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Research Paper

"Susceptibility of Salmonella Bacteria Isolated from Humans to Certain Antibiotics"

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Abstract

In our current study, 4 serotypes of the Salmonella genus were isolated: S.typhimurium, S.typhi, S.paratyphiB, and S.diarizonae from inpatients and outpatients of Salahuddin General Hospital in Tikrit and the model health sector hospital in Al-Dawr, both affiliated with the Salahuddin governorate in Iraq. These samples spanned various age groups ranging from one day to 61 years and older. The results showed the highest infection rate in the age group (21-30) years at 2.20%, while the lowest was in the age groups (7-12) years and 61 years and older with an isolation rate of 0%. Concerning diarrhea affliction, positive isolates from those suffering from diarrhea were 15 isolates, with a rate of 78.95%, while the number of positive isolates from non-diarrhea affected individuals was 4 isolates, at a rate of 21.05%. These isolates exhibited varying sensitivity towards 10 antibiotics from the Turkish company Bioanalyse. They were 100% sensitive to the antibiotics Meropenem, Chloramphenicol, and Azithromycin, while showing moderate resistance to Nalidixic acid, Kanamycin, and Amikacin. They also demonstrated 100% resistance to the antibiotics Cefepime, Ciprofloxacin, Rifampin, and Trimethprime.

Keywords: Salmonella bacteria, antibiotics, resistance

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Introduction

The Salmonella genus is one of the largest genera of the Enterobacteriaceae family. These bacteria cause a wide range of acute and chronic diseases in both humans and animals (Gram et al., 2021). Species of the Salmonella genus are Gram-negative rods, non-spore-forming, and grow either aerobically or anaerobically, meaning they are facultatively anaerobic (Fong, 2021).

Salmonella diseases are classified into typhoidal Salmonella (TS) and non-typhoidal Salmonella (NTS). Typhoidal Salmonella, such as *S. typhi*, causes typhoid fever, while *S. paratyphi* A, B, and C cause paratyphoid fever (Akinyemi et al., 2021). They cause gastroenteritis, septicemia, and are confined to the human host (Ngogo et al., 2020).

On the other hand, non-typhoidal Salmonella, like *S. typhimurium*, *S. enteridis*, and *S. choleraesuis*, have multiple hosts and infect humans mainly through contaminated food and food products, leading primarily to gastroenteritis and colitis accompanied by diarrhea, cramps, and vomiting (Gong et al., 2022).

Antibiotics are the first line of defense in treating Salmonella bacteria, including Ampicillin, Amoxicillin, Chloramphenicol, and Trimethoprim-Sulfamethoxazole. However, their irrational use in therapeutic applications has led to the emergence of drug-resistant Salmonella bacteria (Abdelsattar et al., 2021). Furthermore, the excessive use of poultry growth promoters has contributed to the emergence of multi-drug resistant strains (Abou Elez et al., 2021).

Materials and Methods

The current study involved collecting 408 samples of blood and urine from inpatients and outpatients of Salahuddin General Hospital and the model health sector hospital in Al-Dawr. Specifically, 204 blood samples and 204 urine samples were collected from the beginning of August 2022 to the end of January 2023. The age groups ranged from one day to 61 years and older, encompassing both genders. The samples were taken at different days throughout each month.

Blood samples, 102 from each hospital, were collected as follows: 2 ml of blood was drawn using a tourniquet and medical syringe. Subsequently, the blood was placed into EDTA tubes and then transferred to Brain Heart Infusion broth. The tubes were incubated at 37°C for 24 hours. After that, the samples were transferred using a loop and inoculated onto solid S.S agar and XRD media, followed by incubation at 37°C for another 24 hours.

Urine samples involved collecting 3 ml of urine in a tube, which was later centrifuged. A swab was taken from the sediment and placed into Tetrathionate broth with the addition of 5 drops of iodine solution. The samples were then incubated at 37°C for 24 hours. Afterward, using a metal loop, they were inoculated onto S.S agar and XRD media and incubated at 37°C for 24 hours.

These bacteria were then identified based on morphological and cultural properties, such as colony size, color, and edge (Forbes et al., 2007). Further

identification was based on various biochemical tests, such as the indole test (Leber, 2016), methyl red test (Forbes et al., 2007), Voges-Proskauer test (Leber, 2016), citrate utilization test (Mahon et al., 2011), catalase test (de la Maza et al., 2020), oxidase test (Leber, 2016), urease test (Tille, 2017), growth on Triple Sugar Iron agar (Alfred, 2005), gelatin hydrolysis, carbohydrate fermentation test (CruickShank et al., 1975), and motility test (Mohan et al., 2011).

For a more precise identification at the species level, the VITEK system was used. Sensitivity tests for the Salmonella isolates were conducted against 10 antibiotics from the Turkish company Bioanalyse, following the standard Kirby-Bauer method as described by the Clinical and Laboratory Standards Institute (CLSI, 2020). Bacterial cultures were prepared in tubes containing 5 ml of Nutrient broth and incubated at 37°C for 24 hours. The turbidity of the bacterial suspension was compared to the McFarland standard. If not matching, saline solution was added until it matched the McFarland standard's turbidity. The bacterial suspension was then spread on the surface of the pre-prepared Muller-Hinton agar using a sterile cotton swab. Using sterile forceps, antibiotic discs were placed on the agar surface, with 5 discs per plate. The plates were then incubated at 37°C for 24 hours. Results were interpreted by observing the inhibition zone around the disc and measuring its diameter in millimeters using a calibrated ruler. These measurements were then compared to the standard inhibition zone diameters for antibiotics as per CLSI (2020).

Results and Discussio

Distribution of Salmonella Infections According to Age Groups:

Samples were collected from various age groups, ranging from one day to 61 years and older. The results indicated that the highest rate of infection with Salmonella bacteria was found in the age group (21-30) at 2.20%. The lowest rate of infection was 0% for the age groups (7-12) years and (61 years and older), as illustrated in Table (1) and Figure (1).

Table (1): Distribution of Salmonella Infections by Age Group

Age Groups	Number of Examined Samples	Number of Positive Samples	Percentage of Infection
1 day - 2 years	44	4	0.980%
> 2 years - 6 years	28	1	0.245%
> 6 years - 12 years	28	0	0%
> 12 years - 20 years	44	1	0.245%
21 years - 30 years	86	9	2.210%
31 years - 40	76	1	0.245%

years			
41 years - 50 years	58	2	0.490%
51 years - 60 years	36	1	0.245%
61 years and older	8	0	0%
Total	408	19	4.66%

Chi-Square = 13.267 P-Value = 0.002

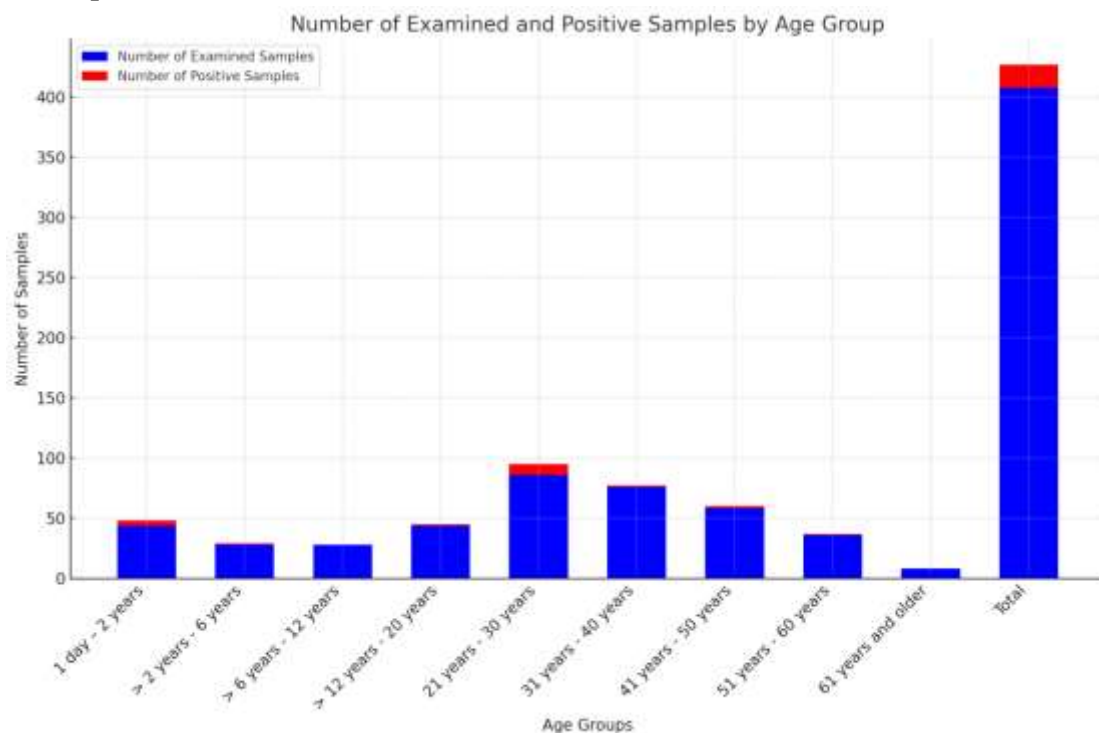


Figure 1: Distribution of Salmonella infections by age groups

The results of the current study align with the findings of the study conducted by Maharjan et al., 2021. The age group of 21-30 years was found to be the most affected by Salmonella bacteria, with an isolation rate of 30.1% out of 1298 samples.

Our current results also coincide with the findings documented by Al-Naimi (2014), indicating that the age group of 60-69 years had the lowest infection rate with Salmonella bacteria, amounting to 0%. This low percentage may be attributed to the small number of samples studied, totaling 8 samples.

Furthermore, our current study's results agree with the study of Al-Douri, 2021. The age group of 21-30 years, with a rate of 8.64%, was the most affected by Salmonella bacteria.

The prevalence of this bacterium in this age group is likely due to their active social lifestyle, where the idea of eating out is highly probable.

Distribution of Salmonella bacterial infections based on their association with diarrhea:

Upon reviewing Table 2 and Figure 2, we can discern the distribution of positive isolates of Salmonella bacteria based on their association with diarrhea. The number of these isolates reached 15, with a rate of 78.95%, while the number of positive isolates not associated with diarrhea was 4, representing 21.05%.

Table 2: Distribution of Salmonella bacterial infections based on their association with diarrhea.

Positive Isolates	Number of Isolates	Percentage (%)
With Diarrhea	15	78.95%
Without Diarrhea	4	21.05%
Total	19	100%

Chi-Square = 12.831 P-Value = 0.002

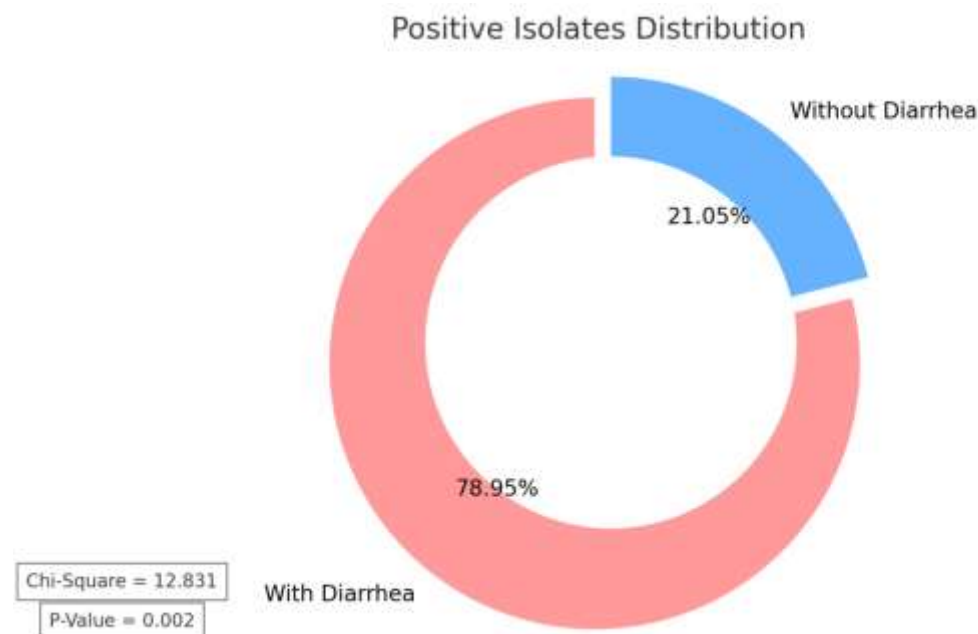


Table (2): Distribution of Salmonella infections based on diarrhea incidence.

Our current study's results, which showed that approximately 78.95% of those infected with Salmonella suffer from diarrhea, align with the findings of Ngogo et al., 2020. Their study found that 25 out of 99 samples (or 25.3%) of individuals with Salmonella infections had diarrhea. In contrast, 21.05% of the subjects in our study were infected with Salmonella but did not have diarrhea. This is consistent with the aforementioned source, which found that 24 out of 198 samples (or 12.1%) were Salmonella positive without showing symptoms of diarrhea.

The infection rate of Salmonella was highest in those with diarrhea, standing at 78.95%, and decreased to 21.05% in those who didn't suffer from diarrhea. This is because sometimes diarrhea is caused by Salmonella infections, while other times, it could be due to other pathogens such as E. coli or rotavirus. In a study conducted by Ahmad in 2020, out of 120 samples taken from the nails

of children under five with diarrhea, 112 samples (or 93.3%) tested positive, while 8 samples (or 6.7%) showed no bacterial growth. Gram-positive bacteria were predominant, making up 75 samples (or 66.96%), while Gram-negative bacteria constituted 37 samples (or 33.04%). When the Gram-negative bacteria were further analyzed based on cultural, microscopic characteristics, and biochemical tests, and confirmed at the species level using Vitck2 compact, no *Salmonella* bacteria were detected.

The World Health Organization (WHO) has stated that over 2,000 children die daily due to diarrhea, which is more than deaths from HIV, measles, and malaria combined. This makes diarrhea the second leading cause of child mortality worldwide (El-Ghazaly, 2021).

Sensitivity of *Salmonella* isolates to antibiotics

Upon examining Table (3) and Figure (3), it is evident that all *Salmonella* isolates were sensitive to the antibiotics meropenem, chloramphenicol, and azithromycin, with a sensitivity rate of 100%. Our findings align with the results of Abu Elez et al., 2021, where all *Salmonella* isolates were sensitive to meropenem (100%). However, another study by Dallal et al., 2020 showed a sensitivity rate of 11.11% to this antibiotic, a moderate resistance rate of 72.22%, and a resistance rate of 16.66%. As for Chloramphenicol, our results concur with those of Maharjan et al., 2021, where all *Salmonella* isolates were sensitive to the antibiotic (100%). On the other hand, Dallal et al., 2020 found a sensitivity rate of 94.44%, a moderate resistance rate of 5.55%, and no resistance. Contrarily, a study by Al-Obaidi, 2022 reported that all *Salmonella* isolates showed resistance to Chloramphenicol (100%).

Regarding the antibiotics Nalidixic acid, Kanamycin, and Amikacin, *Salmonella* isolates exhibited moderate resistance, with rates of 36.84%, 84.21%, and 100% respectively. Our findings somewhat align with those of Gebeyehu et al., 2022, where *Salmonella* isolates showed moderate resistance to Nalidixic acid at 57.5%. In a study by Dallal et al., 2020, the resistance rate was 5.55%. In contrast, Maharjan et al., 2021 reported a resistance rate of 2.5%. As for Kanamycin, the findings of Gebeyehu et al., 2022 were somewhat consistent with our study, showing a moderate resistance rate of 27.5%.

As for the Amikacin antibiotic, all isolates of *Salmonella* bacteria have shown moderate resistance at a rate of 100%. This result somewhat agrees with the findings of the study conducted by Ahmed et al. (2022), where the *Salmonella* bacteria isolates were moderately resistant by 82.35%. Our study's results also demonstrated that all isolates of *Salmonella* were resistant at a rate of 100% towards the antibiotics Cefepime, Ciprofloxacin, Rifampin, and Trimethoprim.

In a study carried out by Vidal et al. (2023), *Salmonella* bacterial isolates exhibited varying sensitivity towards Cefepime. Of the isolates, 93 (69%) were sensitive, 3 (2%) were moderately resistant, and 39 (29%) were resistant to this antibiotic. In another study by Muinde et al. (2023), the resistance rate of *Salmonella* isolates towards Cefepime decreased to 3%. In contrast, our current

study's results were in line with those found in Raji et al. (2021), where all isolates of Salmonella bacteria were resistant to the antibiotic Ciprofloxacin at a rate of 100%.

Our study's results also agreed with those of Mohammed and Shareef (2022), showing that the Salmonella bacteria isolates were resistant to Ciprofloxacin at a rate of 100%. However, our findings did not agree with the results of a study by Al-Obaidi (2022), which showed that Salmonella bacterial isolates were sensitive to Ciprofloxacin at a rate of 100%. In a study by Muinde et al. (2023), the results demonstrated that Rifampin has the highest resistance rate, reaching 96%, which roughly aligns with our study where Salmonella bacteria isolates were resistant to this antibiotic at a rate of 100%.

Meanwhile, our current study's results were somewhat consistent with the findings of Nguyen et al. (2023), where Salmonella isolates were sensitive to the antibiotic Trimethoprim, with 8 out of 34 isolates (23.52%) being sensitive, while 26 out of 34 isolates (76.47%) were resistant to this antibiotic. The emergence of antibiotic-resistant strains can be attributed to incorrect and random use of these drugs without conducting sensitivity tests. The discovery of a new antibiotic often leads to widespread and sometimes arbitrary use, as a result of self-medication, causing many resistant isolates to appear. This poses a serious problem from both health and economic perspectives. Moreover, the excessive use of poultry growth promoters has contributed to the emergence of these multi-drug resistant strains (Abou Elez et al, 2021). As bacteria develop numerous resistance mechanisms against these antibiotics, such as altering the target site of the antibiotic's action, increasing beta-lactamase enzyme production, and changing the permeability barrier, it becomes imperative to raise health awareness among community members. Avoiding arbitrary antibiotic usage, and insisting on performing sensitivity tests to determine the appropriate antibiotic for the patient, is vital (Al-Obaidi, 2022).

Table (3): Sensitivity of Salmonella bacterial isolates towards antibiotics.

#	Antibiotic	Symbol	Diameter of Inhibition zone (mm)	Sensitive Isolates (%)	Moderately Resistant Isolates (%)	Resistant Isolates (%)
1	Meropenem	MEM	23≤, 20-22, 19≥	19 (100%)	0 (0%)	0 (0%)
2	Cefepime	FEP	25≤, -, 18≥	0 (0%)	0 (0%)	19 (100%)
3	Nalidixic acid	NA	19≤, 14-18, 13≥	0 (0%)	7 (36.84%)	12 (63.16%)
4	Ciprofloxacin	CIP	26≤, 22-25, 21≥	0 (0%)	0 (0%)	19 (100%)

5	Chloramphenicol	C	18≤, 13-17, 12≥	19 (100%)	0 (0%)	0 (0%)
6	Azithromycin	AZM	13≤, -, 12≥	19 (100%)	0 (0%)	0 (0%)
7	Kanamycin	K	18≤, 14-17, 13≥	0 (0%)	16 (84.21%)	3 (15.79%)
8	Amikacin	AK	17≤, 15-16, 14≥	0 (0%)	19 (100%)	0 (0%)
9	Rifampin	RA	20≤, 17-19, 16≥	0 (0%)	0 (0%)	19 (100%)
10	Trimethoprim	TMP	16≤, 11-15, 10≥	0 (0%)	0 (0%)	19 (100%)

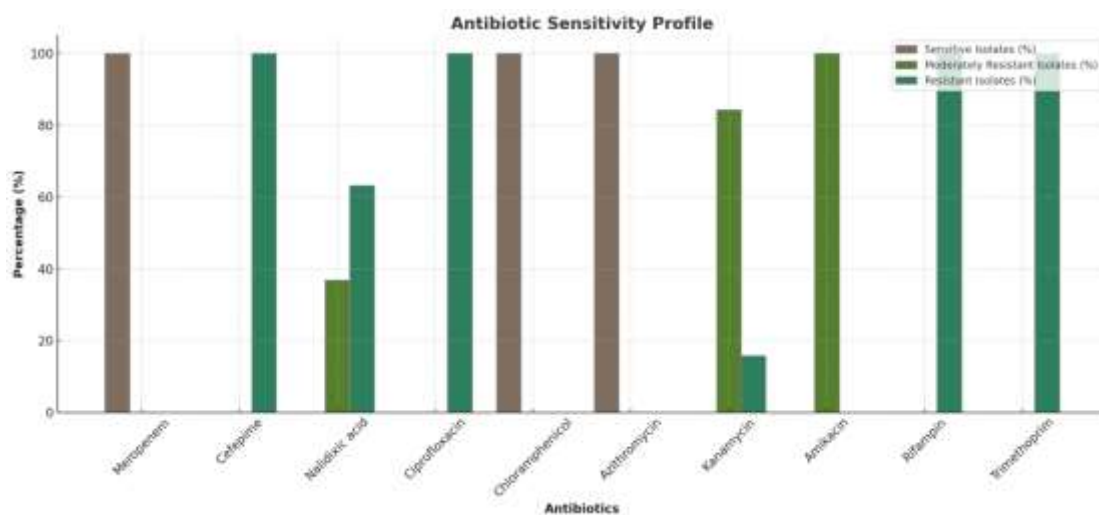


Figure (3): Sensitivity of Salmonella isolates to antibiotics.

Statistical Analysis:

The results were statistically analyzed using the statistical software MINITAB17. The Chi-Square test was used to compare between the groups included in the study, and the ANOVA F-test was applied to determine the significant differences between the treatments using the Duncan's Multiple Range Test at a probability level of 0.05 (Al-Rawi, 2000).

This translation gives an overview of the methods used for the statistical analysis of the data on the sensitivity of Salmonella isolates to different antibiotics.

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