

# PREVALENCE AND CHARACTERIZATION OF NON-TYPHOIDAL *SALMONELLA* IN DOGS, CATS, AND THEIR ENVIRONMENTS

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Non-typhoidal salmonellosis presents a significant threat to humans, dogs, and cats. This study involved conducting Whole Genome Sequencing (WGS) of *Salmonella* isolates from dogs, cats, and their surroundings, coupled with analysis of serotyping, virulence gene repertoire, and antimicrobial resistance genes. A total of 300 samples were collected from drinking water, feed, feces, and beddings of dogs and cats. The overall occurrence of non-typhoidal *Salmonella* was recorded at 13.33%, with 11.49% in cats and their environment and 14.08% in dogs and their environment. Among the cats, the highest incidence of *Salmonella* was detected in cat feces (16.28%), followed by bedding. In dogs, the highest occurrence of *Salmonella* was observed in feces (22.22%), followed by drinking water. Serologically identified strains were *Salmonella* Kentucky (55%), *Salmonella* Infantis (20%), and *Salmonella* Virchow (7.5%). However, WGS analysis revealed the presence of serotypes as Kentucky (70%), Virchow (20%), and Typhimurium (10%) due to differences in sample size. More than 105 virulence genes were found, with all isolates carrying genes for type three secretion systems (T3SS1 and T3SS2) and their effectors. Fimbrial adherence determinants were present in all isolates, except for isolate 148. The *shdA* gene was detected in isolates 156, 192, and 178, while the *ratB* gene was exclusively present in isolate 178. Macrophage Inducible Genes (*mig-14*) were found in all isolates except for 148. Antimicrobial resistance gene profiling revealed six resistance genes against aminoglycosides, trimethoprim, sulphonamides, and tetracycline. The presence of antimicrobial-resistant strains highlights the need for judicious use of antimicrobials in dogs and cats.

**Keywords:** Antimicrobial Resistance, Serotyping, Virulence Genes, Whole Genome Sequencing (WGS),

Non-typhoidal *Salmonella* (NTS) is a major contributor to gastroenteritis in both humans and animals around the world. While the primary concern has traditionally been its effect on human health, recent studies have started to highlight the role of pets as reservoirs and vectors of *Salmonella* transmission to humans and other animals. Household pets, such as dogs and cats, are capable of carrying and shedding *Salmonella*, which can lead to the contamination of their surroundings and pose a health risk to their human caregivers. The purpose of this study was to examine the prevalence, serotypes, virulence factors, and antimicrobial resistance

profiles of *Salmonella* isolates obtained from dogs, cats, and their environments. Use of Whole Genome Sequencing (WGS) allows for an in-depth analysis of the genetic composition of the isolates, which enhances our understanding of their potential public health implications (CDCP, 2020). By analyzing samples from dogs, cats, and various environmental sources within their habitats, researchers sought to quantify the presence of *Salmonella* and identify the specific serotypes involved. This information is crucial, as different serotypes can vary significantly in terms of virulence and the ability to cause disease. Virulence factors are genetic traits that enable the bacteria to infect hosts and evade their immune responses.

\*Part of M.V.Sc. Thesis

Identifying these factors provides insight into how these pathogens cause illness and can inform the development of strategies to mitigate their impact.

Antimicrobial resistance is a growing concern in both human and veterinary medicine, as it can complicate treatment and lead to more severe or prolonged illnesses. By identifying resistance patterns, researchers can better understand the challenges posed by these pathogens and guide the selection of effective treatments. Whole Genome Sequencing (WGS) was the chosen method for this study due to its ability to provide a detailed and comprehensive view of the genetic makeup of the isolates. This can identify specific genes associated with virulence and antimicrobial resistance, as well as track the genetic relationships between different strains.

## **Materials and Methods**

### ***Sample Collection***

A total of 300 samples were collected from various sources associated with dogs and cats, including drinking water, feed, feces, and beddings. The samples were categorized as follows:

- Dog environment: drinking water, feed, feces, and bedding.
- Cat environment: drinking water, feed, feces, and bedding.

### ***Isolation and Identification of Salmonella***

Samples were processed for the isolation of *Salmonella* using standard microbiological methods. Presumptive *Salmonella* colonies were confirmed by biochemical tests and serologically identified using specific antisera.

### ***Whole Genome Sequencing (WGS)***

The Whole Genome Sequencing was performed on confirmed *Salmonella* isolates to determine serotypes, virulence genes, and antimicrobial resistance genes. DNA extraction was followed by library preparation and sequencing on an Illumina platform. The resulting sequences were

analyzed using bioinformatics tools to identify serotypes and genetic determinants of virulence and resistance.

The isolates were examined using the ResFinder database and NCBI AMR FinderPlus, which resulted in the identification of six antimicrobial resistance genes. These genes confer resistance against four distinct groups of antimicrobial agents. Plasmid Finder was used to detect plasmids.

### ***Data Analysis***

Data from WGS were analyzed to determine the presence of virulence genes and antimicrobial resistance genes. The distribution of these genes among the isolates was compared between different sources and species.

## **Results and Discussion**

### ***Prevalence of Salmonella***

The overall occurrence of non-typhoidal *Salmonella* was recorded at 13.33%. In cats and their environment, the prevalence was 11.49%, with the highest incidence in feces (16.28%) followed by bedding. In dogs and their environment, the prevalence was 14.08%, with the highest occurrence in feces (22.22%) followed by drinking water (5.55%).

### ***Serotyping***

For the species of dogs, there were a total of 30 isolates identified. Among these, the *Salmonella* serotype Kentucky was the most prevalent, with 18 isolates, which accounts for 60% of the total isolates from dogs. The serotype Infantis was identified in 4 isolates, representing 13.33% of the total. The Virchow serotype was found in 2 isolates, making up 6.67% of the isolates. Additionally, there were 6 isolates that were untypable, comprising 20% of the total isolates from dogs.

In the case of cats, there were 10 isolates identified in total. The *Salmonella* serotype Kentucky was found in 4 isolates, accounting for 40% of the total isolates from cats. Similarly, the serotype Infantis was also

found in 4 isolates, making up another 40%. The Virchow serotype was identified in 1 isolate, representing 10% of the total. Lastly, there was 1 isolate that was untypable, also comprising 10% of the total isolates from cats.

Serologically identified strains were *Salmonella* Kentucky (55%), *Salmonella* Infantis (20%), and *Salmonella* Virchow (7.5%). However, WGS analysis revealed a different distribution of serotypes: *Salmonella* Kentucky (70%), *Salmonella* Virchow (20%), and *Salmonella* Typhimurium (10%).

### Virulence Gene Repertoire

Over 105 virulence genes were identified in the *Salmonella* isolates. Key findings include the presence of Type Three Secretion Systems (T3SS1 and T3SS2) in all isolates, with fimbrial adherence determinants present in all isolates except for isolate one (Sample no. 148). The *shdA* gene was detected in isolates of sample numbers 156, 192, and 178, while the *ratB* gene was exclusively present in isolate of sample no. 178. Additionally, Macrophage Inducible Genes (*mig-14*) were found in all isolates except in isolate of sample number 148.

**Table 1: VIRULENCE GENES IDENTIFIED IN *SALMONELLA* ISOLATES**

Sample Number	T3SS1	T3SS2	Fimbrial Adherence Determinants	<i>shdA</i>	<i>ratB</i>	<i>mig-14</i>
148	Yes	Yes	No	No	No	No
156	Yes	Yes	Yes	Yes	No	Yes
192	Yes	Yes	Yes	Yes	No	Yes
178	Yes	Yes	Yes	Yes	Yes	Yes

**Note:** All other isolates have expressed all the above six mentioned Virulence Genes

### Antimicrobial Resistance Genes

Six resistance genes were identified, conferring resistance to aminoglycosides, trimethoprim, sulphonamides, and tetracycline. This highlights the presence of antimicrobial-resistant strains in both dogs and cats, underscoring the need for prudent use of antimicrobials in these animals. The Table 5 presents data on the presence of various antimicrobial resistance genes across different bacterial isolates, structured to show the type of antimicrobial, specific resistance genes, isolate numbers, the number of isolates carrying each gene, and the total number of isolates tested for each antimicrobial. For aminoglycosides, the genes *aac(3)-Id* and *aadA7* were found in isolates 50, 169, and 197, each with three isolates carrying the gene, while the *aac(6')-Iaa* gene was present in all 10 tested isolates. The trimethoprim resistance gene *dfrA5* was identified in isolates 156 and 192, with two isolates carrying the gene. The sulphonamide

resistance gene *sul1* was present in isolates 50, 156, 169, 192, and 197, with five isolates carrying the gene. The tetracycline resistance gene *tet(A)* was also found in isolates 50, 156, 169, 192, and 197, with five isolates carrying the gene. Among the aminoglycosides, *aac(6')-Iaa* showed high prevalence by being detected in all 10 isolates, whereas *aac(3)-Id* and *aadA7* were found in three isolates each (50, 169, and 197). The trimethoprim resistance gene *dfrA5* was less prevalent, found in only two isolates (156 and 192). The *sul1* gene was relatively common among sulphonamides, present in five isolates, and similarly, the *tet(A)* gene was found in five isolates. This table highlights the distribution of specific antimicrobial resistance genes among bacterial isolates, indicating varied prevalence rates for different resistance genes, which is crucial for developing targeted strategies to manage and control the spread of antimicrobial resistance. Any plasmid could not be detected in the study.

The findings of this study indicate a significant prevalence of non-typhoidal *Salmonella* in dogs, cats, and their environments. This aligns with previous research by Mukhopadhyay *et al.*, (2023) highlighting the widespread occurrence of *Salmonella* in various animal hosts and their potential to act as reservoirs for human infections. In this study, the overall occurrence of *Salmonella* was 13.33%, with higher prevalence rates observed in dog and cat feces, suggesting that direct contact with animal waste is a major route of transmission.

The serotypes identified in this study, particularly *Salmonella* Kentucky, Virchow, and Typhimurium, are consistent with those reported in other studies involving domestic animals and their environments as also reported by Jajere, 2019. The discrepancies between serological and WGS-based serotyping underscore the importance of using advanced molecular techniques for accurate identification and characterization of pathogens.

The detection of over 105 virulence genes, including type three secretion systems (T3SS1 and T3SS2), fimbrial adherence determinants, and macrophage inducible genes, highlighted the pathogenic potential of these isolates (Keelara *et al.*, 2021). The presence of the *shdA* and *ratB* genes in specific isolates suggested variability in virulence factors, which could influence the severity of infections in different hosts.

One of the most concerning aspects of this study is the identification of antimicrobial resistance genes, particularly those conferring resistance to aminoglycosides, trimethoprim, sulphonamides, and tetracycline. This finding is significant given the increasing prevalence of antimicrobial-resistant *Salmonella* strains globally and the implications for both veterinary and public health (Nguyen, 2023). The presence of antimicrobial-resistant strains in both dogs and cats underscores the need for judicious use of antimicrobials in these animals. Antimicrobial stewardship programs in veterinary practice are essential to mitigate the development and spread of resistance as

also reported by Guardabassi *et al.*, (2015). Moreover, public health agencies, such as the Centers for Disease Control and Prevention (CDC), emphasize the importance of monitoring and controlling *Salmonella* in both human and animal populations to reduce the risk of transmission and associated health burdens as also mentioned by (CDCP, 2020).

Overall, this study underscores the importance of comprehensive monitoring and molecular characterization of non-typhoidal *Salmonella* in pets and their environments. The use of WGS provides valuable insights into the genetic makeup of these isolates, enhancing our understanding of their potential impact on public health. The findings advocate for continued surveillance and prudent antimicrobial use to mitigate the risks associated with *Salmonella* infections in domestic animals and their human companions.

## Conclusion

This study aimed to investigate the prevalence, serotypes, virulence factors, and antimicrobial resistance profiles of *Salmonella* isolates from dogs, cats, and their environments using Whole Genome Sequencing (WGS). WGS offers a detailed genetic analysis, crucial for understanding the public health implications of these isolates. The research assessed the spread of *Salmonella* among pets and their habitats, identified specific serotypes, and examined the genetic traits that enable infection and immune evasion. Furthermore, it explored antimicrobial resistance patterns, a growing concern in medical treatment, providing insights for better management and treatment strategies. The study's findings underscore the importance of proper hygiene practices and monitoring to prevent *Salmonella* spread, emphasizing the need for vigilance in both human and veterinary health sectors.

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