarizonae* infections (Song et al., 2023).

4. Conclusion

In conclusion, *Salmonella enterica diarizonae* poses a significant public health concern due to its antibiotic resistance. The increasing prevalence of multidrug-resistant strains of this bacterium is alarming, as it limits the effectiveness of common treatment options and can lead to more severe and prolonged infections. As such, it is imperative for public health authorities and healthcare providers in Iraq to closely monitor the spread of antibiotic-resistant strains and to implement effective measures to prevent further dissemination. Additionally, promoting responsible antibiotic use and adherence to hygiene and food safety practices is crucial in reducing the risk of *Salmonella enterica diarizonae* infections. Further research and Collaboration between public health authorities, veterinary professionals, and the poultry industry is essential to address this growing threat and safeguard public health. Continued monitoring, research, and public awareness are crucial in mitigating the risks associated with *Salmonella* contamination in chicken meat.

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