PREVALENCE AND CHARACTERIZATION OF NON-TYPHOIDAL *SALMONELLA*

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in

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DECLARATION

I, hereby declare that the presented work in the thesis entitled "**Prevalence and Characterization of Non-Typhoidal Salmonella**" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of **Dr. Gaurav Kumar (19454)**, working as **Associate Professor**, in the **Department of Microbiology, School of Bioengineering & Biosciences** of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigators. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "**Prevalence and Characterization of Non-Typhoidal *Salmonella***" submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the **Department of Microbiology, School of Bioengineering & Biosciences,** is a research work carried out by **Mr. Sandeep Kumar,** Registration No.**41500160** is a bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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ABSTRACT

Salmonella, a Gram-negative bacterial genus, merits distinction within the context of infectious diseases for its remarkable pathogenic versatility. It substantiates its prominence by precipitating a spectrum of clinical entities, encompassing self-limited gastroenteritis to life-threatening systemic infections. This thesis embarks upon an exploration of non-typhoidal *Salmonella* (NTS), a subset of the *Salmonella* genus that accounts for a substantial proportion of global gastrointestinal infections. While typhoidal-*Salmonella* serovars (*S.* Typhi and *S.* Paratyphi), cause the systemic illness known as typhoid fever, NTS serovars present a diverse range of clinical manifestations, including localized gastroenteric issues, bloodstream infections, and associated clinical complications. Most cases of non-Typhoidal salmonellosis are induced by various serovars known for their ability to cause self-limiting diarrhoea across a wide range of hosts. Non-typhoidal *Salmonella* has garnered global attention due to its capacity to provoke various enteric diseases, posing challenges for diagnosis and treatment, especially in less developed regions.

One key strategy for addressing the NTS disease burden is controlling the spread of the causative agent. Therefore, it is crucial to conduct epidemiological investigations using diverse typing methods. Despite being prevalent in Asia and frequently associated with diarrheal illnesses, there exists a dearth of comprehensive data on NTS infections in the region, with limited reports primarily from India and a few other Asian countries. In the Indian context, to develop effective surveillance and control strategies, it is essential to periodically generate accurate epidemiological data on NTS. Hence, this study aims to examine the prevalence and various characteristics of NTS isolates received from different regions of the country.

This study presents a characterization of NTS isolates received from diverse regions in India. The samples were received at the 'National *Salmonella* and *Escherichia* Centre' (NSEC) at the Central Research Institute, Kasauli, a distinguished reference laboratory with over six decades of expertise. This collection of *Salmonella* isolates, originated from hospitals, veterinary research institutes, as well as fisheries and food department laboratories, distributed across various geographical locations in India. These NTS isolates, categorized into human, food, environment, and animal

sources, underwent a comprehensive processing regimen involving purification, biotyping, and serotyping. Moreover, the primary focus of the study entailed examining the antibiogram patterns of the NTS isolates. Subsequent investigations included identifying NTS strains producing extended-spectrum beta-lactamase (ESBL), detecting genes associated with ESBL production, identifying carbapenemase activity, assessing virulence factors such as biofilm formation and colicin production, and finally pinpointing the gene responsible for drug resistance in NTS, notably the blaCTX-M15 gene linked to resistance against third-generation cephalosporins, particularly cefotaxime.

The study involved 999 samples, which underwent further processing for comprehensive identification and subsequent investigations. Among them, 300 (30%) isolates were found to be non-*Salmonella* and thus excluded from the study. The remaining 699 (70%) isolates were identified as *Salmonella* and used in the subsequent study. Of the 699 isolates collected, 539 (77.1%) were confirmed to be non-Typhoidal *Salmonella*, which was the main focus of the study. The remaining isolates were either rough *Salmonella* or Typhoidal *Salmonella* and were not used further. Serotyping categorized these 539 NTS into 17 serovars. *S. enterica* serovar Typhimurium and Lindenburg demonstrated substantial prevalence accounting for 167 (30.98%) and 135 (25.05%) of the isolates, respectively. The remaining 15 NTS serovars, collectively represent nearly 44% of the total.

The study of geographical distribution of these 539 serovar revealed that 313 (58.07%) serovars originated from the southern part of the country; 98 (18.18%) from the Northern region; 89 (16.51%) from the western region; 37(6.86%) from Eastern region and 2 (0.37%) from central part of the country.

A source-wise distribution of the NTS revealed that the majority i.e., 319(58.18%) were of human origin; 99(18.36%) from food sources; 83 (15.4%) from animal sources and 38 (7.05%) from environmental sources. Further, the distribution of NTS across different human and non-human sources revealed that human fecal samples emerged as the dominant reservoir for NTS isolates, representing 63.95% (204 out of 319) followed by 22.8 % from blood, while other sites (urine, cerebrospinal fluid (CSF), pus, tissue, and body fluid samples) collectively yielded < 15% (40/319).

Amongst the 17 NTS serovars acquired in this study, it is notable that 10 of these serovars were present in the samples sourced from human-related origins. A further detailed analysis based on each source sub-category revealed that the serovars Lindenburg (75 out of 204, or 36.76%) and Typhimurium (52 out of 204, or 25.49%) collectively showed their dominance within the samples originating from fecal sources. This study reports the first single incidence where serovar Mathura was isolated from the human blood sample. Among the NTS from animal sources, 43 (51.8%) NTS isolates were derived from animal fecal samples; Poultry feces contributed 11 (13.3%); 7 (8.4%) NTS isolates were sourced directly from poultry specimens; 1 NTS isolate was obtained from calf fetal stomach content; 4 NTS isolates were extracted from poultry diarrhoea samples. Serovar Typhimurium (32/83, 38.6%) dominated among the NTS collection from animals, followed by *S. enterica* serovar Lindenburg (11/83, 13.3%); *S. enterica* serovar Weltevreden (9/83, 10.8%) and *S. enterica* serovar Stuttgart (8/83, 9.6%). NTS Serovars such as Mathura, Anatum, Bazenheid, Tennessee, Enteritidis, and Senftenberg, collectively represented 27.7% (23/83) of the total collection. Among the NTS from food sources, 32 (32.3%), were recovered from raw pork; Seafood contributed 12 (12.1%); Poultry meat contributed 6 (6.1%); Raw cow or buffalo milk samples yielded 13 (13.1%) and Meat (fish, pork, beef) contributed 11 (11.1%) to the total NTS collection from food sources. *S. enterica* serovar Typhimurium stands out as a predominant serovar with 45.4% isolates out of 99; followed by *S. enterica* serovar Lindenburg with an isolation rate of 21.2% (21/99). The contribution of environmental sources to NTS collection was 7.05% with *S. enterica* serovar Mathura dominated with 25 isolates; followed by 5 isolates of Jaffna, one isolate each of Typhimurium and Enteritidis; 2 isolates of Enteritidis, and 4 of Hissar.

The antibiogram profiling of 539 isolates was done using 19 antimicrobial drugs belonging to various classes. The results showed a concerning 44.90% (242 out of 539) of NTS isolates exhibited resistance to Cefotaxime; Followed by Nalidixic acid (39.9%); Piperacillin/tazobactam (27.7%); Ampicillin (27.09%), amoxicillin/clavulanic acid (25.97%); Trimethoprim (19.67%); nitrofurantoin (17.44%); co-trimoxazole (14.47%); Imipenem (13.73%); Kanamycin (12.1%); Ceftriaxone (11.9%);

Ciprofloxacin (11.1%); Cefuroxime (11.1%); Meropenem(10.2%); Gentamicin (6.1%); Norfloxacin (6.1%); Amikacin (5.9%); Cefepime (1.7%) and Chloramphenicol (0.9%). The multi-drug resistance profile of NTS revealed different patterns of resistance. The southern region exhibited the greatest proportion of multidrug resistance across all categories of drugs. 44 out of 83 isolates (53%) from animal sources and 158 out of 319 isolates (49.5%) from human sources exhibit MDR and XDR behaviours.

Further, test results to detect ESBL, depicted 24 (4.45%) isolates as ESBL producers. Among these 24, 11 ESBL Positive NTS confirmed by a single drug Ceftazidime and Ceftazidime-Clavulanic acid; **9** ESBL Positive NTS confirmed by a single drug Cefotaxime and Cefotaxime -Clavulanic acid; and **4** NTS showed overall Positivity towards both the drugs and their combinations with Clavulanic acid. Furthermore. Among 24 ESBL-producing NTS, 16 isolates were of human origin while 4, 3 & 1 isolates were obtained from environmental sources, animal sources, and food items respectively. *S. enterica* serovar Typhimurium dominated in human fecal samples followed by serovars Lindenburg (n=3), Weltevreden (n=2), Choleraesuis (n=1), and Enteritidis (n=1). While a single isolate each of serovars Typhimurium and Weltevreden was isolated from CSF and tissue samples respectively. Samples from environmental; animals; and food sources were found to harbor ESBL-producing NTS serovars such as Mathura (n=3) and Hissar (n=1); Typhimurium (n=1), Anatum (n=1); and Senftenberg (n=1) respectively. In this study, a targeted investigation was undertaken on 24 phenotypically confirmed ESBL-producing NTS serovars to characterize the presence of ESBL genes, specifically $bla_{\text{CTX-M15}}$, $bla_{\text{CMY-2}}$, bla_{SHV}, and bla_{TEM}. Few serovars harbored multiple genes ESBL genes; 7 NTS isolates harbored $bla_{\text{CTX-M15}}$ gene, 12 & 4 isolates harbored the $bla_{\text{CMY-2}}$ gene and bla_{TEM} genes respectively. Two serovars, specifically Lindenburg (n=2) and Weltevreden (n=1), originating from human fecal samples, were identified as hosts for a maximum of three ESBL genes out of the four, excluding bla_{SHV}. Furthermore, a total of 4 isolates, comprising two instances of *S. enterica* serovar Weltevreden and two of *S. enterica* serovar Typhimurium, were found to carry the $bla_{\text{CTX-M15}}$ with $bla_{\text{CMY-2}}$ gene combination. Additionally, one isolate, specifically *S. enterica* serovar Lindenburg, was

identified as carrying the *bla*$_{\text{CTX-M15}}$ with the TEM gene combination. Among the 4 environmental isolates, two isolates specifically *S. enterica* serovar Mathura showed the presence of *bla*$_{\text{CTX-M15}}$ genes only and all other three genes were not detected. One isolate from animal source, namely *S. enterica* serovar Anatum (E11) was found to harbour three genes *bla*$_{\text{CTX-M15}}$, *bla*$_{\text{CMY-2}}$, and *bla*$_{\text{TEM}}$.

In the present study, an in-depth analysis of the antibiogram profiles of 539 NTS isolates unveiled that 96 of these isolates demonstrated resistance to carbapenem antibiotics, particularly imipenem and meropenem, with 41 and 22 isolates respectively, while 33 isolates were resistant to both drugs. None of the isolates exhibited the presence of the carbapenemase enzyme.

In the present study, all 539 NTS isolates were tested to detect the production of biofilm. A substantial portion, specifically 374 (70%) isolates of the NTS pool, exhibited biofilm-producing capabilities with different grades of biofilm production such as ‘strong’, ‘moderate’, and ‘weak’. Among these 374 biofilm-producing NTS, 220 (58.8%) originated from human sources; 77 (20.6%) from food, 53 (14.2%) from animals, and 24 (6.4%) from environmental sources. Further analysis, unveiled that approximately 6.4% (24 out of 374) were classified as strong biofilm producers, while 28.3% (106 out of 374) exhibited a moderate level of biofilm formation. The remaining 65.2% displayed a weak level of biofilm formation. Furthermore, in this study, an association between the biofilm-producing NTS isolates and multiple drug resistance behaviour has also been observed in the NTS isolates under investigation.

Additionally, the results of the colicin detection test indicated a relatively low occurrence of colicin activity among the NTS isolates, with 24 out of 539 isolates (4.45%) demonstrating the capability to produce colicins. The colicin production rate was observed to be higher among NTS isolates obtained from human sources (13/24, 54.16%), followed by those obtained from food items (6/24, 25%) and animals (5/24, 20.83%). Conversely, none of the environmental isolates showed colicin activity.

Additionally, 223 cefotaxime-resistant NTS were subjected to the detection of the *bla*$_{\text{CTX-M15}}$ gene and the results revealed that 65 isolates, constituting 29.1%, were identified as harbouring this gene. The regional breakdown showcased distinct

patterns, with the Southern region demonstrating the highest prevalence rate at 73.8% (48 out of 65). Serovars Lindenburg (16) and Typhimurium (18) were particularly prevalent in this region, collectively accounting for 70% of *bla*$_{\text{CTX-M15}}$ gene-positive isolates. A similar trend was observed in other regions, notably for the occurrence of *bla*$_{\text{CTX-M15}}$ gene-bearing S. Typhimurium. Furthermore, the Southern region exhibited the presence of other *bla*$_{\text{CTX-M15}}$ gene-positive serovars, including Enteritidis (9), Choleraesuis (3), Kentucky (1), and Weltevreden (1). Additionally, a lower occurrence of less common serovars such as Anatum (1), Bazenheid (1), and Mathura (1), was also observed in this study.

Given the significance of NTS serovars in human, environmental, animal, and food, this research underscored the need for continued studies to better understand the epidemiology of NTS as the available data on the prevalence of NTS are limited or inconsistent. The results of this research raise significant public health concerns and underscore the necessity for enhancing nationwide NTS surveillance to identify and manage these highly virulent NTS serovars. This is essential for enhancing knowledge and implementing effective control measures on both regional and national levels to mitigate infections caused by this group of pathogens.

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As I stand at the culmination of this long and transformative journey, I take a moment to acknowledge my own perseverance, resilience, and dedication. The path to this achievement was filled with challenges, sacrifices, and countless hours of hard work, yet each obstacle only strengthened my resolve. This Ph.D. is not just a degree—it is a testament to the passion, patience, and persistence that have shaped me into the scholar I am today. I take immense pride in this accomplishment, knowing that it reflects not only my individual efforts but also the unwavering support of those who stood by me. This milestone is a reminder that perseverance and determination can turn dreams into reality.

Finally, I want to express my appreciation to all those who have directly or indirectly played a role in the completion of this thesis. Thank you all for being a part of this significant chapter in my life.

(Sandeep Kumar)

Dated: 03RD MAR, 2025

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CHAPTER – 1

INTRODUCTION

1. INTRODUCTION

Infectious diseases have persistently afflicted human populations, bearing profound implications for global public health. These illnesses, driven by a multitude of pathogens, exert the capacity to instigate substantial morbidity and mortality rates, while concurrently challenging societal infrastructures and healthcare systems. Among these pathogenic entities, the *Salmonella* genus emerges as a challenging rival within the domain of foodborne illnesses, characterized by its tendency for adaptability, resilience, and the capacity to infect a diverse array of hosts.

This thesis embarks upon an exploration of non-typhoidal *Salmonella* (NTS), a subset of the *Salmonella* genus that accounts for a substantial proportion of global gastrointestinal infections. While Typhoidal *Salmonella* strains, typified by *S.* Typhi and *S.* Paratyphi, cause the severe systemic illness known as typhoid fever, NTS serovars collectively exhibit a broad spectrum of clinical manifestations. These encompass localized gastroenteric pathologies, bloodstream infections, and related clinical sequelae. NTS predominantly propagates through the transmission channels of contaminated consumables and potable water sources, thereby ascertaining its prominence in matters concerning public health, food safety, and the extensive landscape of global health security.

Salmonella, a Gram-negative bacterial genus, merits distinction within the context of infectious diseases for its remarkable pathogenic versatility. It substantiates its prominence by precipitating a spectrum of clinical entities, encompassing self-limited gastroenteritis to severe systemic infections (Sears et al., 2023).

The genus *Salmonella* comprises two species, *S. bongori* and *S. enterica.* The latter is further subdivided into six subspecies. Among these, *S. enterica* subsp. *enterica*, with its extensive diversity, stands out. This subspecies plays a predominant role, accounting for approximately 99% of salmonellosis cases in both human and warm-blooded animal populations (Aleksandrowicz et al., 2023).

Salmonella encompasses a spectrum of clinical manifestations in human hosts, including bacteremia, enteric fever, gastroenteritis, and other extra-intestinal

complications, alongside the potential for a chronic carrier state (Darby & Sheorey, 2008; Eng et al., 2015). The occurrence of these varied clinical syndromes is contingent upon specific host factors and the serotype of *Salmonella* involved.

The enteric fever is a collective term for both Typhoid and Paratyphoid fevers. *S.* Typhi is the causative agent of former while the latter is caused by either of *S.* Paratyphi A, B, and C (Issenhuth-Jeanjean, 2014; Chen et al., 2023). In humans, infection typically arises from the ingestion of food and water contaminated with fecal matter harbouring the pathogen. The disease's incubation period extends to seven days or beyond and manifests with symptoms such as abdominal discomfort, headache, and diarrhoea, often accompanied by fever (Bhan et al., 2005). The outcome of the infection varies among different age groups more specifically children develop diarrhoea more commonly while older persons or patients with immunocompromised states develop constipation (Thielman and Guerrant, 2004). Individuals at the extremes of age and those with underlying comorbidities are at a notably heightened risk of developing bacteremia compared to those with enteric salmonellosis (Ballal et al., 2016). The Enteric fever is described by a distinctive fever pattern with a low-grade fever initially (between 37.5^0C - 38.2^0C) which if untreated slowly develops into a high-grade fever (38.2^0C – 41.5^0C) (Patel et al., 2010). Alongside fever, patients may experience myalgia, bradycardia, and hepatosplenomegaly, manifested as liver or spleen enlargement, as well as the appearance of rose spots on the chest or abdomen (Kuvandik et al., 2009). Patients with recurrent infections may develop some complications like pancreatitis, hepatitis, and cholecystitis. Additionally, severe infections in Peyer's patches or lymphatic nodules, located in the terminal ileum can result in extensive tissue damage, leading to perforations and hemorrhage (Eng et al., 2015).

Non-typhoidal *Salmonella* species are predominantly accountable for foodborne infections, acquired through contact (direct or indirect) with animals or by consuming foods e.g., meat, seafood, poultry products, and leafy vegetables (Antony, 2023). Contributing factors include compromised immune status, inadequate sanitation infrastructure, and suboptimal practices associated with food handling and hygiene (Devleesschauwer et al., 2018).

Gastroenteritis is typified by inflammation of the gastrointestinal tract, presenting symptoms such as diarrhoea, vomiting, nausea, and abdominal cramps, distinct from enteric fever which involves no liver or spleen involvement. (Wang et al., 2023). NTS infections usually do not lead to serious complications, however mild complications like cholecystitis, pancreatitis, and appendicitis have been reported earlier without any haemorrhagic conditions (Acheson and Hohmann, 2001). Symptoms typically manifest within 6-12 hours post-infection and typically resolve on their own within 10 days (Crump et al., 2008). However, NTS infections in infants, young children, aged persons, and immunocompromised individuals have been reported to develop into severe complications (Scallan et al., 2011).

In addition to the clinical diseases described earlier, almost all non-Typhoidal *Salmonella* infections lead to bacteremia and other extra-intestinal complications. Particularly, *S.* Dublin and *S.* Choleraesuis, recognized as highly invasive strains, are linked with bacteremia (Woods et al., 2008; Jones et al., 2008) and are characterized by high-grade fever akin to enteric fever. In certain serious conditions, the bacteremia may also lead to septic shock and death in patients, thereby depicting high mortality rates.

The majority of non-Typhoidal salmonellosis cases arises from various serovars, which are characterized by their wide host range and their ability to trigger self-resolving diarrhoea (Crump et al., 2015). The distribution of these serovars varies widely over time and place (Hendriksen et al., 2011; Van et al., 2012) and has a wide host range (Maguire et al.,1993; Gordon 2011). In addition to the typical diarrheal syndrome observed in human infected with NTS , invasive (bloodstream) disease can also occur by NTS known as iNTS disease (Feasey et al., 2012; Crump and Heyderman, 2015) primarily caused by the serovars Typhimurium and Enteritidis (Kariuki and Onsare, 2015; Feasey et al., 2015), and is presented with a severe systemic infection resembling the typhoid fever (Crump et al., 2011, 2015; Crump and Heyderman, 2015).

NTS infections constitute a significant global health concern, engendering morbidity and mortality across diverse socioeconomic settings (Gong et al., 2022). Globally, *Salmonellae* are one of the four primary causative agents of diarrheal diseases (CDC, 2022). NTS has gained global scientific attention due to its capacity to cause a

wide spectrum of enteric diseases, presenting a challenge for diagnosis and cure, particularly in the developing world. The Institute of Health Metrics and Evaluation (IHME) reported even higher estimates, with 73.9 million cases & associated 61,600 deaths attributed to NTS in 2019. The World Health Organization (WHO) estimates an annual incidence of approximately 93.8 million cases of Salmonellosis attributed to NTS, resulting in an estimated 155,000 mortalities (Mohakud et al., 2022). Further, global estimates for invasive (iNTS) infections showed 535,000 reported cases and 77,500 documented fatalities. In Southeast Asia alone, an estimated 22.8 million cases of Salmonellosis and over 37,000 deaths occur annually, (Majowicz et al., 2010; Van et al., 2012).

Salmonellae are facultative intracellular pathogens, transmitted through food or water contaminated with these microbes. They invade the host cells and survive intracellularly thereby increasing their pathogenic potential by evading host-mediated defense mechanisms. The pathogenic potential of *Salmonellae* is increasing rapidly, posing a growing threat. The commonly employed antimicrobial drugs for the treatment of salmonellosis, have now become ineffective due to the emergence of drug resistance worldwide (Su et al., 2004). The spread of drug-resistance genes amongst different species has further aggravated the problem by introducing multidrug-resistant strains. (Chiu et al., 2002, 2004)

Preventing the spread of NTS-related diseases is crucial, and therefore, epidemiological studies play a key role in achieving this goal. Various typing techniques are employed to investigate NTS, enabling the identification of relationships among isolates from different patients or regions. This helps to uncover common transmission sources of the pathogen, aiding in disease control efforts (Kang and Dunne, 2003).

Numerous phenotypic and genotypic methods are available for typing NTS, including biotyping, serotyping, phage typing, and antimicrobial susceptibility testing. Although these methods may not always provide comprehensive epidemiological information, yet they establish the phenotypic characteristics of the isolates and help in understanding antimicrobial resistance patterns, thus aiding in disease prevention

strategies (Mohanty et al., 2006). Additionally, molecular techniques such as PFGE, conjugation, RFLP analysis, RAPD analysis, and Microarray have been introduced to further elucidate the epidemiological patterns of NTS infections (Aktas et al., 2007; Sirichote et al., 2010; Fernandes et al., 2003; Ogunremi et al., 2014).

Invasive NTS is frequently implicated in cases of diarrhoea in Asia; however, comprehensive data on NTS infections from the region are scarce, with limited reports originating from India (Menezes et al., 2010) in addition to other Asian countries like Taiwan (Chen et al., 2012), Thailand (Kiratisin, 2008; Hendriksen et al., 2012) and Vietnam (Nga et al., 2012). In India, NTS infections have become a serious public health problem, following a global trend and becoming more prevalent (Sudhanthirakodi, 2016). The epidemiology of NTS in India remains relatively under-documented due to the absence of effective surveillance systems and the lack of suitable diagnostic tools (Jain et al., 2020). The burden of NTS in Asia is increasing. However, there is currently a lack of data regarding clinical symptoms, patient demographics, disease outcomes, or the specific infecting serovars associated with NTS infections in India. To develop effective surveillance and rational control strategies for combating the disease, it is essential to generate detailed and accurate epidemiological data on NTS periodically. Therefore, in the present study, we have planned to investigate the prevalence and different characteristics of non-typhoidal *Salmonellae* from the isolates obtained from different parts of the country.

CHAPTER – 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Bacteriology & Cultural Characteristics

Lignieres coined the generic name "*Salmonella* " (Le Minor, 1981) after the name of pathologist Dr. D.E. Salmon who isolated it for the first time from pigs in 1885 (Salmon and Smith, 1885). The genus "*Salmonella* " is described as an intracellular pathogen, which exhibits a great diversity with more than 2600 serotypes or serovars (Issenhuth-Jeanjean et al., 2014; Marzel et al., 2016).

The genus *Salmonella* is defined as Gram-negative bacilli 2-4µm long and 0.6 µm in diameter. They are often motile using peritrichous flagella except for *S.* Gallinarum Pullorum. The majority of *Salmonella* strains possess common fimbriae, with many fimbriated strains exhibiting Type-I fimbriae that are linked to mannose-sensitive adhesive properties except *S.* Paratyphi A and *S.* Gallinarum Pullorum (Duguid et al., 1966, 1975; Duguid and Old, 1980, 1994). Some of them are capsulated. These are easily cultivable on ordinary laboratory media over a wide temperature range from 7 – 48^0C, at pH 4-8, and at water activities above 0.93 (Baird & Parker, 1990). Even the species surviving & proliferating below 4^0C and pH less than 4.0 have been reported (d'Aoust, 1991; Foster, 1992). They are aerobic and/or facultative anaerobes. The colony size on blood agar and nutrient agar ranges in diameter from 2-4 mm, with a circular, low convex, and smooth surface. On MacConkey's agar (a differential and selective media), the colonies are colourless due to the absence of lactose fermentation. Several selective media have been proposed for the isolation purpose namely bile salt agar: deoxycholate citrate agar (DCA) initially suggested by Leifson (1935); Hektoen Enteric agar (King and Metzger, 1968); *Salmonella & Shigella* (SS) agar (Pollock and Dahlgren, 1974), and XLD agar (Taylor, 1965), the brilliant green agars with modifications (Edel and Kampelmacher, 1968: Van Schothorst et al., 1987) and bismuth sulfite agar (Leininger, 1976).

All species ferment glucose, resulting in the production of acid with or without gas. Hydrogen sulfide (H_2S) is produced by most *Salmonella*, but serovars Paratyphi A and Choleraesuis are the exceptions. Citrate is generally utilized except for serovars Typhi and Paratyphi A. They are found typically in the intestine of humans and animals,

though, some species may occur in soil. Many species are pathogenic. The G+C content of DNA is in the range of 48-50 mol% (Holmes, 1998).

2.2. Classification

The classification system used for *Salmonella* has undergone substantial revisions over time and remains subject to ongoing updates and refinements. Before 1983, multiple *Salmonella* species were recognized. However, subsequent experimental evidence, demonstrating a high degree of DNA similarity among *Salmonella* species, led to the classification of all *Salmonella* isolates into a single species namely *S.* Choleraesuis (Crosa et al., 1973; Farmer, 1995). Based on host range and DNA similarity, *S.* Choleraesuis was further divided into seven subgroups. Subgroup-I encompassed all serotypes pathogenic for humans (Tauxe, 1998).

This classification transformed in 1999 when **Euzéby** proposed designating "*Salmonella enterica* " as a new type species of *Salmonella*, replacing *S.* Choleraesuis with *S. enterica* (Euzéby, 1999). Presently, the genus *Salmonella* is recognized as comprising two species: *S. enterica* and *S. bongori* having six and one subspecies respectively. These seven subspecies can now be serotyped into over 2,600 distinct serotypes (Issenhuth-Jeanjean et al., 2014; Marzel et al., 2016). This classification system has been widely accepted and is employed by institutions such as the World Health Organization (WHO) and in publications endorsed by the American Society for Microbiology (Popoff et al., 2004). According to this system, the genus *Salmonella* is classified into two species based on 16S rRNA sequence analysis: *Salmonella enterica* (the type species) and *Salmonella bongori*. Further differentiation within the type species, *S. enterica*, is based on genomic relatedness and biochemical properties, resulting in six subspecies as presented in Table 2.1 (Reeves et al., 1989; Hurley et al., 2014). These subspecies are further differentiated into ‘serovars’ based on the somatic ‘O’ and flagellar ‘H’ antigens (Hurley et al., 2014).

Table 2.1: Classification of *Salmonella* (Hurley et al., 2014).

Genus:	*Salmonella*		
Species:	*S. enterica*		*S. bongori* Subsp. V
	Subspecies:	**Serovar:**	
	I. *enterica*	• Typhoidal : e.g. *S.* Typhi, *S.* Paratyphi. • Non-Typhoidal : e.g. *S.* Typhimurium, *S.* Enteritidis.	
	II. *salamae*		
	IIIa. *arozonae*		
	IIIb. *diarizonae*		
	IV. *houtenae*		
	VI. *indica*		

2.3. Pathogenesis:

The majority of *Salmonella* strains are virulent and possess the ability to cause severe diseases by invading and replicating within host cells. In humans, various serotypes of *Salmonella* can result in a range of illness severity, with factors such as age and the individual's health status playing a significant role. Young children (under 5 years), the elderly individuals, and those undergoing immunosuppressive medications tend to experience more severe *Salmonella* infections compared to healthy adults (Eng et al., 2015). *Salmonella* has developed sophisticated mechanisms that enable it to initiate its phagocytosis, facilitating its entry into host cells (Hansen-Wester et al., 2002). This remarkable capability is attributed to the presence of *Salmonella* pathogenicity islands (SPIs) and clusters of genes located on large chromosomal DNA (Grassl and Finlay, 2008). Upon ingestion of the food or water contaminated by these bacteria, these pathogens adhere to the intestinal wall's lining and enter epithelial cells via SPIs. These SPIs encompass a Type-III secretion system, functioning as a sensory probe to detect their presence in the host cell. Additionally, this system facilitates the secretion of effector proteins directly into the cytoplasm of the host cell. These effector proteins play a crucial role in helping the bacteria infect the host cells, survive, and

replicate intracellularly, thus evading host-mediated immune responses (Takaya et al., 2003). This ability for intracellular persistence is vital for the pathogenicity of *Salmonella* (Bakowski et al., 2008). Generally, after bacteria are phagocytosed, the host's immune system is activated, resulting in the fusion of lysosomes with phagosomes. Digestive enzymes in the lysosomes then degrade the bacteria. However, in the case of *Salmonella*, the effector proteins encoded by the Type-III secretion system cause structural alterations in the phagosome, preventing the fusion of lysosomes with it. Consequently, the bacteria can evade degradation by lytic enzymes (Eng et al., 2015). This ability of intracellular survival and multiplication of the bacteria in macrophages leads to their dissemination to the reticuloendothelial (RE) system (Monack et al., 2004).

2.4. Clinical Manifestations

Salmonella species are extensively distributed in the environment and are commonly present in farm effluents, human sewage, and any materials that have been exposed to fecal contamination (Antony, 2023).

In humans, *Salmonella* infections can result in various clinical presentations, including: enteric fever, gastroenteritis, bacteremia, and other extra-intestinal complications, as well as a chronic carrier state (Darby and Sheorey, 2008; Eng et al., 2015). The specific clinical manifestations depend on host factors and the serotype of the *Salmonella* strain involved.

The term ‘enteric fever’ encompasses both Typhoid and Paratyphoid fevers with *S.* Typhi being the causative agent responsible for Typhoid fever while the latter is caused by *S.* Paratyphi A, B, and C (Issenhuth-Jeanjean, 2014; Chen et al., 2023).

Human infection commonly arises from the ingestion of food or water contaminated with waste from infected individuals. Following an incubation period that can extend beyond seven days, symptoms typically begin with abdominal pain, headache, and diarrhoea, which then progress to fever (Bhan et al., 2005). Age plays a significant role in the clinical presentation, as children are more likely to experience diarrhoea, while constipation is more frequently observed in older adults or

immunocompromised individuals (Thielman and Guerrant, 2004). Those at the extremes of age or with pre-existing health conditions face a higher risk of bacteremia compared to those with enteric salmonellosis alone (Ballal et al., 2016).

Enteric fever has a characteristic fever progression, beginning with a mild fever (37.5–38.2°C) that, if untreated, can escalate to high-grade levels (38.2–41.5°C) (Patel et al., 2010). Patients may present with muscle pain, bradycardia, hepatosplenomegaly (enlargement of the liver or spleen), and, occasionally, rose-coloured spots on the chest or abdomen, in addition to fever (Kuvandik et al., 2009). Recurrent infections can lead to complications such as pancreatitis, hepatitis, and cholecystitis, with more severe infections in Peyer's patches or lymph nodes in the terminal ileum causing tissue damage, hemorrhage, and potentially life-threatening perforations (Eng et al., 2015).

Non-typhoidal *Salmonella* (NTS) species are predominantly linked to foodborne gastroenteritis, typically contracted through animal contact or consumption of contaminated foods, including meat, seafood, poultry, and leafy vegetables (Antony, 2023). Factors that increase susceptibility to NTS infections include immunocompromised conditions, inadequate sanitation, and poor food-handling practices (Devleesschauwer et al., 2018).

Gastroenteritis from NTS involves inflammation of the gastrointestinal tract and presents with diarrhoea, vomiting, nausea, and abdominal cramps, unlike enteric fever, which often affects the liver and spleen (Wang et al., 2023). While NTS infections are generally self-limiting within 6 to 12 hours of symptom onset and resolve within 10 days (Crump et al., 2008), mild complications such as cholecystitis, pancreatitis, and appendicitis may occasionally occur (Acheson and Hohmann, 2001). More severe outcomes, however, are noted in infants, young children, the elderly, and those with weakened immune systems (Scallan et al., 2011).

Beyond these clinical presentations, nearly all non-typhoidal *Salmonella* infections pose a risk for bacteremia and other extra-intestinal complications. The strains *S.* Dublin and *S.* Choleraesuis are especially invasive, commonly associated with bacteremia and high-grade fevers similar to enteric fever (Woods et al., 2008; Jones et

al., 2008). In severe cases, bacteremia may progress to septic shock, significantly increasing the risk of mortality.

As mentioned above, the NTS infections are more serious in terms of the bacteremia they cause compared to Typhoidal *Salmonella* infections. The bacteremia has been reported in 5% of NTS-infected patients. Extra-intestinal complications have also been reported in some patients with NTS infections e.g. cellulitis, urinary tract infection (UTI), pneumonia, meningitis and endocarditis (Shimoni et al., 1999; Arii et al., 2002).

NTS infections represent a significant global health concern, especially for children (Magwedere et al., 2015). NTS typically manifests as gastroenteritis in children (Chen et al., 2023). A study conducted in the United States, focusing on children under the age of 5, found that NTS was responsible for the highest proportion (42%) of isolated bacterial enteric pathogens (Scallan et al., 2013). Furthermore, septicemia associated with NTS gastroenteritis has been reported in young children under 5 years, often presenting with a high fever as the predominant clinical symptom (Shuai and Chaomin, 2023). In Taiwan, NTS has emerged as the predominant pathogen associated with childhood bacterial enterocolitis that requires hospitalization (Su and Chiu, 2007).

While NTS infections are commonly presented with symptoms like gastroenteritis, enteric fever, and bacteremia, a recent study has reported a rare occurrence of bone and joint infections, specifically multifocal osteomyelitis attributed to *S.* Dublin in an immunocompromised patient. This particular case represents a rare subset, accounting for only 0.8% of all *Salmonella* infections and a mere 0.45% of all osteomyelitis cases (Jiang et al., 2023). Furthermore, there is growing evidence suggesting a potential association between NTS and gall bladder cancer (Iyer et al., 2016).

Nosocomial outbreaks of *Salmonella* infections have been documented in the past, although they are relatively infrequent. Various modes of nosocomial transmission have been reported, including cases attributed to the reactivation of latent *Salmonella*

infections (Buchwald and Blaser, 1984; Khuri-Bulos et al., 1994; Delaloye et al., 2004). In a recent study, an unusual case was reported where *Salmonella* bacteremia developed in a patient after intubation resulting into an extended hospital stay (Oxman et al., 2023).

Salmonella bacteremia has been documented to occur without the typical accompanying diarrheal illness. Invasive diseases can manifest in various forms, including osteomyelitis, abscesses, meningitis, endocarditis, septic arthritis, pericarditis, respiratory symptoms, abdominal infections, endovascular infections, and more (Gong et al., 2022). Hence, infections caused by NTS remain a formidable challenge for healthcare systems across the globe, presenting an immediate and critical global public health issue.

2.5. Virulence Factors in non-Typhoidal *Salmonella* :

Virulence factors are the molecular mechanisms that enable pathogenic bacteria to colonize host tissues and cause disease. NTS is a gram-negative bacterium that possesses several virulence factors which help it to establish and maintain an infection in the host. Some of the key virulence factors associated with NTS are:

2.5.1 Lipopolysaccharides (LPS):

It is a constituent of the outer membrane of the bacterial cell wall. About 70% of the outer membrane surface of Gram-negative bacteria is comprised of LPS, playing a pivotal role in safeguarding the bacterial cell against host defense mechanisms and antibiotics. This feature establishes LPS as one of the foremost virulence factors in Gram-negative bacteria (Lodowska et al., 2007). LPS consists of Lipid A, core oligosaccharide, and O-antigen polysaccharide in many bacteria (Raetz and Whitfield, 2002). Lipid A, the most conservative part, is covalently linked to the core oligosaccharide, providing an anchor to the outer membrane and imparting biological activity to LPS. The O-antigen, an immune-dominant component of LPS, forms the basis for serotyping in *Salmonella* species (Thomsen et al., 2003). The O-antigen exhibits variability in composition and the number of polysaccharide subunits (Krzyżewska-Dudek et al., 2022). Notably, in *Salmonella*, O-antigen capsules play a

crucial role in adhesion processes and the persistence of bacteria in the environment (Jacques, M., 1996; Barak et al., 2007; Crawford et al., 2008). LPS serves as a potent activator of the host's immune response and can induce systemic inflammatory response syndrome (SIRS) and sepsis, with potentially fatal outcomes in severe cases. Additionally, LPS plays a significant role in the initial step of biofilm formation, namely, the adhesion of microorganisms to a surface (Williams and Fletcher, 1996).

2.5.2 **Fimbriae**:

Amongst various virulence factors, fimbriae play a major role in the pathogenesis of *Salmonella* sp. and also act as a source of diversity amongst *Salmonella* serovars (Dufresne and Daigle, 2017). Fimbriae are thin protein filaments that can help the bacteria to bind to host cells, and this adherence can enhance invasion and colonization of host cells (Townsend et al., 2001; Humphries et al., 2003; Rehman et al., 2019). In addition, they participate in various other functions, such as interacting with macrophages, forming biofilms, ensuring intestinal persistence, and promoting bacterial aggregation (Edwards et al., 2000; Boddicker et al., 2002; Althouse et al., 2003; Harris et al., 2006; Sabbagh et al., 2010; Sabbagh et al., 2012). The expression of fimbriae in *Salmonella* was first described by Duguid and Gillies (1958), by demonstrating agglutination of RBCs of some species by *Salmonella* fimbriae. A great variety of fimbriae have now been identified and the modern system of classification has broadly classified them based on their assembly pathways as CU, N/P, and T4 fimbriae (Nuccio and Baumler, 2007; Fronzes et al., 2008). The CU assembly is distinguished by interactions among its subunits—a periplasmic chaperone and an outer membrane usher—facilitating the biogenesis of mature fimbriae. Extracellularly, a nucleator initiates the formation of fibre aggregates through the N/P pathway. T4 fimbriae require ATP for their assembly and employ sophisticated machinery for fimbrial formation. Additionally, T4 fimbriae possess the unique capability to retract and reverse the fimbrial assembly process (Fronzes et al., 2008). Fimbriae play a crucial role in virulence by facilitating bacterial interaction with host cells (Low et al., 1996; Edwards and Puente, 1998). The distribution of fimbrial operons among enteric bacteria suggests a role for fimbriae in pathogenesis especially. Broadly distributed fimbrial operons may provide general adhesive functions, whereas those with limited

distribution may offer specific functions required for virulence. For instance, the common type I fimbriae found in many Gram-negative bacteria mediates adherence to the pharynx, intestinal epithelium, and bladder. In contrast, plasmid-encoded fimbriae specific to *Salmonella* bind specifically to M cells in the intestine (Low et al., 1996; Baumler et al., 1997). Most fimbriae, except for type IV pili, share a conserved mechanism for translocation to the bacterial surface. Upon entering the intestinal milieu, *Salmonella* fimbriae adeptly navigate the mucous layer, facilitating their invasion of the host epithelium (Wang et al., 2018). These fimbriae play a pivotal role in engaging non-phagocytic enterocytes within the intestinal epithelium, initiating a robust adhesion process (Dufresne and Saulnier-Bellemare, 2018). Notably, *Salmonella* fimbriae exhibit a predilection for microfold (M) cells—specialized intestinal epithelial cells characterized by diminished mucous coverage, glycocalyx, and shorter microvilli (Tahoun et al., 2012). This preferential adherence, particularly to M-cells, likely facilitates the ingress of *Salmonella* into intestinal epithelial cells, navigating through the basement of these cells. Subsequently, these epithelial cells shed *Salmonella* into the intestinal lumen, providing an environment conducive to bacterial replication—an indispensable step for their continued survival. This finely orchestrated mechanism appears to be instrumental in the effective intestinal colonization of *Salmonella*, ultimately culminating in the establishment of an acute infection (Azriel et al., 2017; Gast et al., 2017).

2.5.3 Flagella:

Flagella are whip-like appendages that protrude from the surface of many bacteria, serving as the primary organelles of locomotion. This movement allows bacteria to navigate their environment, responding to stimuli like temperature gradients, nutrient availability, or chemical signals. *Salmonella*, being a motile bacterium, utilizes its flagella to navigate toward favorable environments, such as the intestinal tract (Wadhwa and Berg, 2022). Beyond locomotion, flagella plays a crucial role in the adhesion and invasion of host cells, contributing significantly to *Salmonella* infection (Jahan et al., 2022). In addition to their role in locomotion, flagella are essential for several other functions. They are required for bacterial movement to surfaces to facilitate attachment and play a crucial role in propelling organisms as they search for

other bacteria (Pratt and Kolter, 1998; Crawford et al., 2010). This multifunctionality underscores the significance of flagella in various aspects of bacterial behaviour and survival.

2.5.4 **Colicins**:

The antibacterial proteins or peptides produced by bacteria are broadly termed bacteriocins. In the case of the members of the family *Enterobacteriaceae,* these are specifically termed ‘colicins’ (Marković et al., 2022). The initial evidence of colicin production dates back to 1925 when it was discovered in *E. coli* (Cascales et al., 2007). Some *Salmonella enterica* serovars especially *S.* Typhimurium is known to produce colicin IB (ColIb) (Nedialkova et al., 2014; Gollan at al., 2024). Some researchers have cloned and expressed colicin-like proteins from *Salmonella* called ‘salmocins’ in plants and found them to be more potent than colicins (Schneider et al., 2018). Colicins are large molecular weight proteins (Cascales et al., 2007) that are often produced under stress conditions (Gordon and O’Brien, 2006). A diverse range of colicins and microcins employ various cytotoxic mechanisms as their mode of action. These mechanisms include pore formation, degradation of peptidoglycan precursors, phosphatase activity, RNase activity (often targeting 16S rRNA and specific tRNAs), and DNase activity (Cascales et al., 2007). Colicin is typically not synthesized under normal physiological conditions; however, a limited number of colicin molecules are consistently present within the cell. The production of colicin undergoes a substantial increase in response to various DNA-damaging agents, such as UV light and the antibiotic mitomycin-C, as well as environmental factors like nutrient deprivation or elevated bacterial population density, triggering an "SOS response" (Herschman and Helinski, 1967). Alternatively, activation of colicin synthesis may also occur through thymine starvation, catabolite repression, and mutation of the ompR gene (Pugsley et al., 1983; Ebina and Nakazawa, 1983). This results in colicin expression and accumulation within the cell cytoplasm, but its release in the extracellular environment requires colicin lysis proteins. These are the small lipoproteins and are co-expressed with colicin proteins and facilitate the colicin release by lysing the host cell envelope and thus causing its death (Cascales et al., 2007; Dekker et al., 1999). Colicins may also possess enzymatic activity such as hydrolases or transferases, targeting phosphodiester

bonds in the DNA (DNase) or RNA (rRNase or tRNase) of the host cell. **DNase colicins** degrade DNA by creating dents in dsDNA through repeated cleavage (Cascales et al., 2007). These colicins are metal-dependent, and while the nature of the required metal ions remains unknown, research suggests that Zn^{2+} and Ni^{2+} serve as necessary cofactors for enzyme activity (James et al., 2002; Ku et al., 2002). **RNase colicins** induce cell death by inhibiting protein synthesis, and interestingly, no cofactors are needed for this activity. The target molecules of RNase colicins include 16S rRNA and anticodon loops of tRNA (Cascales et al., 2007). **Nuclease colicins** are produced and released from the cell in complex with their nuclease-specific immunity proteins, safeguarding the producing cell from lethal activity (Krone et al., 1986).

2.5.5 Secretion systems:

Bacteria have evolved various secretion systems to export proteins and other molecules across their cell membranes. These secretion systems play crucial roles in bacterial pathogenicity, survival, and interactions with their environment (Green and Mecsas, 2016). *Salmonella* possesses several secretion systems, each with specific functions. Here are some of the well-known secretion systems in *Salmonella*:

2.5.5.1 Type I Secretion System (T1SS):

Salmonella utilizes T1SS for the secretion of specific virulence factors. T1SS serves as a direct means of exporting specific proteins from the bacterial cytoplasm to the extracellular milieu. These proteins encompass adenylate cyclases, proteases, lipases, surface layer proteins and toxins (Lenders et al., 2015). In *Salmonella*, two non-fimbrial adhesins secreted by the Type I Secretion System (T1SS) have been identified: BapA and SiiE (adhesion proteins) (Barlag and Hensel, 2015; Wagner et al., 2011). Kirchweger et al. (2019) recently illustrated that the periplasmic domain of SiiA exhibits direct binding to peptidoglycan in-vitro, with this interaction being contingent upon pH levels. The mutation in Arg162 was identified as potentially affecting both the peptidoglycan binding affinity and the invasion of polarized epithelial cells (Kirchweger et al., 2019). Another investigation highlighted the indispensable role of the BapA adhesin in *S.* Enteritidis, showing its requirement for the formation of biofilms and invasion within the murine host (Latasa et al., 2005).

2.5.5.2 Type III Secretion System (T3SS):

The Type III Secretion System (T3SS) is a needle-like apparatus that enables bacteria to directly inject effector proteins into host cells (Notti and Stebbins, 2016). *Salmonella*'s T3SS is a crucial virulence factor that enables the bacterium to invade and manipulate host cells during infection. *Salmonella enterica* is equipped with two gene clusters for Type III Secretion Systems (T3SS), known as T3SS-1 and T3SS-2, situated on *Salmonella* Pathogenicity Island 1 (SPI-1) and SPI-2, respectively. The T3SS-1 cluster plays a pivotal role in the initial phase of invasion, targeting intestinal epithelial cells and microfold (M) cells within the gut lumen. Its activation triggers proinflammatory responses, contributing to the early stages of infection (Bao et al., 2020). In contrast, T3SS-2 is linked to the later phases of infection, encompassing intracellular survival and replication within host phagocyte (Anderson and Kendall, 2017; Hensel et al., 1998). Following internalization into the host cell, *Salmonella* can establish *Salmonella* - Containing Vacuoles (SCVs). These vacuoles serve as protective compartments, shielding the bacterium from degradation and providing an environment conducive to its intracellular survival and growth (Bao et al., 2020).

2.5.5.3 Type IV Secretion System (T4SS):

T4SS is involved in the transfer of DNA between bacteria (conjugation) and the delivery of effector proteins to host cells. T4SSs can be categorized into three functional types. The first type involves conjugation systems, facilitating the transfer of DNA from one bacterial cell to recipient cells in a contact-dependent manner. The second type is responsible for mediating the uptake or release of DNA into or from the extracellular milieu. The third type of T4SSs, primarily found in pathogenic bacteria, is dedicated to transferring proteins and other molecules into eukaryotic targets (Backert and Meyer, 2006; Alvarez-Martinez and Christie, 2009; Christie, 2016). While T4SS is often associated with plasmid transfer, some pathogenic strains of *Salmonella* may employ T4SS to enhance virulence. Presently, only a limited number of *Salmonella enterica* serotypes have been documented to harbor a Type IV Secretion System (T4SS) cluster. Notable examples include *S. enterica* serotype Enteritidis (Chen et al., 2009) phage type 34, certain isolates of *S. enterica* serotype Montevideo (Delgado-Suárez et al., 2018), *S. enterica* serovar Typhimurium strain ST1660/06 (Li et al., 2012), and a

majority of *S. enterica* serovar Heidelberg (Deblais et al., 2018). These T4SS clusters are located on various plasmids.

2.5.5.4 Type VI Secretion System (T6SS):

T6SS is a complex secretion system involved in the delivery of effector proteins into both prokaryotic and eukaryotic cells (Bao et al., 2020). It plays a role in bacterial competition and interactions with other microorganisms in the environment. The Type VI Secretion System (T6SS) is a contact-dependent nanomachine of Gram-negative bacteria (Boyer et al., 2009). The T6SS apparatus is a needle-like structure spanning the cell envelope, resembling the tail of inverted T4 bacteriophage (Cascales and Cambillau, 2012). Initially identified in *Vibrio cholerae* in 2006 (Pukatzki et al., 2006; Ho et al., 2014), this system has the capability to transport toxins directly into both prokaryotic and eukaryotic cells (Basler et al., 2013). Presently, three primary types of T6SSs have been characterized (Type I, II, & III). Type I T6SS is ubiquitous across most bacteria and is further categorized into five subtypes. Type II T6SS is exclusive to *Francisella* species, while Type III T6SS is encoded solely in *Bacteroidales* (Russell et al., 2014). *Salmonella* encompasses five phylogenetically distinct Type I T6SSs encoded on different *Salmonella* Pathogenicity Islands (SPIs), including SPI-6, SPI-19, SPI-20, SPI-21, and SPI-22. (Blondel et al., 2009; Fookes et al., 2011). T6SSs are distributed in various *Salmonella enterica* serotypes and are identified as important virulence-related factors in breaching the microbiota barrier for efficient colonization. SPI-6 has been implicated in the pathogenesis of non-Typhoidal *Salmonella* serovars (Haneda et al., 2009), while the virulence of *S.* Typhimurium in mouse models is associated with T6SS (Liu et al., 2013). Additionally, *Salmonella enterica* serovars Typhi and Dublin, exhibit T6SS-related virulence (Wang et al., 2011; Pezoa et al., 2014). In *Salmonella enterica* serotype Gallinarum, SPI-19 and the associated T6SS cluster contribute to intestinal and organ colonization in chickens (Blondel et al., 2010). The precise control of T6SS gene clusters by *Salmonella* allows adaptation to different environments (Brunet et al., 2015; Wang et al., 2019). *Salmonella* strains possessing T6SSs encoded on SPI-19 are primarily isolated from warm-blooded hosts, indicating a possible role of SPI-19-derived T6SS in host adaptation (Bao et al., 2019).

These secretion systems collectively contribute to *Salmonella*'s ability to establish infection, evade the host immune response, and survive within host cells. Understanding the specific mechanisms and proteins involved in these secretion systems is crucial for unravelling the pathogenic strategies of *Salmonella* and developing targeted interventions for prevention and treatment.

2.5.6 **Biofilm**:

Biofilms represent intricate communities of microorganisms, predominantly bacteria, that adhere to surfaces and are enveloped in a viscous matrix comprising extracellular polymeric substances (EPS). These substances include polysaccharides, proteins, nucleic acids, and other molecules synthesized by the microorganisms dwelling within the biofilm (Harrell et al., 2021). The intricate cell network formed by biofilms represents a predominant mode of bacterial growth in various environmental settings. The formation of biofilms occurs as microorganisms affix themselves to a surface, generating a defensive and adhesive matrix (Harrell et al., 2021). Within a biofilm matrix or microenvironment, bacteria benefit from a multifaceted protective shield, conferring resilience against environmental stressors such as UV exposure (Espeland and Wetzel, 2001), action of disinfectants and immune responses from hosts (Jensen et al., 2010), and the actions of antibiotics (Mah and O'Toole, 2001; Stewart and Costerton, 2001; Mah, 2012) and therefore are difficult to eradicate (Burmolle et al., 2010). This biofilm is often implicated in the development of numerous infections and outbreaks. Biofilm contributes to the resistance and persistence of bacteria on diverse biotic and abiotic surfaces (Steenackers et al., 2012). Robust evidence from different studies underscores the predominant role of biofilms in approximately 80% of bacterial infections (Davies, 2003; Hall-Stoodley and Stoodley, 2009). Given their tenacity on abiotic surfaces like medical devices (prosthetics, stents, catheters, implants, dentures, etc.), biofilms are linked to approximately 50% of nosocomial infections (Paredes et al., 2014; Percival et al., 2015). Additionally, the presence of biofilm on food and food processing equipment (Flemming, 2016; Galie et al., 2018), contamination of water pipelines (Wingender and Flemming, 2011), corrosion of underwater metal surfaces (de Carvalho, 2018), can cause adverse effects. This capability underscores the paramount significance of biofilms across varied domains,

encompassing environmental contexts, industrial settings, and medical applications. *Salmonella*, Typhoidal and Non-Typhoidal, both exhibit the capability to colonize human and other animal hosts and have the potential to induce disease (Harrell et al., 2021). *Salmonella* biofilm can occur on any biotic or abiotic surface (Steenackers et al., 2012). The biofilm, which is known to be highly conserved, provides *Salmonella* an additional advantage during the initiation of infection as well as their transmission cycle (Römling et al., 2003; MacKenzie et al., 2017; MacKenzie et al., 2019). Like other biofilm-forming bacteria, biofilm-associated *Salmonella* is enveloped within a self-secreted matrix of extracellular polymeric substances (EPSs) comprising proteins, carbohydrates, and extracellular DNA (eDNA) (Maruzani et al., 2019). These EPSs, collectively enable cells to securely adhere to surfaces and other cells within the biofilm, retain moisture in dry environments, and potentially impede the diffusion of detrimental molecules, such as antimicrobial peptides and antibiotics (Dieltjens et al., 2020). *Salmonella* biofilm has been documented on various abiotic surfaces such as plastics, glass, stainless steel, cement, rubber, etc. (Obe et al., 2022; Thames et al., 2023; Ormsby et al., 2024) which are commonly used in households, industry, agriculture. Evidence over the years has made it clear that *Salmonella* can colonize various parts of a diverse range of plant species. This colonization extends from seeds (Mahon et al., 1997) to sprouts (O'Mahony et al., 1990), leaves (Campbell et al., 2001; Lapidot et al., 2006), roots (Klerks et al., 2007), and even fruits (Guo et al., 2001). This highlights the significance of plants as important vectors for the transmission of *Salmonella* between hosts. Several researchers have conducted studies demonstrating the isolation of biofilms from diverse environments and hosts. For instance, Stepanovic et al. (2004) investigated the biofilm formation by *Salmonella* isolated from humans, animals, and food on plastic (polystyrene) microplates. Giaouris and Nychas (2006) focused on demonstrating biofilm formation on stainless steel surfaces. Vestby et al. (2009) provided insights into the biofilm formation by *Salmonella* isolated from animal feed within factory environments. Additionally, Barker and Bloomfield (2000) explored the association between *Salmonella* infection in a family and its survival and persistence in their toilet and bathroom, despite the regular use of disinfectants. *Salmonella* demonstrates the capacity to adhere to and form biofilms on epithelial cells, playing a crucial role in the initiation and perpetuation of mucosal infections in

susceptible hosts which may result in intestinal carriage (Althouse et al., 2003; Morgan et al., 2004). In a study by Boddicker et al. (2002), an in-vitro model utilizing Hep-2 cells was employed to replicate infections across different hosts, elucidating the genetic elements critical for *S.* Typhimurium biofilm formation on epithelial cells. Subsequently, these same determinants were demonstrated to be essential in an in-vivo model involving poultry (Ledeboer & Jones, 2005; Ledeboer et al., 2006).

The significance of biofilms in pathogenicity is notable, as they play a pivotal role in pathogen establishment, persistence within host environments, and resilience against various host defences and antimicrobial therapies. Here are some key aspects of the role of biofilms in pathogenicity:

- Biofilms enable pathogens to adhere to host tissues and surfaces more effectively than free-floating (planktonic) cells. This initial adhesion is a critical step in the establishment of infections (Sauer et al., 2022).
- Biofilm formation is frequently linked to chronic infections, as biofilm-associated bacteria can resist clearance by the immune system and survive prolonged antibiotic treatments. Biofilms create a protective niche for pathogens, enhancing their resistance against host immune responses, antibiotics, and other antimicrobial agents. The matrix of extracellular polymeric substances (EPS) in biofilms acts as a physical barrier that limits the penetration of immune cells and drugs (Harrell et al., 2021).
- Biofilms contribute to the persistence of infections by allowing pathogens to evade the host's immune system. Cells within a biofilm can adopt dormant or slow-growing states, making them less susceptible to the host's defense mechanisms and antimicrobial treatments (Jensen et al., 2010).
- Microorganisms within biofilms can communicate through a process known as quorum sensing, facilitating coordinated gene expression. This communication enhances pathogens' virulence and facilitates biofilms’ formation and maintenance (Zhang et al., 2022).
- Biofilms are commonly linked to infections associated with medical devices such as catheters, ventilators and prosthetic implants etc. Pathogens can form biofilms on these surfaces, leading to persistent infections that are challenging to treat (Percival et al., 2015).

- Bacteria within biofilms might display heightened virulence in contrast to their free-floating counterparts. This increased virulence can be attributed to gene expression changes and the biofilm's overall microenvironment (Franco et al., 2022).
- Another critical pathogenic role of biofilms is evident in the development of drug resistance among bacterial species capable of producing biofilms. The resistance of *Salmonella* biofilms against antibiotics poses a significant challenge in combating infections (Steenackers et al., 2012). The association between biofilm production and resistance to antimicrobial drugs has been consistently demonstrated in numerous studies conducted over an extended period (Olson et al., 2002; Linares et al., 2006; Parry & Threlfall, 2008; Papavasileiou et al., 2010; Turki et al., 2012; Dai et al., 2021; Lenchenko et al., 2021; Chen et al., 2022; Aleksandrowicz et al., 2023).

It's worth noting that, understanding these virulence factors is crucial for developing effective therapies and vaccines against *Salmonella* infections. Studying these virulence factors can offer valuable insights into the mechanisms driving bacterial pathogenesis and provide insights into the development of new interventions and treatments for NTS infections.

2.6. Mode of Transmission

Salmonella is transmitted through the faeco-oral route, with the main vectors being contaminated food, water, animal contact, and less frequently, human-to-human contact (Li et al., 2012). An estimated 94% of salmonellosis cases result from the consumption of contaminated food (Ehuwa et al., 2021). Given its prevalence in various food production environments, *Salmonella* can infiltrate the food supply at any stage, spanning from the initial stages of farming to the final stages of food consumption.

The diverse reservoir of NTS infection poses a challenge for public health to effectively control the spread to the human population. (Swanson et al., 2007; Dione et al., 2011). Salmonellosis results from a combination of factors, including those connected to food, the environment, vectors, humans, machinery, animal transportation, and animal reservoirs (Koutsoumanis et al., 2019). *Salmonella* has been

identified in humans, animals, and environmental samples, serving as significant pathogens associated with various environmental reservoirs and transmission pathways (Liu et al., 2018; Ehuwa, et al., 2021; McLure et al., 2022). Many biotic and abiotic variables contribute to the organism's prevalence in the environment (Harrell et al., 2021; Ramatla et al., 2022). It is crucial to understand the potential roles that natural environments may play in harbouring *Salmonella* for ultimate transmission to food, animals, and humans (Kurtz et al., 2017). Water and soil are two potential environmental *Salmonella* reservoirs that people may come into direct contact with or ingest (Greene et al., 2008; Li et al., 2021).

Animals are the major reservoirs of *Salmonella,* especially the NTS. The main mode of infection in humans involves consuming food or beverages contaminated with waste from infected animals, coming into contact with infected animals, or eating undercooked meat from such animals (Eng et al., 2015; Dudhane et al., 2023). Poultry and products derived from poultry, are prominently recognized as the main sources often linked to NTS infections in the human food chain (Bangera et al., 2019). India, as one of the leading global poultry producers (Mottet and Tempio, 2017), holds considerable importance in this regard. Chicken meat and eggs, among animal-based food sources, have a widespread acceptance, for a considerable portion of the impoverished population in India (Fourat et al., 2018). The modernization and globalization of the poultry industry have introduced novel and intricate pathways for the dissemination of *Salmonella,* exacerbating the potential for its transmission (Gast, 2003; Gast et al., 2013). The poultry industry faces significant challenges in eradicating, eliminating, and controlling foodborne and zoonotic diseases linked to poultry (Al-Ansari, 2021). Furthermore, pork and beef have been highlighted as primary contributors to salmonellosis (Ferrari et al., 2019; Soliani et al., 2023).

The use of polluted water, sourced from compromised water reservoirs, in irrigation poses a potential route for contaminating fruits and vegetables with *Salmonella* (Liu et al., 2018; Cho et al., 2020). Consumption of unpasteurized milk has been identified as a significant means of transmitting NTS to humans (Gebeyehu et al., 2022; Wang et al., 2023). *Salmonella* can persist on surfaces in contact with food for extended durations, increasing the risk of cross-contamination incidents involving food

handlers, food products, and surfaces within food establishments. (Agarwal et al., 2022). Such persistence heightens the risk of outbreaks associated with food prepared in restaurants.

In conclusion, the transmission of NTS is multifaceted. These factors collectively contribute to the heightened risk of cross-contamination events, underscoring the importance of stringent hygiene practices and preventive measures within various stages of food production, processing, and consumption. It is essential to tackle these pathways of transmission to effectively reduce the impact of outbreaks related to NTS and to guarantee the safety of the food chain.

2.7. Epidemiology

NTS infections constitute a significant global health concern, engendering morbidity and mortality across diverse socioeconomic settings (Gong et al., 2022). Worldwide, *Salmonella* has been recognized as one of the top four main contributors to diarrheal illnesses. In US, NTS stands out as the leading cause of hospitalizations and deaths, and ranks second in causing sickness among foodborne pathogens (Scallan et al., 2011). The impact of this disease burden is NTS considerable, emphasized by a concerning statistic indicating that annually, one in every ten people fall victim to this ailment, leading to the deprivation of more than 33 million healthy years of life (Havelaar et al., 2010; Lai et al., 2020).

During the period from 2001–2005, the prevailing serovars responsible for non-typhoidal *Salmonella* infections globally were *S.* Enteritidis (accounting for 65%), *S.* Typhimurium (12%), and *S.* Newport (4%) (Galanis et al., 2006; Fashae et al., 2010; Hendriksen et al., 2011; Van et al., 2012). Notably, *S.* Enteritidis emerged as the most frequent serotype in Asia (38%), Latin America (31%), and Europe (87%) (Galanis et al., 2006). Certain serovars have been observed to dominate specific regions, such as *S.* Weltevreden and *S.* Stanley in several Southeast Asian nations (Lee et al., 2009; Hendriksen et al., 2011; Van et al., 2012).

The Foodborne Diseases Active Surveillance Network (FoodNet) has documented a significant occurrence of NTS infection in the USA, with an annual

incidence rate exceeding 17% per 100,000 population. While this rate may appear comparatively lower than in underdeveloped nations, NTS remains a prominent cause of fatalities, contributing to approximately 40% of all foodborne pathogen-related deaths in the United States (Barton Behravesh et al., 2011). In 2010, an outbreak occurred across 16 states in the USA, wherein the CDC reported >1900 cases of NTS infection by ingesting eggs contaminated with *S.* Enteritidis (CDC, 2010). The food products commonly related to the outbreak include milk, poultry, eggs, etc. and the major contributory factors responsible for such outbreaks include eating undercooked food materials, improper storage of raw food and handling practices (Lynch et al., 2006). In China, NTS is responsible for a significant portion, specifically 70% to 80%, of reported food poisoning incidents (Scallan et al., 2011). In Southeast Asia, there is a notable prevalence of *Salmonella spp.* reported in both retailed chicken and pork products (Inthavong et al., 2006; Luu et al., 2006; Vo et al., 2006; Van et al., 2007; Lay et al., 2011).

The World Health Organization (WHO) estimates that around 93.8 million instances of Salmonellosis caused by NTS occur annually, leading to an estimated 155,000 deaths (Mohakud et al., 2022). Moreover, global figures for invasive non-Typhoidal *Salmonella* (iNTS) infections indicated 535,000 reported cases with 77,500 confirmed deaths. Stanaway et al. (2019) emphasized that sub-Saharan Africa experiences the highest prevalence of invasive NTS illness, which poses a significant burden in terms of both morbidity and mortality on a global scale.

Salmonella is known to manifest in various clinical presentations in humans, including enteric fever, bacteraemia, gastroenteritis, and other extra-intestinal complications, along with the potential for developing of a chronic carrier state (Darby and Sheorey, 2008; Eng et al., 2015). Gastroenteritis, being the most widespread form of *Salmonella* infection globally, accounting for over 90 million cases and nearly 150,000 deaths annually. In Southeast Asia alone, more than 20 million cases result in 37,600 deaths each year (Majowicz et al., 2010). Evidence has revealed a staggering 96.1 million instances of *Salmonella* enterocolitis, which has been responsible for 50,800 documented deaths globally (Stanaway et al 2019).

Despite improved efforts to maintain good health and sanitation, NTS infections continue to occur globally (Majowicz et al., 2010). Reports indicate that there are approximately 700 instances of NTS-related illnesses per 100,000 people in Europe and 100 cases per 100,000 people in Israel (Weinberger & Keller, 2005). In developing nations, especially in Sub-Saharan Africa, where mortality rate exceeding 20% has been observed among children under three years old and HIV-positive patients (Gordon et al. 2008), invasive NTS infections are more common compared to developed nations in Asia (Khan et al., 2010; Phu Huong et al., 2016).

2.8. NTS infections in India:

In India, NTS infections have emerged as a critical public health issue, mirroring a worldwide pattern and growing in prominence. Research carried out in Kolkata emphasizes a noteworthy occurrence of NTS in both poultry and meat samples, highlighting the potential hazard of foodborne illnesses in humans (Sudhanthirakodi, 2016).

In India, diarrhoea ranks as the 3rd leading cause of paediatric mortality, and contributing to 13% of deaths annually in children under the age of 5 (Lakshminarayanan and Jayalakshmy, 2015). Among the bacterial etiologies of diarrhoea, NTS stands out as a noteworthy zoonotic pathogen frequently encountered in children. NTS typically spreads through the ingestion of contaminated food or water or contact with animals or poultry (Scallan et al., 2013; Li et al., 2014; Wen et al., 2017). Predisposing factors such as compromised immunity, immature indigenous gut microbiota, and reduced gastric acidity in children may augment susceptibility to NTS infection (Harb et al., 2017).

In India, there's a lack of extensive documentation regarding the epidemiology of NTS gastroenteritis in children due to ineffective surveillance systems and inadequate diagnostic tools (Jain et al., 2020). In a recent investigation by Jain et al. (2020), efforts were made to comprehensively assess the prevalence of NTS among children under 5 years old who presented with acute gastroenteritis. The study sought to ascertain the frequency of NTS isolation, the prevalence of particular serovars, antimicrobial resistance (AMR) patterns, and molecular subtypes of NTS among this paediatric

cohort. The results revealed an NTS isolation rate of 1.0%, identifying 17 distinct *Salmonella* serovars among the participants. These findings offer valuable insights into the epidemiology and diversity of NTS infections among young children.

In summary, the global epidemiology of NTS reflects a widespread concern with diverse transmission routes. While global incidences continue to challenge public health systems, it's imperative to note the specific context in India, where the burden of NTS is notable. Comprehensive surveillance and interventions are crucial on a global scale, with tailored strategies required to address the specific challenges posed by NTS in the Indian context, ensuring a concerted effort to minimize the impact of outbreaks and enhance food safety worldwide.

2.9. Treatment of NTS infection:

Gastroenteritis caused by NTS typically resolves spontaneously in young adults globally (Eng et al., 2015). Antibiotic therapy is generally not recommended in most cases (Jacob et al., 2020) because it does not help in reducing the duration of illness but also can prolong the bacterial shedding i.e. carrier state (Onwuezobe et al., 2012; Wen et al., 2017; CDC, 2024). However, paediatric, geriatric, immunocompromised, or atherosclerosis patients, who are more prone to developing invasive disease, may require antibiotic treatment until the final cure (Galanakis et al., 2007; Tsai et al., 2011). Bacteremic patients typically necessitate a minimum of seven days of antimicrobial drug therapy, coupled with an investigation to identify potential sites of infection. In cases of extraintestinal infections, prolonged therapy, consultation with specialists, and surgical involvement may be necessary. Immunocompromised patients, due to their heightened vulnerability, face an increased risk of recurrent invasive disease and, consequently, often require an extended duration of therapy to effectively manage their condition (CDC, 2024). Certain antibiotics, notably fluoroquinolones and third-generation cephalosporins, are categorized as critically important by the WHO (Angulo et al., 2009). Fluoroquinolones have traditionally been recommended for treating salmonellosis in adults, while third-generation cephalosporins are favoured for paediatric cases in the event of fluoroquinolones resistance (Sood et al., 1999). Presently, fluoroquinolones are preferred as the first line of treatment for adult

travellers; ceftriaxone can be used to treat children or adults presenting invasive illnesses due to *Salmonella*. Azithromycin can be used in children as an alternative therapy (CDC, 2024). Some studies have recommended the usage of macrolides (e.g. azithromycin) and carbapenems (e.g. meropenem), in case resistance to first-line antibiotics occurs (WHO, 2019; Tack et al., 2020).

As of now, there is no available vaccine against NTS, underscoring the importance of preventive measures. Adopting routine practices, including thorough handwashing before meals and after contact with animals, is vital. Avoiding the consumption of raw vegetables or meat, opting for properly cooked food, and drinking water from safe sources can significantly reduce the risk of infection. Individuals with illness should abstain from preparing food for others, and even after recovery, maintaining safe food handling practices is essential, as they may continue to shed bacteria for an extended period. These measures collectively contribute to curtailing the chances of acquiring NTS infection and safeguarding public health. (CDC, 2024)

2.10. Antimicrobial Resistance in NTS – The Global Scenario:

Antibiotics, whether natural or synthetic compounds, serve a pivotal role in preventing infections. However, the rise of antibiotic resistance has significantly impeded the effective use of these medications. The pervasive presence of *Salmonella* strains exhibiting resistance to multiple antibiotics represents a pressing global public health concern. According to findings from Su et al. (2004), the prevalence of antimicrobial resistance among *Salmonellae* has markedly escalated, surging from 20%–30% in the early 1990s to as high as 70% in specific countries. Since the occurrence of MDR *S.* Typhimurium DT104 strains in 1990, there has been a considerable increase in the appearance of different MDR strains (Helms et al. 2005; Kim and Wei, 2007, 2009).

Earlier drugs such as ampicillin, chloramphenicol and co-trimoxazole were considered to be the drugs of choice for treatment against NTS infection. However, indiscriminate use of antibiotics, amongst humans as well as in domestic animals and poultry has resulted in emergence of MDR *Salmonella* strains globally (Lee et al., 2009;

Vo et al., 2010; Meng et al., 2011; Wannaprasat et al., 2011; Chen et al., 2013, Crump et al., 2015; Adesiji et al., 2018). This global emergence of drug resistance has complicated the challenges associated with the containment strategies of NTS (Vo et al., 2010; Aouf et al., 2011).

In a study conducted by Kuo et al. (2014), it was observed that 96% of 110 isolates obtained from diseased pigs in 2011 and 2012 exhibited multidrug resistance (MDR), indicating resistance to three or more antimicrobial drug classes. Notably, 21% of these isolates were resistant to ciprofloxacin, while 44% demonstrated resistance to cefotaxime. Reports from regions with a high prevalence of MDR isolates suggest heightened resistance to fluoroquinolones (Hasan et al., 2008). Additionally, increased incidence rates of nalidixic acid resistance reported in countries such as India, Vietnam, and Pakistan (Ochiai et al., 2008) have raised concerns regarding management and infection control & prevention (Crump et al., 2011), as nalidixic acid resistance is indicative of decreased susceptibility to fluoroquinolones (Eng et al., 2015).

In a study by Vlieghe et al. (2012), elevated resistance to azithromycin was observed in *Salmonella* enterica-induced bloodstream infections among Cambodian adults infected with HIV. In the United States in 2014, a modest level of resistance to ceftriaxone was detected in 2.4% of human NTS isolates, with 0.4% showing resistance to ciprofloxacin and 0.1% to azithromycin (McDermott et al., 2018). Conversely, in Europe in 2020, significantly elevated resistance to ciprofloxacin was reported in 14.1% of human NTS isolates, while resistance to cefotaxime and ceftazidime was noted at 0.8% each, with azithromycin resistance also standing at 0.8% (EFS Authority, 2022).

In another study conducted in Taiwan spanning from 1998 to 2002, exceedingly high rates of resistance were documented for various antibiotics. Specifically, resistance rates were reported as follows: ampicillin (48.5%), sulfamethoxazole (68%), streptomycin (59.0%), chloramphenicol (55.3%), and tetracycline (67.8%) (Lauderdale et al., 2006).

In a study conducted in China from 2014 to 2016 (Liang et al., 2019), NTS strains isolated from faecal samples displayed significantly elevated resistance rates to various

antibiotics. The research reported notably high levels of resistance, with the highest rates observed for ampicillin (76.61%), sulfamethoxazole (29.95%), cefotaxime (29.93%), chloramphenicol (29.77%), ceftazidime (23.20%), ciprofloxacin (7.51%), and cefoperazone/sulbactam (7.18%). These findings underscore a substantial level of resistance, particularly against 3rd-generation cephalosporins and quinolones, highlighting the growing challenge of antimicrobial resistance in NTS isolates.

A comparative analysis of NTS drug resistance between 2009-2013 and 2014-2018 revealed an increasing trend in resistance rates to cefazolin, cefotaxime, levofloxacin, ciprofloxacin, and imipenem. While resistance rates to ampicillin, chloramphenicol, cefepime, ceftriaxone, and compound sulfamethoxazole slightly decreased, they still maintained high levels. Among children with NTS infections, 13.7% were identified as having multi-drug resistant (MDR) isolates. This comprehensive analysis underscores the evolving patterns of NTS infections and the dynamic landscape of antibiotic resistance observed over the studied periods (Wu et al., 2021).

In another study conducted in sub-Saharan Africa from 2015 to 2017 by Tack et al (2020), multidrug resistance was prevalent, affecting a significant 87.4% of the 864 NTS isolates analysed. The findings further revealed decreased susceptibility to ciprofloxacin in 7.3% of cases, while ceftriaxone resistance was observed in 15.7%, and azithromycin resistance in 14.9%.

The extensive utilization of antibiotics in poultry and other livestock farming has been widespread. Developing countries, in particular, are experiencing high incidences of drug resistance due to the inappropriate use of antibiotics in farming practices (Van et al., 2007; Van et al., 2008; Fashae et al., 2010; Yang et al., 2010). This issue is exacerbated in developing nations where there is inadequate control over the direct sale of drugs through medical stores or pharmacies without a doctor's prescription (Ogasawara et al., 2008). Moreover, the easy availability and low cost of certain antimicrobials aggravate the situation still further (Nguyen et al., 2005; Wannaprasat et al., 2011). In a recent study conducted in Nigeria, a concerning trend of widespread multiple drug resistance (MDR) among NTS isolates from poultry was observed. A

substantial 48% of the NTS isolates exhibited a multidrug-resistant profile. Furthermore, 73% of these isolates demonstrated high resistance rates to key antibiotics, including ciprofloxacin, gentamicin, nalidixic acid, sulfamethoxazole, and tetracycline. Notably, ciprofloxacin resistance was nearly ubiquitous among the tested NTS isolates. The study identified the indiscriminate use of antibiotics by poultry farmers as the root cause of this alarming situation, underscoring the need for targeted interventions and improved antimicrobial stewardship practices in the poultry farming sector to address the escalating issue of antibiotic resistance (Fagbamila et al., 2023).

Over the years, experimental evidence has consistently underscored the concerning link between antimicrobial usage in animals and the rising crisis of bacterial antimicrobial resistance (AMR). This association poses a significant threat to public health, highlighting the critical need for prudent and responsible antimicrobial use in both human and veterinary medicine to address the impact of antimicrobial resistance.

In a recent study by Delgado-Suárez et al. (2021), among NTS isolates obtained from beef in Mexico, several prevalent resistant phenotypes were identified. These included tetracycline resistance in 40.3% of cases, carbenicillin resistance in 26.0%, amoxicillin-clavulanic acid resistance in 20.8%, chloramphenicol resistance in 19.5%, and trimethoprim-sulfamethoxazole resistance in 16.9%. Additionally, over 55% of the isolates exhibited reduced susceptibility to ciprofloxacin, and 26% were categorized as MDR. Furthermore, the MDR phenotypes were strongly associated with NTS serovar Typhimurium accounting for nearly 40% of MDR strains.

In India, the prevailing data on the prevalence and antibiotic susceptibility patterns of NTS are limited or inconsistent. A study from North India, conducted by (Taneja et al., 2004), reported resistance rates among NTS isolates. The results revealed resistance rates of 62.5% to amoxicillin, 66.7% to nalidixic acid, 34.6% to cotrimoxazole, 48.1% to cefotaxime, 37% to chloramphenicol, and 18.5% to ciprofloxacin. This study highlighted the existence of antibiotic resistance among NTS strains in the region and emphasized the importance of continued research and investigation to better understand and address the dynamics of antibiotic resistance in India. In a study by Shahane et al (2007) from Pune, India, NTS isolates from paediatric

and adult patients showed high resistance to commonly used drugs, including ampicillin (86%), chloramphenicol, cefotaxime, and gentamicin (72.4% each). Another study by Ballal et al in 2016 reported minimal resistance, with only 3% resistance each to ampicillin and ciprofloxacin among NTS isolates. These findings highlight variations in antibiotic resistance patterns among NTS strains in India and underscore the necessity for continuous surveillance to address the dynamic nature of antimicrobial resistance. Notably, the study also reported a unique finding in the Indian context – the isolation of a Ciprofloxacin-resistant *Salmonella enterica* serovar Kentucky, marking the first instance of such resistance reported in India. This highlights the introduction of a new facet of drug resistance in NTS in the country, underlining the evolving landscape of antibiotic resistance.

In Southern India, an 18-year study (2000–2018) revealed that, 5% of NTS isolates were MDR in addition, resistance to nalidixic acid was most prevalent being 26%. The resistance rates to other drugs was also found notable viz. ampicillin (18.5%), chloramphenicol (3.6%), ciprofloxacin (12%), ceftriaxone (6.3%), and cotrimoxazole (13.5%) with a notable rate subset at 10.52%. (Jacob et al., 2020). These results underscore the persistent challenge of antimicrobial resistance in NTS over the studied period, highlighting the need for ongoing surveillance and interventions to address evolving resistance patterns.

The global emergence of drug resistance has complicated the challenges associated with the containment strategies of NTS. Enough evidence is available that depicts the acquisition of human infection by multidrug-resistant strains acquired through international travel and trade activities (Aarestrup et al., 2007; Hendriksen et al., 2008; Sirichote et al., 2010). The spread of clinically important resistance amongst NTS has also been associated with travel across different countries (Barlow et al., 2014). The global prevalence of antibiotic resistance in NTS isolates is linked to the unrestricted use of antibiotics in livestock animals (Kim et al., 2012). Historically, third-generation cephalosporins and fluoroquinolones have been effective in treatment of the infection. However, there is a concerning rise in resistance to these medications, as well as to first-line antimicrobial agents (Sethuvel et al., 2015). *Salmonella* isolated from humans has shown co-resistance to fluoroquinolones and 3rd

& 4th -generation cephalosporins (Kulwichit et al., 2007; Zhang et al., 2014). This growing resistance presents a significant challenge to effectively treating NTS infections. It underscores the urgent necessity for comprehensive measures to regulate antibiotic usage, bolster surveillance efforts, and explore alternative therapeutic strategies.

The rise of antimicrobial-resistant strains has severely limited treatment choices and heightened the global disease burden (Chen et al., 2013). The rising prevalence of bacteria that are resistant to commonly prescribed antibiotics presents a substantial public health concern, complicating the treatment of bacterial infections and contributing to elevated rates of illness and mortality on a global scale. Addressing the issues related to antimicrobial resistance demands collaborative actions at local, national, and international scales to establish efficient surveillance systems, implement stewardship programs, and conduct research endeavours aimed at creating alternative treatment approaches and reducing the impact of resistant strains on global health.

2.11. Mechanisms involved in Drug resistance:

In *Salmonella*, antibiotic resistance primarily arises from mutations in target genes, such as those encoding Topoisomerase IV & DNA gyrase, and the increased expression of efflux pumps. Nevertheless, other factors also contribute to resistance, including changes in the cell envelope, reduced expression of membrane porins, elevated levels of lipopolysaccharide (LPS) components in the outer cell membrane, biofilms formation, and the quorum sensing (Martins et al., 2011). The diverse array of mechanisms involved in antibiotic resistance highlights the intricate nature of addressing infections caused by this pathogen.

As previously indicated, no treatment is needed in mild gastrointestinal infections caused by NTS. However, in severe cases of invasive infections, the administration of first-line antibiotics such as ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, alongside fluoroquinolones or third-generation cephalosporins, is critical for saving lives. Unfortunately, the emergence of resistance to these antimicrobials posed serious treatment challenges.

In *Salmonella spp*., resistance to ampicillin is attributed to lactamases, while chloramphenicol resistance is primarily caused by the production of inactivating enzymes such as chloramphenicol acetyltransferase (CATs) (Arcangioli et al., 2000). Fluoroquinolones resistance is typically a result of mutations occurring in DNA gyrase and topoisomerase IV, the target enzymes of quinolones (Su et al., 2004). Resistance typically emerges spontaneously due to point mutations that cause amino acid substitutions in topoisomerase genes. This is frequently accompanied by decreased expression of outer membrane porins and heightened expression of multidrug efflux pumps. These mechanisms collectively contribute to the reduced susceptibility of bacteria to fluoroquinolones and pose challenges for effective treatment (Hopkins et al., 2005).

In the case of 3rd-Generation cephalosporins, resistance is primarily due to the extended-spectrum cephalosporinases production, majorly extended-spectrum beta lactamases (ESBLs) e.g. CTX-M and AmpC beta-lactamases e.g. CMY-2 (Miriagou et al., 2004; Karthikeyan et al., 2011). Other types such as ACC, DHA, OXA, PER, SHV, and TEM have also been reported in *Salmonella* (Su et al., 2004). Horizontal transfer of these genes is mainly responsible for the spread of resistance (Allen and Poppe, 2002) and thereby allow *Salmonella* to disseminate to a new genetic lineage (Guerra et al., 2001; 2002) resulting in increased prevalence in both humans and animals (Llanes et al., 1999). The increased prevalence of these enzymes in *Salmonella* spp., along with reduced susceptibility to quinolones, poses a significant concern from public health point of view, compromising the efficacy of these drugs in treating *Salmonella* infections and underscoring the need for vigilant surveillance and antibiotic stewardship.

The primary cause of carbapenem resistance in *Salmonella* isolates lies in mobile genetic elements that carry diverse classes of β-lactamases, including IMP, KPC, NDM, OXA, TEM, and VIM (Sahuquillo-Arce et al., 2015). Recent studies suggest that these carbapenemase genes are often harboured on plasmids within *Salmonella* serovars, facilitating their transfer between different Enterobacteria (Sarkar et al., 2015). The potential transfer of these resistance genes through plasmids raises concerns about the possible dissemination of carbapenem resistance among different bacterial strains

(Nhu et al., 2010). This underscores the urgent requirement for rigorous surveillance and control strategies to mitigate the spread of antibiotic resistance in *Salmonella* and similar pathogens.

2.12. Diagnostic methods for *Salmonellae*:

The laboratory diagnosis of *Salmonella* infections encompasses several approaches, each with unique strengths in speed, accuracy, and application context. Traditional culture-based techniques have long been considered the gold standard for *Salmonella* identification, particularly in clinical settings where specificity is crucial. By using selective media such as Xylose Lysine Deoxycholate (XLD) agar or Salmonella-Shigella (SS) agar, microbiologists can isolate *Salmonella* colonies from clinical specimens, such as stool or blood, as these media inhibit non-*Salmonella* growth (Bhunia, 2019). These media also facilitate preliminary identification through colony morphology, with *Salmonella* colonies often appearing with a distinctive black centre due to hydrogen sulphide production. Following isolation, biochemical assays, provide further species-level differentiation by revealing metabolic characteristics unique to *Salmonella* (Andrews et al., 2019).

Serotyping is a valuable diagnostic tool in identifying and differentiating *Salmonella* serovars, crucial for effective clinical management and epidemiological tracking. Using the Kauffmann-White classification scheme, *Salmonella* serotyping categorizes serovars based on surface antigens: the O (somatic), H (flagellar), and Vi (capsular) antigens. This method allowed the classification of *Salmonella* into more than 2,600 serovars, each associated with specific disease profiles and transmission routes (Grimont and Weill, 2007; Issenhuth-Jeanjean et al., 2014; Marzel et al., 2016). Though, serotyping offers high specificity but requiring skilled personnel and is labour-intensive (Ibrahim and Morin, 2018; Diep et al., 2019).

Recent advancements in molecular techniques, such as PCR-based serotyping and whole-genome sequencing (WGS), enabling quicker and more comprehensive profiling of *Salmonella* serovars, especially valuable in outbreak settings (Deng et al., 2016). PCR enables the amplification of *Salmonella*-specific DNA sequences directly from clinical samples, allowing for rapid detection and significantly shortening

turnaround time (Siala et al., 2017). This method not only reduces detection time but also enhances sensitivity, making it effective in identifying low bacterial loads that might be undetected by traditional methods. Real-time PCR (qPCR) offers additional advantages by quantifying bacterial DNA and differentiating between various serotypes or strains through genetic markers (Kasturi and Drgon, 2017). Furthermore, PCR allows for the detection of antibiotic resistance genes, providing critical insights for treatment (Nazari et al.,2023; Pavelquesi et al., 2023).

Advanced techniques like whole-genome sequencing (WGS) are also emerging in *Salmonella* diagnostics, offering comprehensive genetic data on virulence factors, phylogenetic relationships particularly for understanding the genetic diversity, epidemiology, and antibiotic resistance of various *Salmonella* serovars (Akinyemi et al., 2023). Although WGS is currently applied more in research and public health surveillance due to its high costs and technical requirements, it has proven invaluable for outbreak management (Brummer et al., 2022). By identifying strain-specific genetic markers, WGS aids in pinpointing infection sources and understanding the spread and evolution of specific strains, thus enhancing diagnostic precision and informing public health actions.

Additionally, point-of-care testing (POCT) is helping bring Salmonella diagnosis closer to patients. Loop Mediated Isothermal Amplification (LAMP) technology has emerged as an effective tool for rapid diagnosis of Salmonella infections (Chu et al., 2021; Vinayaka et al., 2022). This technology has been found to be more sensitive than PCR methodology (Mei et al., 2019) and has been proven to be effective in detection of S. typhi in blood samples (Kaur et al., 2018). Therefore, POCT methods, can serve a critical role in preliminary patient management, particularly in outbreak-prone areas.

In conclusion, advancements in Salmonella diagnostics are progressing swiftly, with culture, molecular, and immunological methods collectively improving the speed and accuracy of detection. As diagnostic technology continues to evolve, the primary aim is to create tools that are faster, more accessible, and highly precise, ultimately supporting prompt treatment, effective outbreak management, and well-informed public health strategies.

CHAPTER – 3

OBJECTIVES

3. OBJECTIVES

The current study was designed to assess the prevalence and varied characteristics of Non-Typhoidal *Salmonella* (NTS) derived from various sources (including humans, animals, environment, and food) across different geographical regions of India. To accomplish the primary aim of this investigation, the following objectives were outlined:

1. To identify the Non-Typhoidal *Salmonella* (NTS) isolates, received at 'National *Salmonella* and *Escherichia*' Centre (NSEC) from different parts of India, by biochemical and serological techniques.
2. To Study the antibiogram profile of the confirmed NTS isolates.
3. To study the Extended-Spectrum β –Lactamase (ESBL) and Carbapenemase production in NTS isolates.
4. Characterization of gene(s) responsible for antimicrobial resistance in NTS.
5. Determination of virulence factors like biofilm and colicins production among NTS isolates.
6. Analysis of the results obtained from the above studies to correlate prevalence of NTS in India on the basis of distribution, drug resistance patterns, virulence factors, and relevant demographic data.

CHAPTER – 4

MATERIALS
&
METHODS

4. MATERIALS AND METHODS

4.1 SAMPLES COLLECTION:

This study aimed to characterize Non-Typhoidal *Salmonella* (NTS) isolates received, during the period from January, 2016 to December, 2018, from different regions of India at the National *Salmonella* and *Escherichia* Centre (NSEC) located at the Central Research Institute (CRI) in Kasauli, Himachal Pradesh. The NSEC is a world-renowned reference laboratory that has been serving the country for over six decades by serotyping *Salmonella* isolates submitted to the centre.

This study included 999 *Salmonella* isolates (both Typhoidal and non-Typhoidal) received at the NSEC lab for serotyping. These isolates were received from various sources such as hospitals, laboratories of fisheries and food departments, agricultural and veterinary research institutes located across different regions in India. These isolates were categorized into four groups based on their origin, namely humans, food, environment, and animals. Further sub-grouping was performed based on the specific origin of NTS within each of these four main groups. For instance, among human NTS isolates, sub-groups included blood, body fluids, urine, stool, pus, and tissue samples. In animal sources, sub-groups covered poultry and livestock specimens, including diarrheal, fecal, and post-mortem samples. Similarly, the food and environmental group comprised raw cow and buffalo milk, raw pork, animal feed, chicken meat, eggs, fish, and shrimp.

Upon receipt, the isolates were further processed for identification through biotyping and serotyping.

4.2 SAMPLE PROCESSING & IDENTIFICATION:

The samples, received in intact condition and following specified transportation conditions (WHO, 2021) such as stab culture in nutrient agar medium without any leakage or breakage and packed within a three-layered packaging, were processed further for identification using a combination of biochemical and serological techniques.

The 999 intact samples were further processed for thorough identification and subsequent investigations. For identification, these 999 samples were sub-cultured onto fresh MacConkey Agar (Hi Media Labs. Pvt. Ltd., Mumbai, India) plates. With the help of a sterile wire loop, the non-lactose fermenting (NLF) and well isolated colony was picked and streaked onto fresh nutrient agar (NA) slants, in duplicates.

4.2.1 BIOTYPING:

The 999 samples, mentioned above, were further subjected to morphological and biochemical characterization. To achieve this, the isolates (on NA slants) were identified based on morphological characteristics i.e., Gram's staining, motility, and biochemical characteristics as described by Edward and Ewing (1986). The biochemical characterization was based on various tests viz:

1. Test for Enzyme detection: Catalase, Oxidase, Urease production
2. Test for Sugar Fermentation: Glucose, Lactose, Sucrose, Mannitol.
3. Test for Substrate Utilization: Citrate, Triple Sugar Iron
4. Test for Decarboxylase: Lysine, Arginine, Ornithine
5. Other tests: Indole production, Methyl-Red test, Vogus Proskauer Test, Cragie's Tube, Hugh, and Leifson's O/F test.

The details of the various biochemical tests performed are as under:

4.2.1.1 Enzyme detection tests:

a. Catalase test (Reiner, 2010):

Took a clean glass slide and put a drop of 3% H_2O_2 and mixed a small amount of bacterial culture using a clean Pasteur pipette. The appearance of catalase was confirmed by the production of gas bubbles immediately or within 10 seconds of mixing the bacterial culture with H_2O_2.

b. Oxidase Test (Shields and Cathcart, 2010; Oladeinde et al., 2011):

Took a strip of sterile Whatman's No. 1 Filter paper and soaked it in the oxidase reagent (1% solution of tetramethyl-p-phenylene diamine dihydrochloride). Placed the strip on a clean glass slide. Took an isolated colony of the bacterium with the help of a sterile Pasteur pipette and smeared it over the moistened area of the filter paper strip. The absence of deep-purple colour indicated a negative reaction.

c. Urease production test (Brink, 2010; Oladeinde et al., 2011):

Inoculated the entire surface of the medium (Christensen's medium) in a tube with the pure culture of the bacterium. Incubated the medium at 35±2°C for overnight. A urease negative result was indicated by the absence of production of pink colour around the bacterial growth.

4.2.1.2 Sugar Fermentation Tests (Hummers-Pradier et al., 2005):

Inoculated the test organism in a tube of sugar fermentation medium. Incubated the tube at 35±2°C for overnight. Observed the results after completion of incubation. Acid production was indicated by the change in the colour of the indicator (Andrade's Indicator) to red while gas production was detected by the appearance of a gas bubble in the inverted Durham's tube placed inside the sugar solution.

4.2.1.3 Substrate Utilization Tests:

a. Citrate (Eltahawy and Khalaf, 1988):

Took a tube of Simmon's Citrate medium and inoculated its surface with the test organism. After incubation at 35 ± 2°C for overnight, observed the tube for any colour change next day. The appearance of blue colour in the medium around the bacterial growth indicated a positive test.

b. Triple Sugar Iron (TSI) (Tankeshwar, 2022):

Took a tube of TSI agar and a straight wire loop and inoculated the test sample by streaking the surface as well as stabbing the medium to the bottom. Incubated the tube at 35±2°C overnight. The appearance of red colour was considered as sugar fermentation, black colour indicated H_2S production, while cracks in the medium indicated CO_2 production.

4.2.1.4 Amino Acid Decarboxylase & Arginine Dihydrolase tests (Lal and Cheeptham, 2015):

Inoculated the medium containing amino acid decarboxylase using a straight wire, then placed the tubes under incubation at a temperature of 35±2°C for overnight. Observed the result after the completion of incubation. The appearance of yellow colour indicated glucose fermentation and acid production; however, violet colour indicated decarboxylation reaction. The control tube remained yellow.

4.2.1.5 Other tests:

a. Nitrate Reduction (NR) Test (Buxton, 2011):

The test culture of bacteria was inoculated into the nitrate broth medium using a sterile inoculation loop. After incubation overnight at 35±2°C, added few drops of α-Naphthylamine (NR reagent I)) followed by Sulfanillic acid (NR reagent II) into the media tubes. The tubes were observed for any changes in the colour of the medium. The emergence of a red coloration in the medium was construed as a positive indication of nitrate reduction.

b. Indole production (MacWilliams, 2012):

Inoculated a tube of Tryptone broth with pure culture of the test bacterium and incubated at 35±2°C overnight. After completion of incubation, checked for the production of Indole by adding 4 -5 drops of Kovac's reagent directly on the medium in the tube. A negative test result was indicated by the absence of a cherry-red ring (and

instead the appearance of yellow-coloured cloudiness) at the interface between the medium and the reagent.

c. Methyl-Red (MR) & Vogus Proskauer (VP) Tests (S. McDevitt, 2009):

Inoculated a freshly prepared Glucose Phosphate Medium (GPM) with the pure culture of the test organism and incubated overnight at 35±2°C. On completion of incubation, divided the growth in GPM into two parts aseptically into two sterile tubes. To one portion, 4-5 drops of MR reagent were added. A positive MR test was indicated by the appearance of a red colour. For the second portion, a few drops of Barritt's reagent A (40% w/v KOH) were added, followed by 4 drops of Barritt's reagent B (5% v/v α-Naphthol). The mixture was gently mixed and allowed to stand for a few minutes for the reaction to complete. A negative VP test was indicated by the absence of a copper-red coloured ring at the interface.

d. Motility detection by Craigie's Tube (Aygan and Arikan, 2007):

Inoculated the Craigie's Tube (a glass tube containing a semi-solid agar (0.2% - 0.4%w/v) having a narrow tube embedded in it, which is open at both ends and extends above the agar surface of agar) with the test sample into the inner tube with the help of a straight wire and incubated at 35±2°C for overnight. Observed the result after the completion of incubation. There was a visible turbidity (i.e. growth) on the surface of the semisolid agar outside of the inner tube, indicating that the organism was motile.

e. Hugh and Leifson's O/F test (Hutson 2008):

Inoculated the test organism into two tubes of O/F Media (one tube has a mineral oil overlay than the other) by stabbing and incubated overnight at 35±2°C. Observed the result after the completion of incubation. Both the tubes showed a change in color to yellow, indicating that the organism had used both the oxidative as well as fermentative pathways for sugar utilization.

4.2.2 SEROTYPING:

Following this initial identification through biotyping, a subsequent step was to confirm the different serovars amongst the genus *Salmonella*. This was accomplished through slide agglutination tests, wherein all the 699 *Salmonella* isolates were tested using polyvalent and monovalent sera specifically targeting the somatic 'O' and flagellar 'H' antigens of *Salmonella*.

The serotyping of the *Salmonella* isolates was done as per the standard method as described originally by the Kauffmann-White-Le Minor (KW) scheme, with minor modifications (Hendriksen and Larsen (2004); Grimont and Weill, 2007; Guibourdenche et al., 2010). Serotyping is based on the interaction between an antigen (Ag) and its specific antibody (Ab) resulting in the formation of Ag-Ab complexes which are visible to the naked eye thereby help in easy determination of O- and H-antigen types by a simple slide agglutination test. Some bacterial isolates carry monophasic- H antigen and thus can be directly serotyped, whereas in some isolates H-antigen exists in two phases (i.e. diphasic). In such a situation, the second phase is determined after phase inversion. After complete serotyping of the *Salmonella* cultures, the names of the serotypes were determined by using the Kauffmann-White Scheme.

4.2.2.1 Testing for somatic ‘O’ antigen:

Took a clean and grease-free glass slide. With the help of a sterile wire loop, took a drop of normal saline on the slide and gently mixed a small quantity of the pure culture of the NTS. [***Note****: First checked for autoagglutination of the isolate. The isolates that showed auto-agglutination were not serotyped and not used further*]. Added a drop of the polyvalent antisera (pool of different monovalent antisera against specific ‘O’ antigen) to the bacterial suspension. Tilted the slide side by side for 5-10 seconds to facilitate the antigen-antibody reaction. The positive reaction was indicated by the appearance of a white chalky deposit within 10 seconds. The absence of chalky deposits indicated a negative reaction. Noted down the pool of the polyvalent antisera which showed a positive slide agglutination reaction. Thereafter the above process was repeated by testing with all individual mono-specific (monovalent) antisera

incorporated in the pool. The reaction with mono-specific antisera helped in determining the exact 'O' serotype of the bacterial isolate.

4.2.2.2 Testing for flagellar H-antigen:

Next, the isolate underwent testing with polyvalent H antiserum using a method akin to the one previously described. Each individual H-antiserum (monovalent) was then employed to identify the specific H-antigen. This process involved testing the isolate with each Monovalent-H antiserum available in the pool.

The majority of *Salmonella* strains possess two separate sets of genes that encode for H-antigens. As a result, they are described as diphasic, with one phase being specific (Phase-I) and the other non-specific (Phase-II). Nevertheless, only one phase is active at a time, as elucidated by Wattiau et al. in 2011. Therefore, to detect the other phase, a method known as "phase inversion" was used wherein the dominant phase of the H-antigen was inhibited by incorporating a specific antiserum in a special medium called "Sven-Gard medium". This prevented the growth of the dominant phase, allowing testing of the isolate bearing flagella of the second phase using specific antisera via slide agglutination, if present. The detailed procedure is mentioned below.

Took 18-20ml of the molten Sven Gard's agar and cooled to near 45 - 47 °C. In a petri dish added 2-3 drops of the antiserum against the H antigen which was detected in Phase I. Poured the molten Sven Gard's agar into the Petri plate and mixed gently to distribute the antisera homogeneously into the medium. Allowed the medium to cool further and solidify into a semi-solid state. Inoculated the NTS isolate tested above into the middle of the Petri plate. Incubated the plate at 35 ± 2°C for overnight. The next day checked the growth on the plate. A swarming type of growth occurred due to semi solid nature of the medium. Then picked the culture from the periphery of the growth and performed slide agglutination with the H-antiserum as mentioned above. The occurrence of agglutination indicated the presence of Phase II H-antigen.

4.3 PREPARATION OF MASTER STOCK (MS) AND WORKING STOCK (WS):

All the confirmed NTS isolates were preserved on Nutrient agar slants as working stock (WS) and Stab Cultures as master stock (MS). The MS cultures were kept under refrigerated conditions (2-8°C) in a cold room. The working cultures (WS) were prepared from time to time from the master stock (MS) as per requirements during the entire study period.

4.4 ANTIBIOGRAM PROFILING:

All identified NTS isolates underwent antibiogram profiling utilizing the Kirby Bauer's Disc Diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI, 2014, 2022). A total of 19 antimicrobial drugs representing different antimicrobial classes (Table 4.1) were used :

Table 4.1: List of 19 antimicrobial drugs used in the study.

S. No.	Name of the antimicrobial agent	Concentration (µg/disc)
1	Amikacin (AK)	30
2	Amoxycillin/clavulanic acid (AMC)	30
3	Ampicillin (AMP)	10
4	Cefepime (CPM)	30
5	Cefotaxime (CTX)	30
6	Ceftriaxone (CTR)	30
7	Cefuroxime (CXM)	30
8	Chloramphenicol (C)	30
9	Ciprofloxacin (CIP)	5
10	Co-trimoxazole (COT)	25
11	Gentamicin (GEN)	10
12	Imipenem (IPM)	10
13	Kanamycin (K)	30

S. No.	Name of the antimicrobial agent	Concentration (µg/disc)
14	Meropenem (MRP)	10
15	Nalidixic acid (NA)	30
16	Norfloxacin (NX)	10
17	Nitrofurantoin (NIT)	300
18	Piperacillin/tazobactam (PIT)	100/10
19	Trimethoprim (TR)	5

The NTS isolates were sub-cultured from the working stock (WS) onto freshly prepared nutrient agar plates and then incubated at 35±2°C overnight. The following day, an isolated colony from the nutrient agar media plate was inoculated into a tube containing Luria Bertani (LB) broth. The LB broth was then incubated in a shaker incubator at 35±2°C for 5-6 hours to promote rapid bacterial growth. The turbidity (OD) was adjusted using LB broth to match the 0.5 McFarland standards, containing approximately 1.5 x 10^8 CFU/ml. A sterile cotton swab soaked in the NTS culture in LB broth was used to evenly apply a lawn culture on the surface of an Mueller-Hinton agar (MHA) plate. Three to four plates were utilized for each NTS isolate to test sensitivity to each of the 19 antimicrobial discs. The agar surface of the MHA plates was allowed to dry at room temperature for 3 to 5 minutes with the lid in place. Using sterile forceps, each of 19 antimicrobial disc were firmly applied onto the surface of the inoculated MHA plate, ensuring their centres were approximately 24mm apart, with a maximum of five discs used per plate. The MHA plates were then incubated at 35±2°C overnight. After incubation, the diameter of the zone of inhibition of bacterial growth around the discs was measured and compared them with the interpretive chart according to CLSI guidelines, to determine sensitive, intermediate, or resistant drugs (CLSI, 2014, CLSI 2022).

4.5 EXTENDED SPECTRUM BETA LACTAMASE (ESBL) DETECTION:

4.5.1 Phenotypic method:

The detection of Extended Spectrum β-Lactamase (ESBL) production was conducted using a phenotypic confirmatory test following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (CLSI 2014, CLSI 2022, Philippon, 1989). The antimicrobial drugs used are mentioned in the Table 4.2 below:

Table 4.2: List of drugs used for phenotypic detection of ESBL production.

S. No.	Name of the antimicrobial agent	Concentration (µg/disc)
1.	Ceftazidime	30
2.	Cefotaxime	30
3.	Ceftazidime with clavulanic acid	30/10
4.	Cefotaxime with clavulanic acid	30/10

The NTS isolates were sub-cultured from the working stock (WS) onto freshly prepared nutrient agar plates, incubated at 35±2°C overnight. Following day, an isolated colony was inoculated into a tube containing LB broth and incubated at 35±2°C for 5-6 hours in a shaker incubator. The turbidity (OD) of the culture was adjusted using LB broth to match the 0.5 McFarland standards, containing approximately 1.5 x 10^8 CFU/ml.

A sterile cotton swab soaked in the NTS culture in LB broth was used to evenly apply a lawn culture on the surface of an Mueller-Hinton agar (MHA) plate. The agar surface of the MHA plate was allowed to dry for 3 to 5 minutes with the lid in place. Using sterile forceps, each of the four antimicrobial discs mentioned earlier was firmly applied onto the surface of the agar plate, ensuring their centres were approximately 24mm apart. The plates were then incubated at 35±2°C overnight. The diameter of the zone of inhibition of bacterial growth around the antimicrobial discs was measured, noting any difference in the diameters around the drugs with clavulanic acid

(combination drug) and those without clavulanic acid (single drug). The detection of ESBL production was confirmed by observing a zone of inhibition diameter increase of more than 5mm around combination drugs compared to single drugs.

4.5.2 Genotypic Assay:

NTS isolates that exhibited positive results in the phenotypic assay for ESBL production underwent additional confirmation through genotypic analysis, which involved detecting specific genes associated with ESBL production (*bla*$_{CMY-2}$, *bla*$_{CTX-M15}$, *bla*$_{SHV}$, *bla*$_{TEM}$) using polymerase Chain Reaction (PCR) based assay as described by Taneja et al (2014) with some modifications (Table 4.3).

Table 4.3: List of different ESBL genes used.

Gene (size)	Primers	Reference
bla$_{CMY-2}$ (332bp)	f – 5′-AACACGGTGCAAATCAAACA-3′ r – 5′-CCGATCCTAGCTCAAACAGC-3′	Taneja et al., 2014
bla$_{CTX-M15}$ (587bp)	f –5′-CCAGAATCAGCGGCGCACGA-3′ r –5′-GCGCTTTGCGATGTGCAGCA-3′	
bla$_{SHV}$ (319bp)	f –5′-AAGATCCACTATCGCCAGCAGG-3′ r –5′-ATTCAGTTCCGTTTCCCAGCGG-3′	
bla$_{TEM}$ (859bp)	f –5′-ATGAGTATTCAACATTTCCG-3′ r –5′-ACCAATGCTTAATCAGTGAG-3′	

4.5.2.1 Plasmid DNA extraction:

The bacterial plasmid DNA extraction was done using the plasmid DNA extraction kit (QIAprep Spin Miniprep kit, QIAGEN GmBH, Germany). The method as described by the manufacturer was followed without deviation. In brief, 1.5ml of an overnight LB broth culture of NTS was transferred to a sterile micro-centrifuge tube and centrifuged at 8000rpm at room temperature for 3-5 minutes to pellet the bacterial

suspension. After discarding the supernatant, 250 µl Buffer-P1, which contains RNase A at a concentration of 100 µg/ml (stored at 2-8°C), was added to the pellet and gently mixed the contents to resuspend the pellet. Then, 250 µl Buffer-P2 (pre-warmed at 37°C) was added and inverted the tubes 4-6 times for proper mixing.

Following this, 350 µl of Buffer-N3 (pre-warmed at 37°C) was added and gently mixed by inversion of the tube 4-6 times. The mixture was then subjected to centrifugation at 13,000 rpm for 10 minutes at room temperature, and 800 µl of the resulting supernatant was carefully applied to the provided Spin column from the kit, while discarding the pellet. The Spin column was centrifuged at 13,000 rpm for 1 minute, and the flow-through was discarded. Washing of the Spin column was performed twice by adding 500 µl of Buffer-PB and 750 µl of Buffer-PE, followed by centrifugation at 13,000 rpm for 1 minute each time at room temperature. Following the final wash, an additional centrifugation step for 1 minute at 13,000 rpm was conducted to ensure removal of any residual wash buffer. The Spin column was then transferred to a 1.5ml microcentrifuge tube, and 50 µl of Buffer-EB (for elution) was added. After incubation for 1-2 minutes, the tube was centrifuged for 1 minute at 13,000rpm to elute the plasmid DNA. The eluate containing the plasmid DNA was carefully collected, and the Spin column was discarded. Finally, the plasmid DNA was stored at -20°C until further use.

4.5.2.2 PCR Methodology:

The plasmid DNA obtained above was subjected to detect ESBL-specific genes using a Polymerase Chain Reaction (PCR) method. The specific ESBL genes utilized in this study are outlined in Table 4.3. Primers for the PCR reactions were synthesized by Eurofins Genomics India Pvt. Ltd. The PCR procedure, adapted from Taneja et al. (2014) with some modifications, was employed for the analysis. PCR reactions were carried out in thin-walled tubes (0.2 ml capacity) with a final volume of 25µl, (Table 4.4).

Table 4.4: Recipe for PCR Reaction Mixture (per reaction).

S. No.	Reaction Components	Quantity used (µl)
1.	Master Mix (FIREPol®) containing 0.4mM Tris-HCl, 0.1% w/v Tween-20, 0.1M $(NH_4)_2SO_4$, 1.5mM $MgCl_2$, , 0.2 mM of each of the four dNTPs and FIREPol® DNA polymerase	2
2.	Forward Primer	1
3.	Reverse Primer	1
4.	Plasmid DNA	4
5.	Nuclease free water	17
	Total volume	**25**

4.5.2.3 PCR Protocols:

The PCR procedure was carried out in a thermocycler (Eppendorf, Hamburg, Germany). The specific conditions for each set of primers are detailed below (Table 4.5 to 4.8).

Table 4.5: PCR Protocol followed for *bla*$_{CMY-2}$ gene.

Reaction Step	Temperature	Time	No. of Cycles
Initial denaturation	94°C	3 min	1
DNA denaturation	94°C	30 s	25
Primer annealing	58 °C	30 s	
Primer extension	72 °C	1 min	
Final extension	72 °C	7 min	1
Hold	4°C	-	-

Table 4.6: PCR Protocol followed for *bla*$_{CTX-M15}$ gene.

Reaction Step	Temperature	Time	No. of Cycles
Initial denaturation	94°C	3 min	1
DNA denaturation	94°C	30 s	25
Primer annealing	64 °C	30 s	
Primer extension	72 °C	1 min	
Final extension	72 °C	7 min	1
Hold	4°C	-	-

Table 4.7: PCR Protocol followed for *bla*$_{SHV}$ gene.

Reaction Step	Temperature	Time	No. of Cycles
Initial denaturation	94°C	3 min	1
DNA denaturation	94°C	30 s	25
Primer annealing	59 °C	30 s	
Primer extension	72 °C	1 min	
Final extension	72 °C	7 min	1
Hold	4°C	-	-

Table 4.8: PCR Protocol followed for *bla*$_{TEM}$ gene.

Reaction Step	Temperature	Time	No. of Cycles
Initial denaturation	94°C	3 min	1
DNA denaturation	94°C	30 s	25
Primer annealing	55 °C	30 s	
Primer extension	72 °C	1 min	
Final extension	72 °C	7 min	1
Hold	4°C	-	-

4.5.2.4 Gel Electrophoresis:

The PCR products were electrophoresed on agarose gel to visualize the amplicons produced by the reaction.

Agarose Gel Preparation: Took 100ml of 1x TAE Buffer (pH 8.0) in a volumetric flask. Weighed 2.0 gm of agarose powder using a calibrated weighing balance. Mixed the agarose in the measured volume of 1xTAE buffer and heated gently to melt the agarose and form a homogeneous solution. Allowed the mixture to cool to near 50°C and then added approx. 2-3 µl ethidium bromide (10 mg/ml), mixed the contents gently. Poured the mixture into a gel casting tray with a comb to mold the well for loading the samples. Allowed the mixture to cool at room temperature and set into the gel.

Sample loading: The gel tray was merged in the 1x TAE buffer in an electrophoresis tank. Loaded 10-15µl of the PCR amplicons into each well of the gel. A 50-1500 bp DNA ladder was also loaded in a well which served as a marker for amplicon size determination. Connected the power cables from the electric power supply unit to the electrophoresis tank and started the electrophoresis to proceed at 100mA current. After completion of the electrophoresis, visualized the gel using a UV trans-illuminator. Captured the image of the results for records.

4.6 DETECTION OF CARBAPENEMASE ACTIVITY:

The Modified Hodge's Test (MHT) by Girlich et al. (2012), was employed for the detection of carbapenemase activity in NTS. A total of 96 NTS isolates exhibiting resistance to meropenem and/or imipenem were included in the study.

Initially, the *E. coli* ATCC 25922 culture was sub-cultured into LB broth and then incubated at 35±2°C for 5-6 hours in a shaker incubator. Following incubation, the turbidity (OD) of the culture was adjusted using LB broth to correspond with the 0.5 McFarland standards, containing approximately 1.5 x 10^8 CFU/ml. Subsequently, the culture was diluted 1:10 in sterile normal saline. A sterile cotton swab was soaked in the diluted *E. coli* culture, and it was then evenly applied as a lawn culture on the surface of an MHA plate. The agar surface of the MHA plate was allowed to dry for 3 to 5 minutes. With the help of a sterile forcep, the meropenem disc was firmly placed onto

the surface of the agar plate at the centre. Following this, the test culture of NTS isolates was streaked in a straight line starting from the edge of the disc till the edge of the plate. Four NTS isolates were used per plate. The plates were then incubated at 35±2°C overnight and afterwards examined for a cloverleaf-type indentation of growth of the test organism and the *E. coli* ATCC 25922, within the zone of inhibition of the meropenem disc.

4.7 DETECTION OF GENE RESPONSIBLE FOR DRUG RESISTANCE IN NTS:

The 223 NTS isolates displaying resistance to Cefotaxime (CTX) underwent screening for the presence of the drug resistance gene $bla_{\text{CTX-M15}}$ (Table 4.3) using a Polymerase Chain Reaction (PCR) based assay. The procedure was adapted from Taneja et al. (2014) with some modifications.

4.7.1 Plasmid DNA extraction:

The bacterial plasmid DNA extraction was performed using the QIAprep Spin Miniprep kit (QIAGEN GmBH, Germany). The detailed procedure has already been mentioned under section 4.5.2.1 above.

4.7.2 PCR Methodology:

The plasmid DNA obtained earlier underwent detection of specific genes using Polymerase Chain Reaction (PCR) based method. The detailed procedure has been mentioned under section 4.5.2.2 & 4.5.2.3 above.

4.7.3 Gel Electrophoresis:

The amplicons, generated by the PCR assay, were subjected to gel electrophoresis for visualization as per the procedure described earlier under section 4.5.2.4 above.

4.8 DETECTION OF BIOFILM PRODUCTION:

Biofilm production was identified utilizing the Tissue Culture Plate (TCP) method outlined by Christensen et al. (1985), with minor variations. Overnight broth cultures of various NTS isolates in Trypticase Soya Broth (TSB) with 1% glucose were prepared. These NTS cultures were then diluted 1:100 in freshly prepared TSB. Subsequently, 200 µl of the diluted cultures were dispensed in triplicate into the wells of a sterile 96-well, flat bottomed, sterile- tissue culture plate, with appropriate labelling to maintain isolate identification. Triplicate positive and negative controls were also established. *E. coli* K-12 culture served as the positive control, while sterile TSB medium acted as the negative control. The plate was then incubated at 35±2°C overnight.

Following incubation, the medium from each well were removed, and the wells were washed 2-3 times with sterile PBS (pH 7.2) to eliminate free-floating bacteria. The biofilm was subsequently stained by adding 200 µl of safranin dye (0.1%) and incubating for 15 minutes. Afterward, the wells were washed to remove excess dye and air-dried. To elute the stain adhered to the biofilm, 200 µl of 30% v/v acetic acid was added. The plate was further incubated at 35±2°C for 10 minutes to facilitate the elution process. Finally, the optical density (OD) of the eluate was measured at 490nm using a micro-ELISA reader. The results were interpreted based on the criteria outlined by Stepanovic et al. (2007), as detailed in Table 4.9.

Table 4.9: Interpretation criteria for biofilm.

Mean OD of the Test sample (OD_t)	Class
$OD_t \leq OD_c$	Negative
$OD_t \leq 2 \times OD_c$ and $> OD_c$	Weak
$OD_t \leq 4 \times OD_c$ and $> 2 \times OD_c$	Moderate
$OD_t > 4 \times OD_c$	Strong

Where ODc refers to the cut-off value.; ODc = mean OD of negative control + 3 SD

4.9 DETECTION OF COLICIN PRODUCTION:

The assessment of colicin production by NTS isolates was done by the 'phenotypic soft agar overlay' technique described by Parreira et al. (1998). Initially, the NTS isolates were inoculated into Luria Bertani (LB) broth and incubated at 35±2°C overnight. Following incubation, the overnight culture was streaked (or spotted) onto an LB agar plate and incubated again at 35±2°C overnight. This allowed any produced colicin to diffuse into the LB agar medium.

After completion of incubation, to eliminate bacterial cells, the plate was exposed to chloroform vapours for 10-15 minutes. Meanwhile, soft agar (0.4%) was melted and cooled to approximately 50-55°C. This soft agar was then mixed with an overnight culture of the Colicin-sensitive strain *E. coli* K12. The resulting mixture was overlaid onto the test organism in the Petri plate. After allowing the soft agar to settle undisturbed for 20 minutes at room temperature, the plates were incubated at 35±2°C overnight.

The following day, the plates were examined for the presence of an inhibition zone of growth of the colicin-sensitive strain *E. coli* K-12 around the test organism's growth. A positive result was confirmed by the presence of an inhibition zone, while the absence of such a zone indicated a negative result.

4.10 STATISTICAL ANALYSIS:

IBM SPSS Statistic Ver. 22 was used for the determination of mean, percentages, standard deviation, and the feature Descriptive statistics > Cross-tabulation for comparison of various variables. MS Excel 2019 was used for the routine management of data and preparation of graphs such as pie charts, and bar charts.

CHAPTER – 5

RESULTS
&
DISCUSSION

5 RESULTS AND DISCUSSION

5.1 Identification of the isolates:

The 999 isolates were bio-typed based on various morphological and biochemical tests. Table 5.1 provides a reference for the biochemical test results required to confirm a bacterial isolate as *Salmonella*.

Table 5.1: Results of biochemical test performed.

Characteristic	Reaction
1. Morphology:	
Gram's staining	Gram-negative, bacilli, approx. 2-3µm in length.
Motility	Motile bacilli
2. Biochemical Tests:	
Nitrate Reduction	Positive
Enzyme detection tests:	
Catalase	Positive
Oxidase	Negative
Urease production	Negative
IMViC Test:	
Indole	Negative
Methyl Red (MR)	Positive
Vogus Proskauer (VP)	Negative
Citrate	Positive
Sugar Fermentation Test:	
Glucose (A/G)	Acid/Gas Positive
Sucrose	Negative*
Lactose	Negative
Mannitol	Positive*
Substrate Utilization Tests:	
Triple Sugar Iron	K/A, H_2S positive, CO_2 positive*
Decarboxylase tests:	
Lysine	Positive*
Arginine	Negative*
Ornithine	Positive*
Miscellaneous:	
Cragie's Tube	Positive
Hugh and Leifson's O/F test	Positive/Positive

* Variable characteristics with different NTS.

The result of morphological and biochemical characterization confirmed the presence of 699 (70%) *Salmonellae* isolates whereas the remaining 300 (30%) isolates belonged to other genera and therefore were not used further in the study.

Further, serotyping was done for the 699 isolates which were confirmed as *Salmonella* based on the results of biotyping. Serotyping facilitated the further categorization of these *Salmonella* isolates, wherein a substantial majority, approximately 539 (77.1%) isolates fall under the category of Non-Typhoidal *Salmonella* (NTS) (Table 5.2); while 98 (14.0%) isolates, were identified as Typhoidal *Salmonella*. Interestingly, the remaining 62 (8.9%) isolates, were found to be auto-agglutinable *Salmonella* isolates. The presence of Typhoidal *Salmonella* is notably lower, constituting only 15.38% of the total isolates. Remarkably there is a comparatively higher prevalence of NTS isolates which signifies a notable public health concern, reflecting the ubiquitous presence and implications, especially NTS infections in India. In contrast, the presence of Typhoidal *Salmonella* is relatively lower. This lower count, while reflecting a relative rarity, does not diminish the significance of Typhoidal *Salmonella* within the context of infectious diseases in the region. The lower counts may be due to the non-reporting of Typhoidal *Salmonella* to the serotyping centres due to the easy availability of diagnostic tools at the respective healthcare centres. Prevalence of Non-Typhoidal *Salmonella* emphasizes the necessity of targeted interventions and public health strategies tailored to the specific epidemiological dynamics associated with this *Salmonella* category.

Table 5.2: Differentiation of *Salmonella* based on serotyping.

Type of *Salmonellae*	**No. of isolates N(%)**
Rough *Salmonella*	62 (8.9)
Typhoidal *Salmonella*	98 (14.0)
Non-Typhoidal *Salmonella*	539 (77.1)

The scope of the present thesis exclusively includes Non-Typhoidal *Salmonella* (NTS). As such, the Typhoidal *Salmonella* category was not included in the detailed analysis or investigation. The objective of this research is to provide an in-depth understanding of NTS, encompassing their epidemiology and antimicrobial resistance profiles within the Indian context. The decision to focus solely on NTS reflects a deliberate choice to provide a comprehensive exploration of this specific *Salmonella* category, acknowledging that the distinct Typhoidal *Salmonella* category falls outside the purview of this study.

Furthermore, Table 5.3 present a comprehensive overview of the distribution of 539 NTS isolates, the main focus of this study, meticulously categorized into 17 unique serovars. Each serovar is accompanied by its count, offering valuable insights into the prevalence and diversity of NTS serovars within our dataset. This detailed breakdown sheds light on the relative abundance of each serovar, enabling a deeper understanding of the epidemiological scenario of NTS in our study.

Within our dataset, it is significant to note that specific NTS serovars have emerged as predominant, with conspicuous prevalence percentages. Notably, *S. enterica* serovar Typhimurium and Lindenburg have demonstrated substantial prominence, accounting for 167 (30.98%) and 135 (25.04%) of the isolates, respectively, making them the most prevalent serovars. Additionally, *S. enterica* serovar Enteritidis accounted for 56 (10.39%) of the total isolates. These findings emphasize the notable prevalence and dominance of these specific serovars within the *Salmonella* population under this study.

Furthermore, it is crucial to highlight the diversity observed among the remaining 14 NTS serovars, collectively representing nearly 33% of the isolates. This diversity underlines the multifaceted nature of NTS, with a spectrum of serovars exhibiting relatively lower prevalence rates. These less frequent serovars, while individually contributing to a smaller portion of the dataset, collectively enrich our understanding of the broader NTS landscape. Their presence within the dataset prompts inquiries into their unique ecological niches, potential sources, and transmission dynamics. While not predominant, these serovars are, on the other hand, vital components of NTS

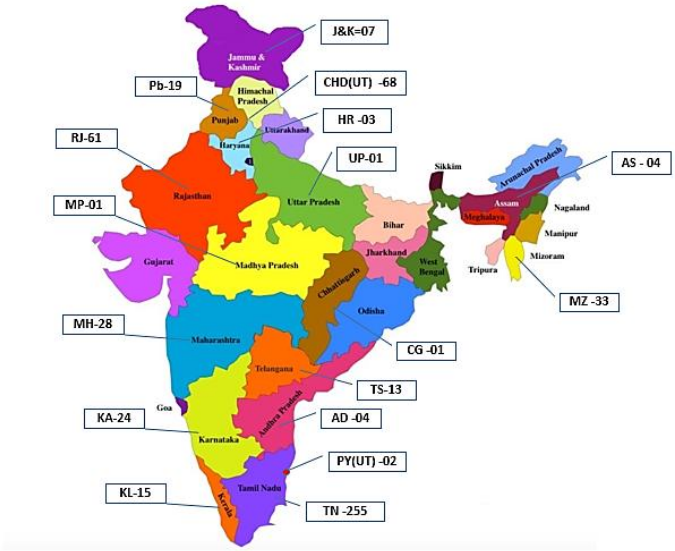
epidemiology, warranting meticulous scrutiny as they may hold insights into emerging patterns, host specificity, or regional variations in NTS distribution.

Table 5.3: List of confirmed Non-Typhoidal *Salmonella* serovars (n=17) identified by serotyping. (Data already published Kumar et al., 2022)

S. No.	Name of organism	Antigenic structure	No. of isolates N (%)
1	*S. enterica* serovar Typhimurium	4,12:i:1,2	167 (30.98)
2	*S. enterica* serovar Lindenburg	6,8:i:1,2	135 (25.04)
3	*S. enterica* serovar Enteritidis	9,12:gm:-	56 (10.39)
4	*S. enterica* serovar Weltevreden	3,10 : r : Z_6	44 (8.16)
5	*S. enterica* serovar Choleraesuis	6,7:c:1,5	41 (7.60)
6	*S. enterica* serovar Mathura	9, 46:i:e,n,Z_{15}	33 (6.12)
7	*S. enterica* serovar Anatum	3,10: e, h:1,6	12 (2.23)
8	*S. enterica* serovar Jaffna	9,12:d:Z_{35}	10 (1.86)
9	*S. enterica* serovar Tennessee	6,7:Z_{29}:-	9 (1.67)
10	*S. enterica* serovar Stuttgart	6,7:i:Z_6	8 (1.48)
11	*S. enterica* serovar Kentucky	8, 20: i: Z_6	6 (1.11)
12	*S. enterica* serovar Bazenheid	8,20: Z_{10}:1,2	4 (0.74)
13	*S. enterica* serovar Hissar	6,7:c:1,2	4 (0.74)
14	*S. enterica* serovar Virchow	6,7:r:1,2	4 (0.74)
15	*S. enterica* serovar Poona	13, 22: z: 1,6	3 (0.56)
16	*S. enterica* serovar Senftenberg	1,3,19:g,t:-	2 (0.37)
17	*S. enterica* serovar Ughelli	3, 10: r : 1,5	1 (0.19)
TOTAL			**539**

5.2 Geographical Distribution of the NTS isolates collected under this study:

As mentioned earlier, amongst the various bacterial isolates received from diverse locations across India (Fig. 5.1), a total of 539 isolates were confirmed as NTS.



S. No.	State(15)/U.T.(2)	No. of NTS
1	Andhra Pradesh (AD)	4
2	Assam (AS)	4
3	Chandigarh UT (CHD)	68
4	Chhattisgarh (CG)	1
5	Haryana (HR)	3
6	Jammu & Kashmir (J&K)	7
7	Karnataka (KA)	24
8	Kerala (KL)	15
9	Madhya Pradesh (MP)	1
10	Maharashtra (MH)	28
11	Mizoram (MZ)	33
12	Puducherry UT (PY)	2
13	Punjab (Pb)	19
14	Rajasthan (RJ)	61
15	Tamil Nadu (TN)	255
16	Telangana (TS)	13
17	Uttar Pradesh (UP)	1
	Total	**539**

Fig. 5.1: Geographic distribution of the source of bacterial isolates across India.

The collection of NTS isolates covered 15 states and 02 union territories (UTs) of India. This extensive geographic coverage was instrumental in capturing the diversity of NTS serovars prevalent in different regions of the country. Samples were received from various sources, including clinical specimens, animals, and environmental samples, representing a broad spectrum of potential NTS hosts and transmission pathways. This extensive and diverse collection provided a comprehensive understanding of the epidemiology of NTS in India, shedding light on regional variations, prevalent serovars, and potential sources of infection.

5.2.1 Distribution of 539 NTS across different regions:

Further analysis of Table 5.4 provides an all-inclusive view of the epidemiology of NTS across different sources viz. humans, animals, food items, and environment.

Table 5.4: NTS Collection from Different Source Categories.

REGION	SOURCE CATEGORY				TOTAL
	Animal	Environment	Food Item	Human	
CENTRAL	0	0	0	2	**2 (0.37%)**
NORTH	45	0	29	24	**98 (18.18%)**
SOUTH	21	0	3	289	**313 (58.07%)**
EAST	17	4	16	0	**37 (6.86%)**
WEST	0	34	51	4	**89 (16.51%)**
G. TOTAL	**83 (15.4%)**	**38 (7.05%)**	**99 (18.36%)**	**319 (59.18%)**	**539**

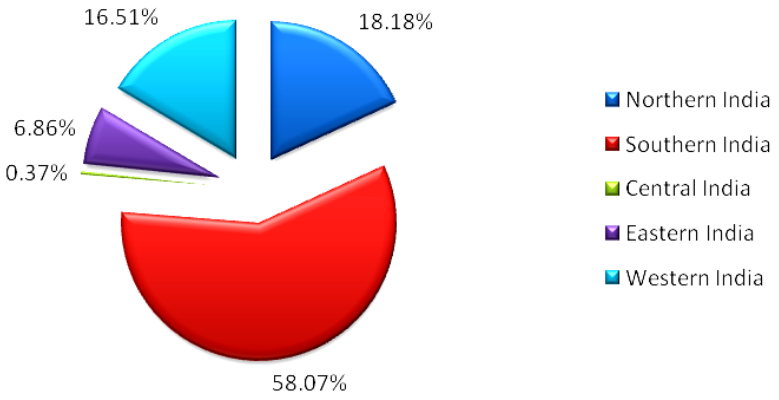


Fig. 5.2: Percentage distribution of the NTS isolates based on Geography.

The distribution pattern of these NTS serovars reveals a notable concentration of isolates originating from the southern region of the country (Fig. 5.2), surpassing the proportions observed in other regions. Notably, there exists a lack of uniformity in the rate of NTS isolate submissions from various regions across the country. Some laboratories have consistently forwarded their suspected NTS isolates for serovar confirmation, while others have not exhibited the same consistency. Furthermore, the elevated rates of collection from the southern regions may be attributed to a greater number of surveillance centres and laboratories in this geographical area compared to other regions.

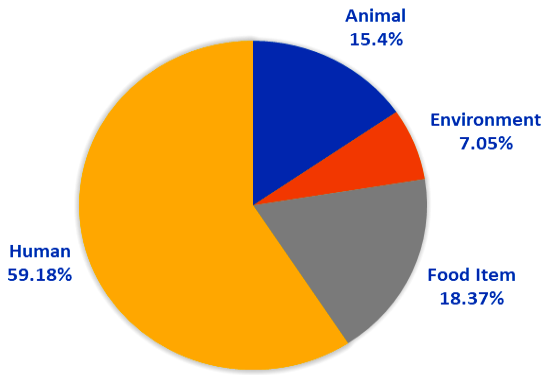


Fig. 5.3: Graphical presentation of NTS associated with different sources

Notably, the consistently high number of NTS isolates originated from human sources i.e., 319/539 (59.18%). Fig. 5.3, highlights the enduring public health challenge posed by NTS-related illnesses. This observation suggests that NTS infections in humans remain an ongoing concern, necessitating continuous efforts in terms of diagnosis, surveillance, and prevention strategies. These efforts are crucial not only for managing individual cases but also for curbing potential outbreaks and reducing the overall burden of NTS-related diseases in the population. The multifaceted variations in NTS distribution across different regions of India can be attributed to a complex interplay of ecological, environmental, host-related, healthcare-related, and cultural factors. Comprehensive epidemiological studies are essential to further elucidate the specific drivers behind these regional differences, enabling the development of targeted interventions for NTS control and prevention in each region.

Additionally, the occurrence of NTS in food items has significant implications for food safety protocols. The sizeable number of NTS isolates recovered from food items (99/539, 18.37%) (Fig. 5.3) highlights the pivotal role of safe food handling, processing, and distribution in preventing foodborne transmission. This observation underscores the critical need for stringent food safety regulations, robust monitoring of food supply chains, and public awareness campaigns to minimize the risk of NTS contamination in food items. Moreover, the collection of NTS isolates from animal sources (83/539, 15.4%) (Fig. 5.3) underlines the zoonotic potential of NTS infections. These isolates may originate from various animal reservoirs, including livestock and poultry. To effectively control the spread of disease, it's essential to grasp how NTS is transmitted between animals and humans, ensuring the implementation of comprehensive disease control measures. Strategies aimed at reducing NTS prevalence in animal populations and enhancing biosecurity measures in animals are crucial components of zoonotic disease prevention.

While the number of NTS isolates from environmental sources was comparatively lower (38/539, 7.05%) (Fig. 5.3), it emphasizes that environmental factors can still play a role in NTS transmission dynamics. This may involve contamination of water bodies, soil, or other ecological elements contributing to NTS transmission pathways. These findings accentuate the significance of considering environmental factors in the broader context of NTS epidemiology.

In summary, the multifaceted observations derived from the analysis of NTS isolates highlight the complex and interconnected nature of NTS infections. This complexity stresses the need for interdisciplinary approaches that encompass human health, food safety, animal health, and environmental factors. A holistic understanding of NTS transmission dynamics across these source categories is pivotal for shaping effective public health interventions, guiding policy decisions, and directing future research efforts in the field of NTS epidemiology and control.

5.2.2 Distribution of NTS Isolates across Different Human And Non-Human Sources

As mentioned earlier, the 539 NTS isolates were primarily sourced from various origins, categorically falling into human, animal, and food/environmental categories. Human sources encompassed a range of specimens, including blood, body fluids, urine, stool, pus, and tissue samples. Animal sources spanned samples obtained from poultry and livestock, including diarrheal, fecal, and post-mortem specimens. In parallel, food/environmental sources encompassed diverse origins, including raw milk from cows and buffaloes, raw pork, animal/poultry feed, and chicken meat, as well as eggs, and seafood such as fish and shrimp.

In Table 5.5, the distribution of Non-Typhoidal *Salmonella* (NTS) among various source categories has been presented, a central focus of this study. The data indicates a significant prevalence of distinct serovars, with *S. enterica* serovar Typhimurium emerging as the predominant serovar, constituting nearly 31% (n=167) of the total NTS (n=539) collection. Following closely, *S. enterica* serovar Lindenburg secures the second position, displaying a noteworthy isolation rate of 25% (n= 135), underscoring its prevalence in the sampled sources. Further examination of the table reveals that *S. enterica* serovar Enteritidis represents 10.4% (n=56) of the isolates, positioning it as the third most prevalent NTS serovar. Additionally, *S. enterica* serovar Weltevreden (n=44) and *S. enterica* serovar Choleraesuis (n=41) exhibit isolation rates of 8.2% and 7.6%, respectively, emphasizing their presence in the studied samples. Remarkably, the remaining 12 NTS serovars collectively contribute to nearly 22% of the total distribution.

Further, in-depth examination of serovar distribution revealed that *S. enterica* serovar Lindenburg (n= 103) and Typhimurium (n=89) were prominently represented in the isolates derived from human samples, exhibiting a substantial isolation rate of 32.3% and 27.9% (Table 5.5). This particular serovar prevalence within human contexts may suggest a specific association or transmission pattern relevant to human health. Further, the dominance of *S. enterica* serovar Typhimurium was also observed in both animal sources and food products, with isolation rates of 38.6% (n= 32) and 45.5%

(n=45), respectively. The prevalence of Typhimurium in these contexts raises questions about potential reservoirs, transmission routes, and food safety practices, warranting further investigation into the specific factors contributing to its prevalence in animals and food products. Meanwhile, the environmental samples painted a distinct picture, with *S. enterica* serovar Mathura taking centre stage and commanding an impressive isolation rate of 65.8%. This high prevalence in environmental samples raises concerns about potential contamination sources and highlights the need for targeted measures to mitigate the environmental spread of this particular serovar.

The nuanced variations in serovar prevalence across these diverse sample categories underscore the complexity of *Salmonella enterica* dynamics. Understanding the specific patterns within each context is crucial for implementing effective preventive measures tailored to the unique challenges presented by human, animal, food, and environmental sources. These findings not only contribute to the broader understanding of *Salmonella* epidemiology but also provide valuable insights for developing targeted strategies to enhance public health and food safety.

Table 5.5. Distribution of NTS isolates across different sources.

Name of the NTS isolates	No. of NTS across Different Source Categories N(%)				Total no. of NTS isolates N(%)
	Human	Animal	Environment	Food product	
S. enterica serovar Anatum	0	4 (4.8)	0	8 (8.1)	12 (2.2)
S. enterica serovar Bazenheid	0	4 (4.8)	0	0	4 (0.7)
S. enterica serovar Choleraesuis	40 (12.5)	0	0	1 (1.0)	**41 (7.6)**
S. enterica serovar Enteritidis	**50 (15.7)**	3 (3.6)	3 (7.9)	0	**56 (10.4)**
S. enterica serovar Hissar	0	0	4 (10.5)	0	4 (0.7)
S. enterica serovar Jaffna	5 (1.6)	0	5 (13.2)	0	10 (1.9)
S. enterica serovar Kentucky	6 (1.9)	0	0	0	6 (1.1)
S. enterica serovar Lindenburg	**103 (32.3)**	11 (13.3)	0	21 (21.2)	**135 (25)**
S. enterica serovar Mathura	1 (0.3)	7 (8.4)	**25 (65.8)**	0	33 (6.1)
S. enterica serovar Poona	3 (0.9)	0	0	0	3 (0.6)
S. enterica serovar Senftenberg	0	1 (1.2)	0	1 (1.0)	2 (0.4)
S. enterica serovar Stuttgart	0	8 (9.6)	0	0	8 (1.5)
S. enterica serovar Tennessee	0	4 (4.8)	0	5 (5.1)	9 (1.7)
S. enterica serovar Typhimurium	**89 (27.9)**	**32 (38.6)**	1 (2.6)	**45 (45.5)**	**167 (31)**
S. enterica serovar Ughelli	1 (0.3)	0	0	0	1 (0.2)
S. enterica serovar Virchow	0	0	0	4 (4.0)	4 (0.7)
S. enterica serovar Weltevreden	21 (6.6)	9 (10.8)	0	14 (14.1)	**44 (8.2)**
Total	**319 (59.2)**	**83 (15.4)**	**38 (7.1)**	**99 (18.37)**	**539**

5.2.2.1 NTS Across Human samples (n=319):

In this study, a detailed examination of 319 NTS isolates was conducted with meticulous attention to source distribution (Fig. 5.4). Notably, human fecal samples emerged as the dominant reservoir for NTS isolates, representing 63.9% (204 out of 319). Additionally, a substantial contribution of 22.8% (73 out of 319) from blood samples emphasizes the significance of understanding the clinical dimensions of NTS infections. The remaining sources amongst this group (urine, cerebrospinal fluid (CSF), pus, tissue, and body fluid samples) collectively yielded < 15% (40/319) of the total isolates. Notably, NTS from the cervical abscess and sputum samples presented the lowest count, contributing just a single isolate each. These diverse sources and distribution of NTS within human samples highlight the importance of source-specific analysis in understanding the epidemiology of NTS infections. These revelations lay a robust foundation for further exploration into the sources of contamination and potential routes of disease transmission.

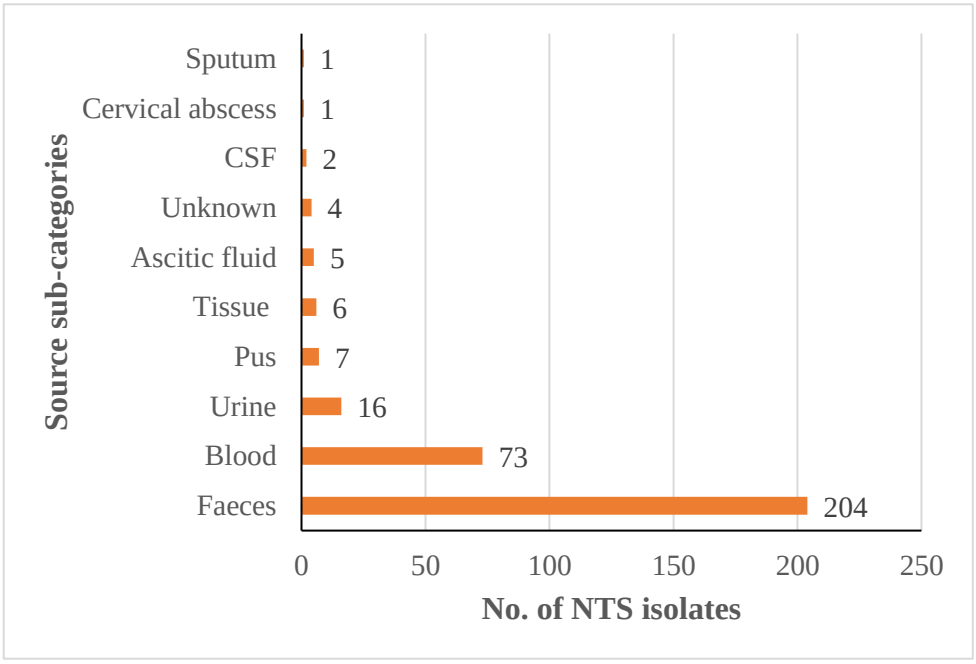


Fig. 5.4: Distribution of NTS across Human samples.

Amongst the 17 NTS serovars acquired in this study, it is notable that 10 of these serovars were present in the samples sourced from human-related origins (Fig. 5.5). Among these, two serovars, namely Lindenburg (103 out of 319, or 32.28%) and Typhimurium (89 out of 319, or 27.89%), stood out as the dominant serovars, collectively representing nearly 60% of the entire NTS collection from human sources. In contrast, serovars Enteritidis (50 out of 319, or 15.67%) and Choleraesuis (40 out of 319, or 12.54%) held a comparatively lesser pronounced presence, while the remaining six serovars collectively contributed to just under 11.6% (37 out of 319) of the total NTS collection from human sources.

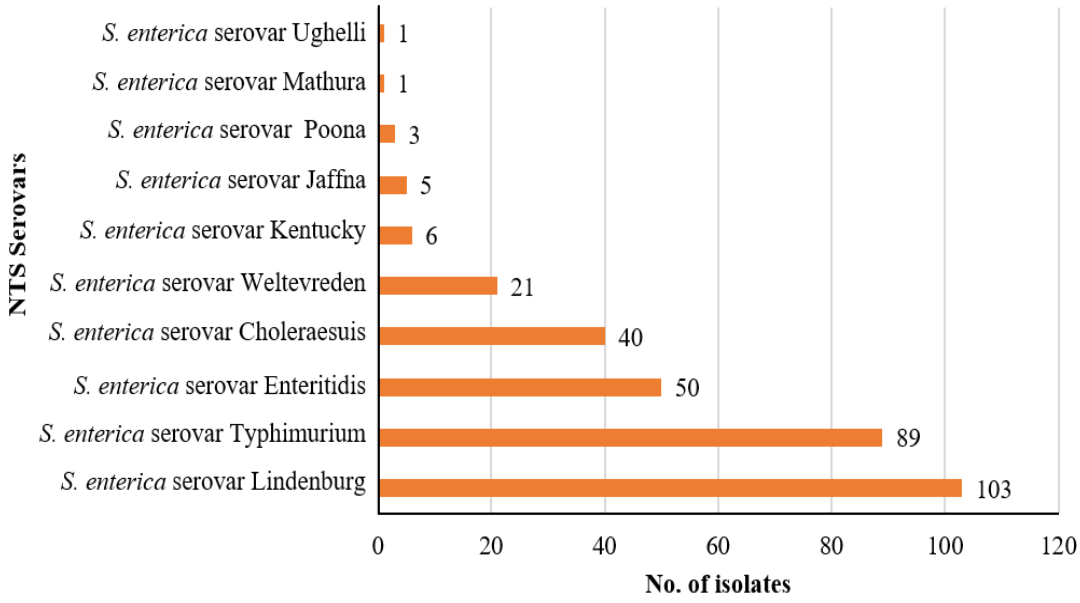


Fig. 5.5: Major NTS serovars isolated from Human Samples.

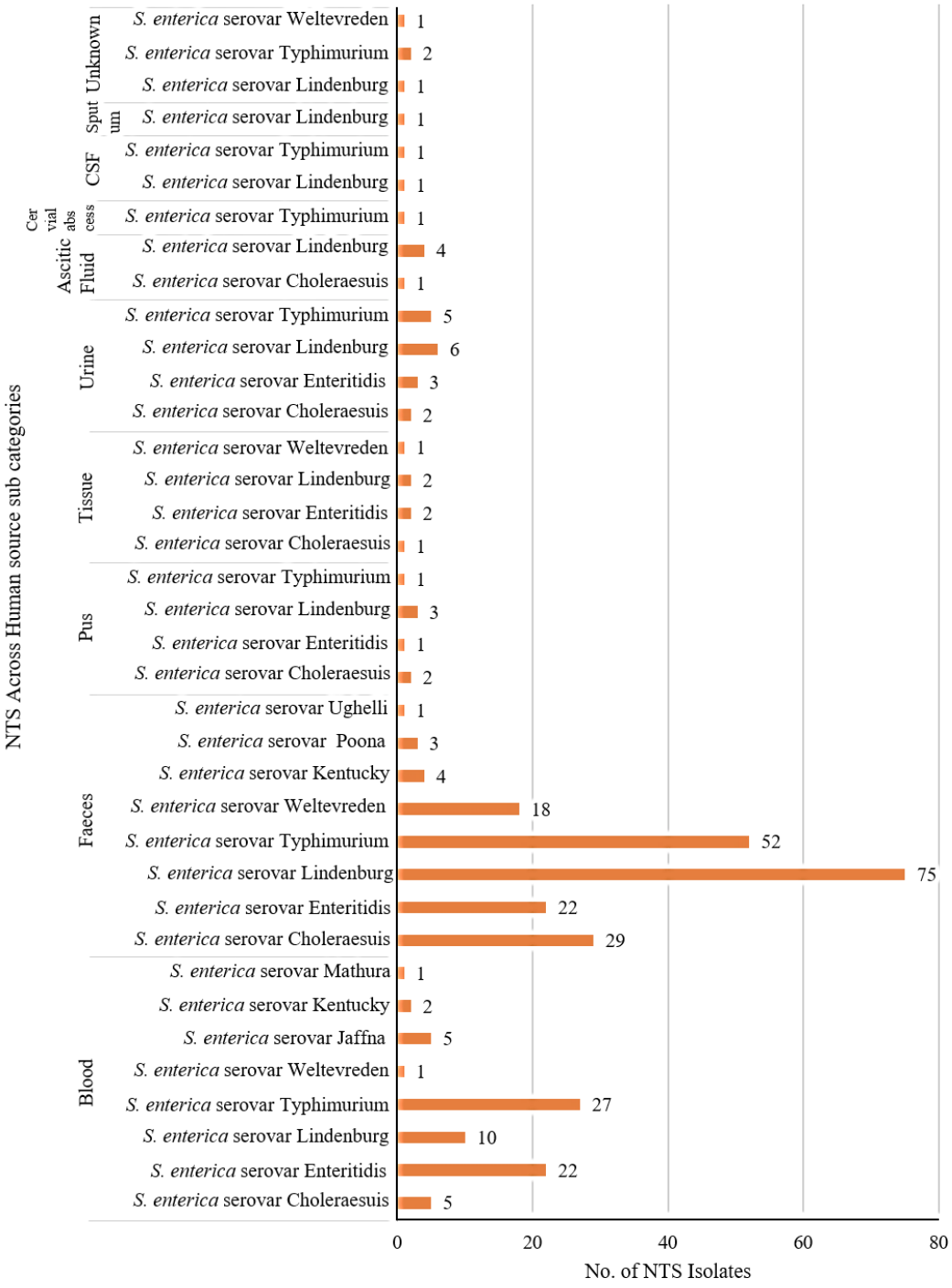


Fig. 5.6: Breakdown of Human NTS isolates across various sub-categories.

A further detailed analysis based on each source sub-category revealed that the serovars Lindenburg (75 out of 204, or 36.76%) and Typhimurium (52 out of 204, or 25.49%) collectively showed their dominance within the samples originating from fecal

sources (Fig. 5.6). These two serovars consistently exhibited their presence across a spectrum of sub-groups, encompassing blood, feces, pus, tissue samples, urine, ascitic fluid, cervical abscess, cerebrospinal fluid (CSF), and sputum. This remarkable consistency underscores their versatility in terms of different infection sites within the body and varied sources of isolation. Consequently, these findings illuminate the diverse nature of these serovars, which exhibit a propensity to thrive and manifest in a multitude of clinical and isolation contexts.

Notably, a single isolate of *S. enterica* serovar Mathura has been isolated from human blood (Fig. 5.6). This is a very crucial finding of isolation of *S. enterica* serovar Mathura in human cases. Despite this, the available data does not offer conclusive insights into the transmission sources of this serovar to humans. Remarkably, there is no prior documentation in the literature, either from India or other countries, about the isolation of *S. enterica* serovar Mathura from humans. Although the incidence rate observed in this study is relatively low, it emphasizes the imperative need for additional investigations to comprehensively explore the epidemiological significance of this particular NTS serovar in human infections. Future studies should aim to unravel the potential sources, transmission dynamics, and clinical implications associated with *S. enterica* serovar Mathura in human populations.

5.2.2.2 NTS from Animal sources (n=83):

Non-Typhoidal *Salmonella* (NTS) isolates obtained from various animal sources are shedding light on the diverse origins of these isolates (Fig. 5.7). Among these, 43 (51.8%) NTS isolates were derived from animal fecal samples, underlining the presence of NTS in the gastrointestinal tracts of animals. Poultry feces contributed 11 (13.3%) NTS isolates, emphasizing the relevance of avian sources in NTS epidemiology. Additionally, 7 (8.4%) NTS isolates were sourced directly from poultry specimens, indicating the potential for NTS transmission from poultry to humans. Notably, 01 NTS isolate was obtained from calf fetal stomach content, suggesting the presence of NTS in young livestock. Furthermore, 4 NTS isolates were extracted from poultry diarrhoea samples, highlighting the importance of monitoring and addressing NTS infection in the poultry populations.

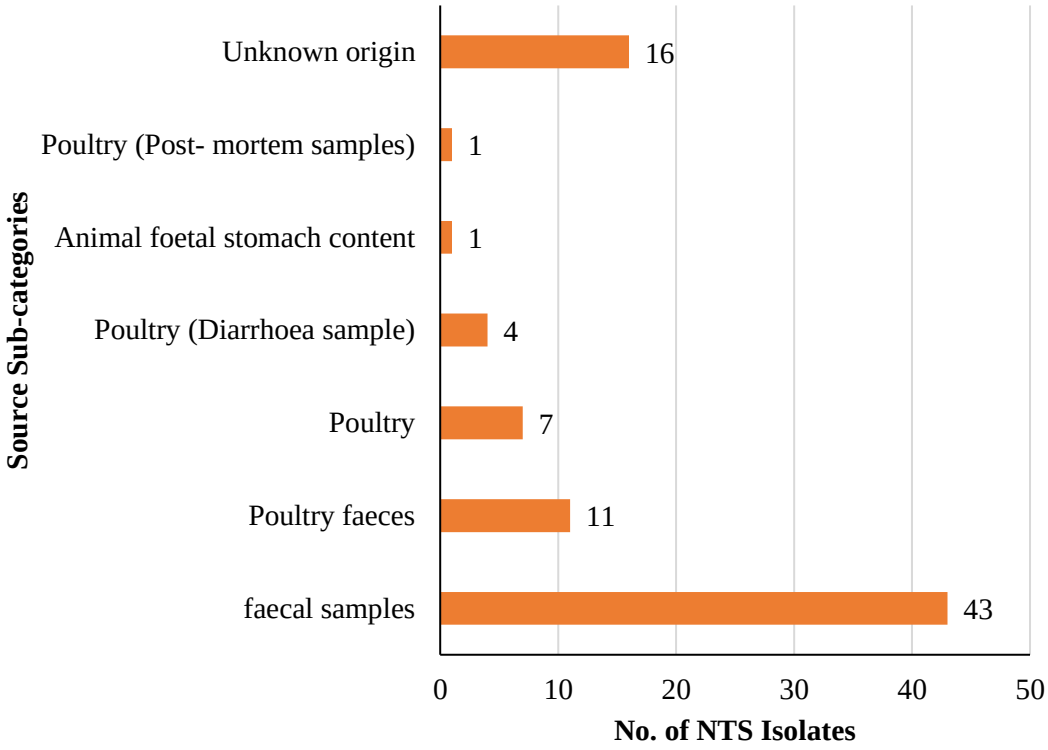


Fig. 5.7. Distribution of NTS across samples from Animal sources.

It's worth mentioning that 16 (19.3%) NTS isolates originated from samples with an unknown site within the animal category, which underscores the need for precise sample tracking and source identification in epidemiological studies. Collectively, these findings illustrate the diverse animal sources of NTS and emphasize the significance of understanding NTS prevalence and transmission dynamics within both domestic and wild animal populations.

The analysis revealed the isolation of a diverse range of NTS serovars from animal sources, with a total of 10 out of the 17 NTS serovars identified in the present study. The analysis also revealed a very high prevalence of *S. enterica* serovar Typhimurium (32/83, 38.6%) (Fig. 5.8), raising significant public health concerns due to its known zoonotic potential and association with severe human infections.

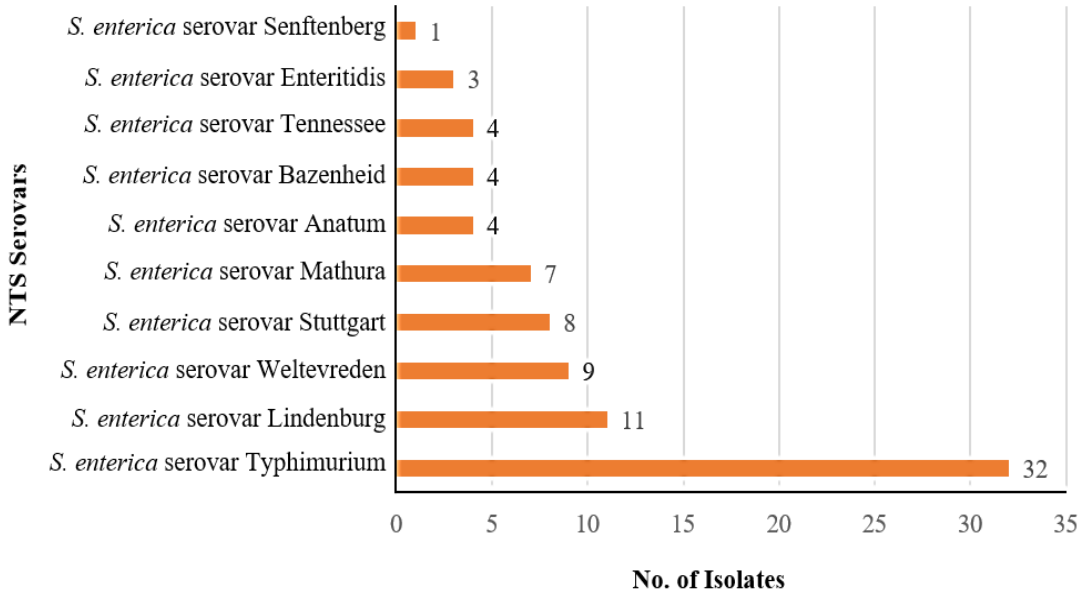


Fig. 5.8. Distribution of NTS serovars across animal sources.

The presence of *S. enterica* serovar Lindenburg (11/83, 13.3%) as the second most common, alongside notable occurrences of *S. enterica* serovar Weltevreden (9/83, 10.8%) and *S. enterica* serovar Stuttgart (8/83, 9.6%), highlights the diversity of *Salmonella* serovars circulating in the animals. In addition to the prominent serovars discussed above, the analysis revealed the presence of a few more NTS serovars associated with the animal samples. Noteworthy among them were *S. enterica* serovar Mathura, Anatum, Bazenheid, Tennessee, Enteritidis, and Senftenberg, collectively representing 27.7% (23/83) of the total NTS serovars from animals. Notably, a majority of these were traced back to samples of animal fecal origin (Fig. 5.9). This comprehensive insight into the prevalence and diversity of *Salmonella* serovars emphasizes the importance of ongoing surveillance and targeted measures to mitigate the potential hazards associated with zoonotic transmission. Understanding the distribution of these specific NTS serovars is pivotal for a comprehensive assessment of *Salmonella* diversity within the animal samples. Each serovar may possess distinct characteristics and potential public health implications, necessitating further investigation to elucidate their prevalence, sources, and transmission dynamics.

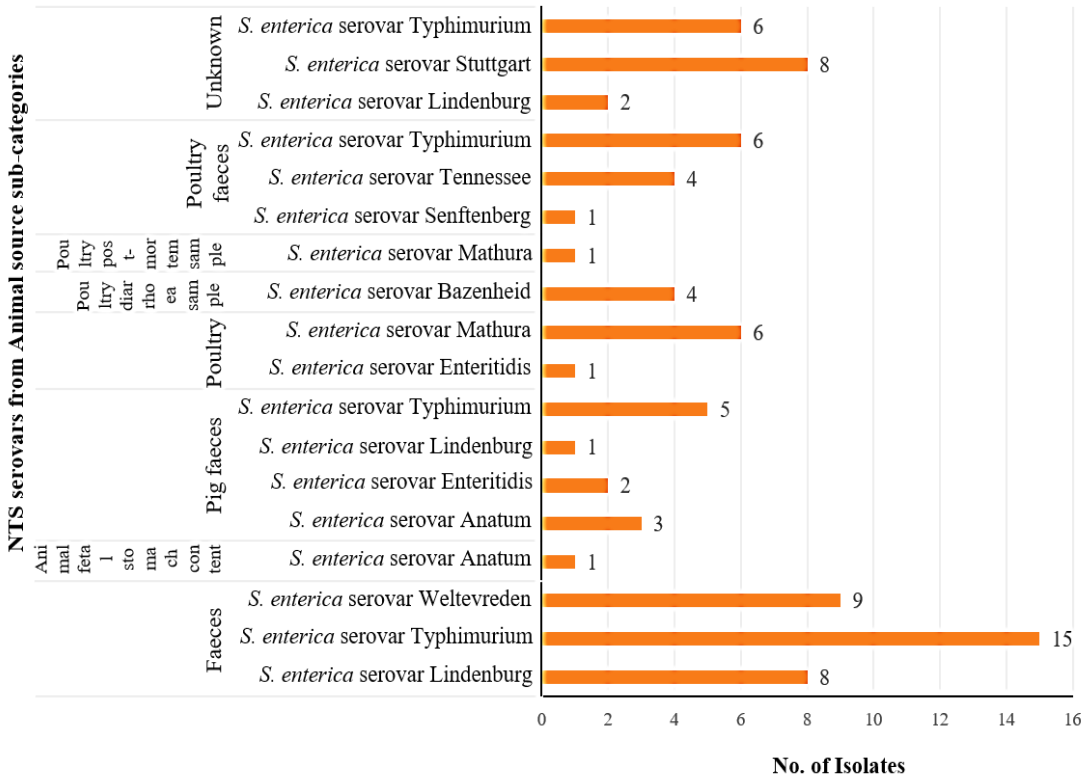


Fig. 5.9. Categorization of NTS isolates based on different animal sub-categories.

This all-inclusive insight into the spectrum of NTS serovars contributes valuable information for targeted surveillance and the development of tailored strategies aimed at mitigating the risk of *Salmonella* infections in both animal and human populations.

5.2.2.3 NTS from Food (n=99):

The analysis of NTS isolates from different food items revealed distinct patterns in terms of their distribution (Fig.5.10). Notably, the highest number of NTS isolates, totaling 32 (32.3%), were recovered from raw pork, signifying a notable presence of NTS in this meat source. Raw milk samples from cows or buffalo yielded 13 (13.1%) NTS isolates, underscoring the significance of pasteurization and stringent hygiene practices in milk production.

Seafood, another significant category, contributed 12 (12.1%) NTS isolates, emphasizing the need for vigilant handling and safety practices in the seafood industry. Animal feed contributed 12 (12.1%) NTS isolates, emphasizing the need for quality control in animal nutrition. Furthermore, the category "Meat (fish, pork, beef)"

encompassed 11 (11.1%) NTS isolates, reflecting the diversity of meat sources that may harbor NTS and the importance of proper handling and cooking. Poultry meat samples were found to contribute 6 (6.1%) NTS isolates, highlighting the importance of thorough cooking to eliminate potential pathogens. Intriguingly, one NTS isolate was found in poultry feed, suggesting a possible route for NTS contamination in poultry production through feed sources, necessitating rigorous quality control measures.

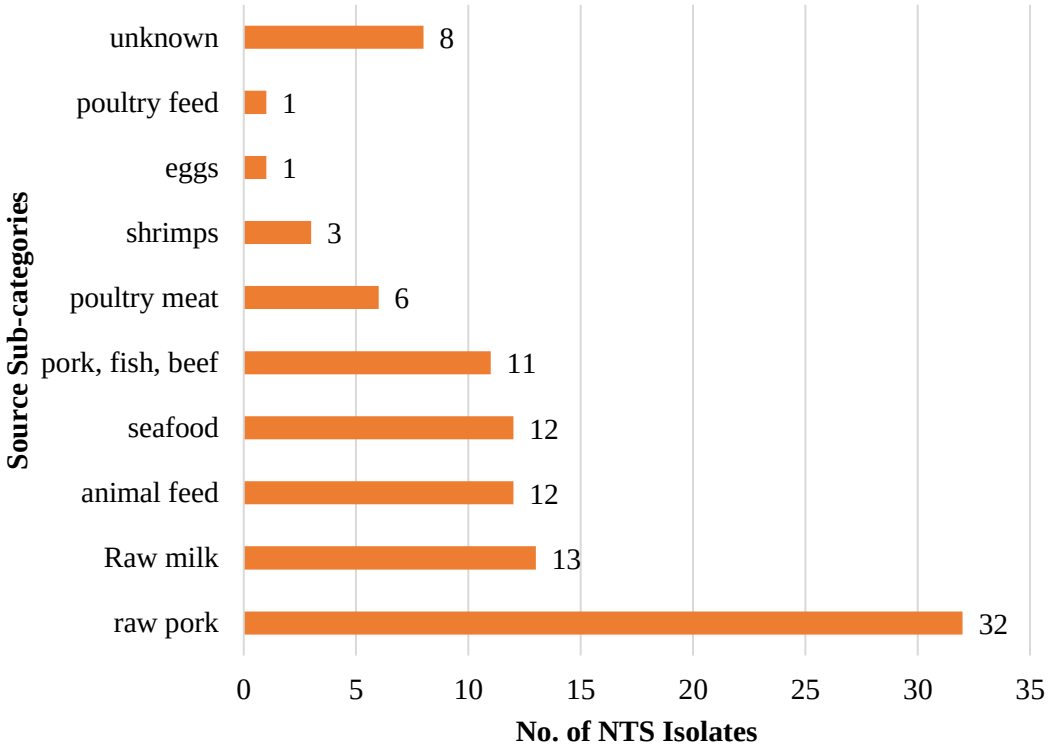


Fig. 5.10: Distribution of NTS across Food Samples.

Shrimp samples accounted for 3 NTS isolates, while a single NTS isolate was identified in eggs, emphasizing the importance of safe handling and cooking practices, particularly for raw or undercooked eggs.

These findings highlight the critical role of food safety measures, such as thorough cooking, hygiene, and quality control in food production, to mitigate the risk of NTS-related infections associated with various food items. Regular monitoring and surveillance of NTS contamination in food sources are paramount for public health protection and safety.

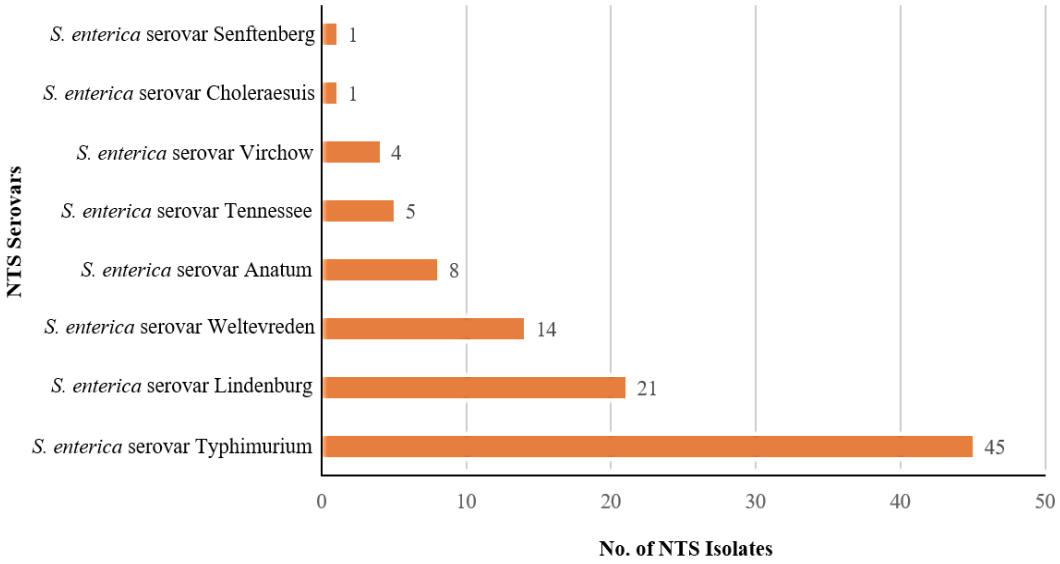


Fig. 5.11. NTS serovars isolated from Food Samples.

Upon analyzing the NTS data gathered from food samples, a notable trend emerged, indicating that *S. enterica* serovar Typhimurium stands out as the predominant serovar. As illustrated in Fig. 5.11, this serovar displayed a significant occurrence rate, representing 45.4% (45 out of 99 samples) of the total isolates. Remarkably, *S. enterica* serovar Typhimurium showcased a widespread presence across a diverse spectrum of food items. This included eggs, shrimps, animal feed items, pork, fish, beef, and various seafood, suggesting a versatile contamination pattern (Fig. 5.12). The prevalence of serovar Typhimurium in such a broad range of food products underscores the importance of thorough food safety measures to address and mitigate the impact of this particular *Salmonella* serovar across diverse food categories.

S. enterica serovar Lindenburg emerged as the second most prevalent serovar, with an isolation rate of 21.2% (21/99) (Fig. 5.11). This serovar was notably found in food items such as raw milk, poultry meat, and seafood. While, in 8 instances of *S. enterica* serovar Lindenburg isolates, the specific origin remained unknown or unspecified (Fig. 5.12). This lack of information regarding the source or origin of the isolate highlights a gap in the data, indicating a need for further investigation and detailed surveillance to determine the potential reservoirs and transmission pathways of this NTS serovar.

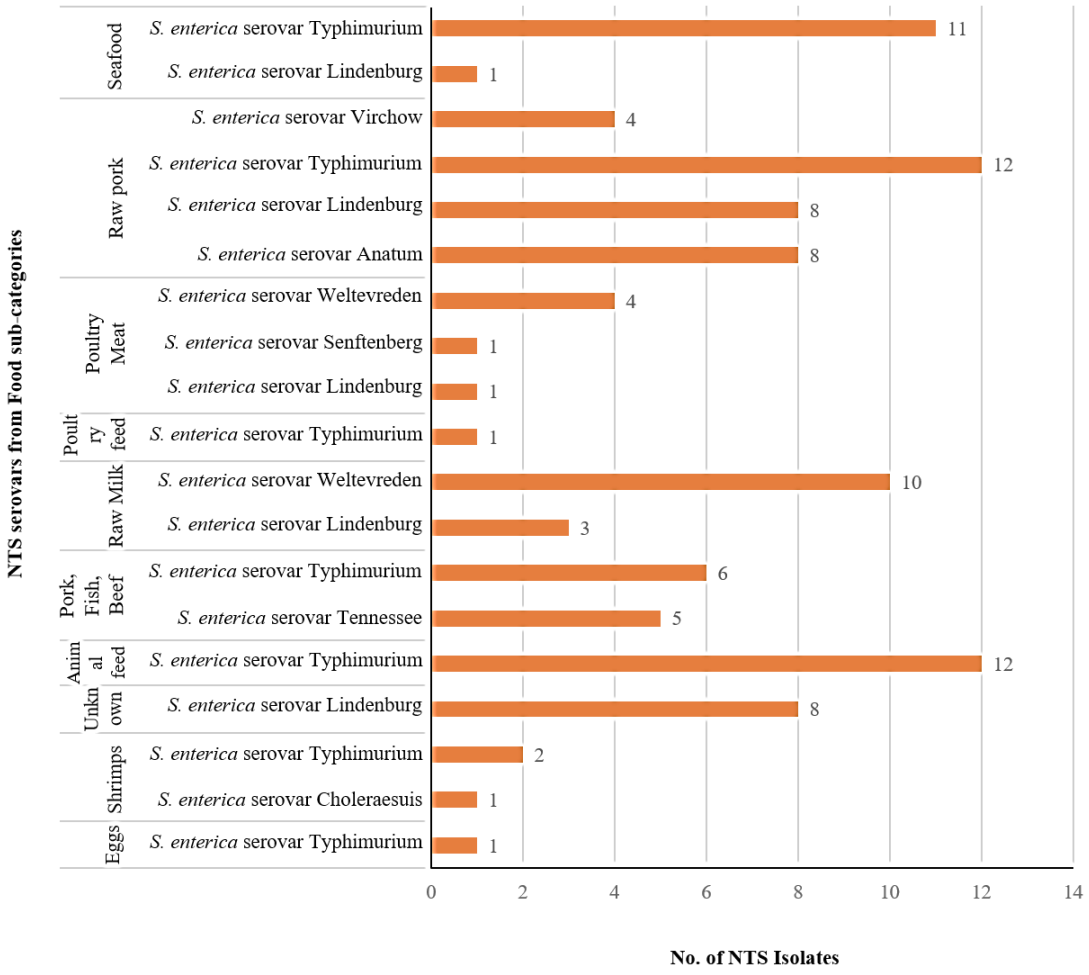


Fig. 5.12. Breakdown of NTS serovars across various food sub-categories.

5.2.2.4 NTS from Environment (n= 38):

Among the 539 NTS isolates examined, a subset of 38 isolates was derived from environmental sources, constituting approximately 7.05% of the total isolates. While this percentage may appear relatively low, still it carries significant implications for food safety and public health. Within the environmental sources, specific categories unveiled distinct patterns (Fig. 5.13).

Fig. 5.13: Distribution of NTS across Environment Samples.

Notably, the presence of NTS in poultry environments, with four isolates identified, suggests that conditions within poultry settings may facilitate NTS survival and transmission. Also, two NTS isolates were recovered from meat shop floor samples, emphasizing the importance of meticulous hygiene in food retail establishments. The presence of NTS on meat-cutting knives (two isolates) underscores the risk of cross-contamination during handling of meat, necessitating stringent sanitation practices. Most notably, the category of animal fodder environments yielded the highest number of NTS isolates, totalling 30. This finding highlights the significance of animal feed environments as a reservoir for NTS, potentially affecting livestock health and food production chains. These diverse origins of NTS contamination highlight the need for comprehensive surveillance and mitigation strategies in various environmental settings to ensure food safety and protect public health.

The investigation delved into the isolation of various *Salmonella enterica* serovars from a range of environmental sources associated with food handling and storage. Notably, a substantial prevalence was observed in locations linked to animal food storage, with 25 isolates identified as *S. enterica* serovar Mathura and 5 isolates as *S. enterica* serovar Jaffna (Fig. 5.14). The presence of these serovars in areas pertinent to animal food storage signals potential sources of contamination, necessitating targeted interventions to uphold food safety standards in these environments.

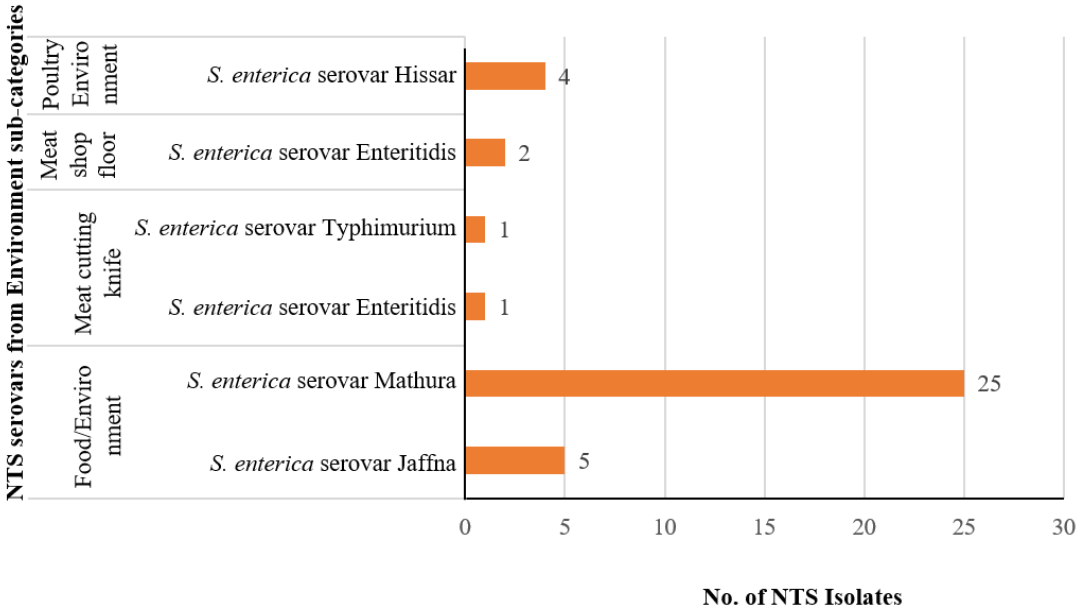


Fig. 5.14: NTS serovars isolated from Environmental sources.

Within meat-cutting areas, intriguing findings emerged. A single isolate of each of the two serovars viz *S. enterica* serovar Typhimurium and Enteritidis was isolated from meat cutting knives. This discovery prompts inquiries into the hygiene practices of meat processing and raises concerns about the potential introduction of specific serovars through equipment or handling procedures. A comprehensive understanding of *Salmonella* contamination dynamics in meat processing environments becomes imperative for instituting effective control measures to forestall the dissemination of these pathogens.

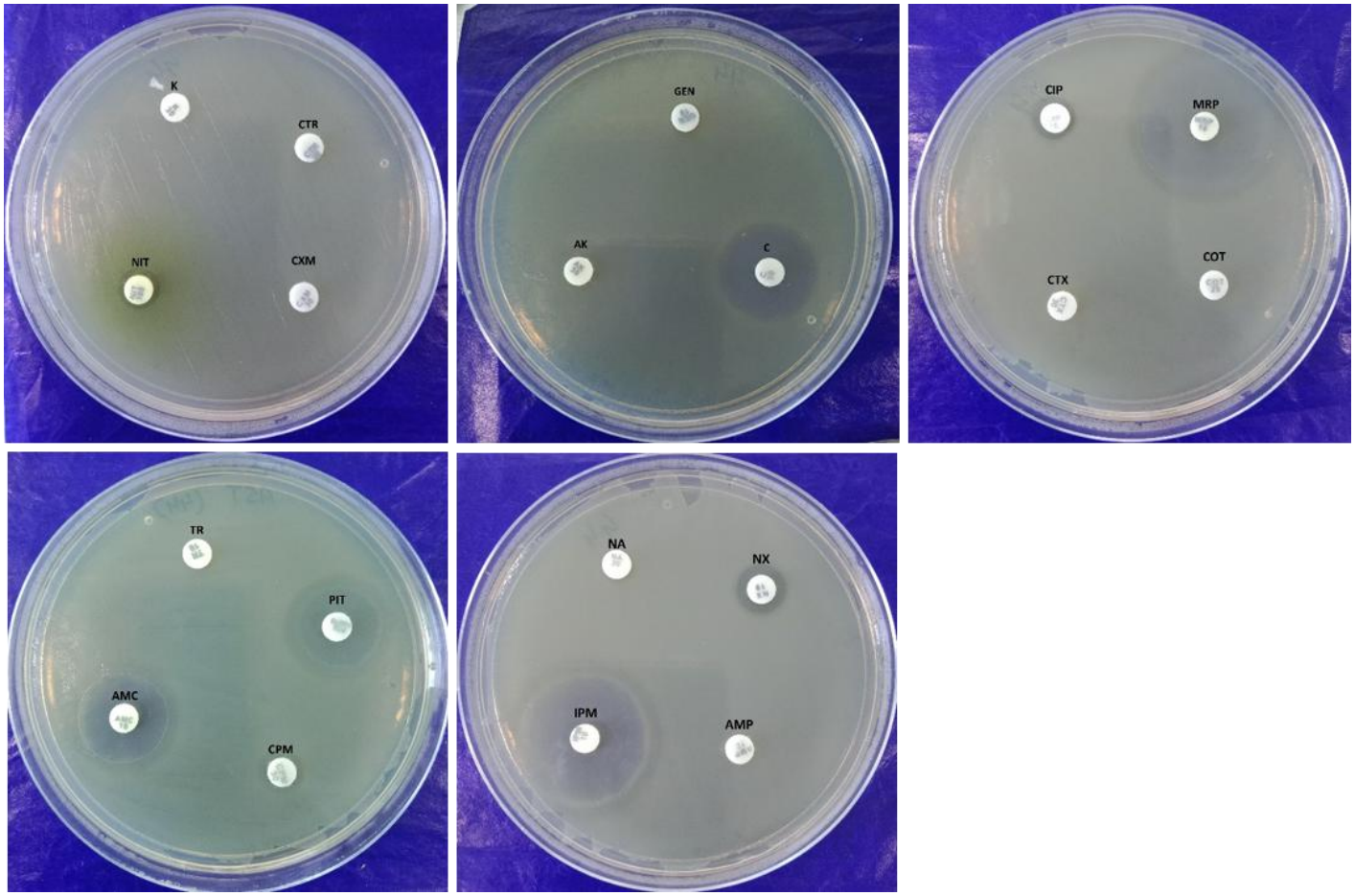
Further exploration of meat shop environments revealed additional insights. Two samples from the meat shop floor were found to harbour *S. enterica* serovar Enteritidis. The identification of this serovar on the floor of a meat shop suggests the potential for cross-contamination, emphasizing the critical need for stringent hygiene standards throughout the meat handling process. This finding emphasizes the importance of targeted sanitation measures and employee training to minimize the risk of *Salmonella* contamination. in retail settings.

In the poultry setting, the research revealed the detection of serovar Hissar in four samples. The presence of this serovar in samples linked to poultry raises concerns regarding biosecurity measures and the potential spread of *Salmonella* within the poultry production process. Considering the public health implications of *Salmonella* contamination in poultry products, it is crucial to understand and address the factors contributing to the presence of *S. enterica* serovar Hissar in poultry environments to mitigate risks to both animal and human health.

In summary, the study offers a comprehensive examination of the distribution of various *Salmonella enterica* serovars across various environmental sources. The results highlight the importance of implementing improved hygiene practices in specific food handling and storage facilities to enhance overall food safety. The identification of specific serovars in each environment provides valuable insights for future research and intervention strategies aimed at reducing the prevalence of *Salmonella* in the food supply chain.

5.3 Antibiogram Profiling of NTS:

The antibiogram profiling of the 539 NTS isolates was done using the standard disk diffusion method (Photograph 1) as per CLSI guidelines (CLSI 2014, CLSI 2022). In a comprehensive analysis of antibiotic resistance patterns in Non-Typhoidal *Salmonella* (NTS), a concerning trend emerged, reflecting a substantial level of resistance among the studied strains. Table 5.6 provides a clearer perspective on the varying degrees of resistance among the 19 antibiotics tested against 539 NTS isolates.



Photograph 1: Antimicrobial susceptibility testing of NTS by disk diffusion method.

As is evident from Table 5.6, cefotaxime emerged with the highest resistance, occupying the primary position. A concerning 44.90% (242 out of 539) of Non-Typhoidal *Salmonella* (NTS) strains exhibited resistance to this crucial antibiotic. Nalidixic acid and piperacillin/tazobactam followed closely, with resistance rates of 39.89% and 27.27%, respectively, indicating a widespread challenge in combating NTS infections.

Table 5.6: Antibiotic Susceptibility Profile of NTS Isolates: Ranking Based on Drug Resistance.

S. No.	Name of the antimicrobial agent	Resistant N(%)	Intermediate N(%)	Sensitive N(%)	Rank
1.	Cefotaxime	242 (44.9)	207 (38.4)	90 (16.7)	1
2.	Nalidixic acid	215 (39.9)	59 (10.9)	265 (49.***2***)	2
3.	Piperacillin/tazobactam	147 (27.***3***)	260 (48.2)	132 (24.***5***)	3
4.	Ampicillin	146 (27.***1***)	54 (10.0)	339 (62.***9***)	4
5.	Amoxycillin/clavulanic acid	140 (25.9)	58 (10.***8***)	341 (63.***3***)	5
6.	Trimethoprim	106 (19.7)	18 (3.3)	415 (76.9)	6
7.	Nitrofurantoin	94 (17.4)	88 (16.3)	357 (66.***2***)	7
8.	Co-trimoxazole	78 (14.***5***)	21 (3.90)	440 (81.6)	8
9.	Imipenem	74 (13.7)	141 (26.***2***)	324 (60.1)	9
10.	Kanamycin	65 (12.***1***)	184 (34.1)	290 (53.8)	10
11.	Ceftriaxone	64 (11.***9***)	167 (30.9)	308 (57.1)	11
12.	Ciprofloxacin	60 (11.1)	108 (20.0)	371 (68.8)	12.5
13.	Cefuroxime sodium	60 (11.1)	136 (25.2)	343 (63.6)	12.5
14.	Meropenem	55 (10.2)	159 (29.5)	325 (60.3)	14
15.	Gentamicin	33 (6.1)	38 (7.***1***)	468 (86.8)	15.5
16.	Norfloxacin	33 (6.1)	44 (8.***2***)	462 (85.7)	15.5
17.	Amikacin	32 (5.9)	71 (13.***2***)	436 (80.***9***)	17
18.	Cefepime	9 (1.***7***)	29 (5.***4***)	501 (92.9)	18
19.	Chloramphenicol	5 (0.9)	51 (9.***5***)	483 (89.6)	19

The resistance profile extended to commonly prescribed antibiotics, such as ampicillin (27.1%), amoxicillin/clavulanic acid (25.9%), and trimethoprim (19.7%). Further down the list, nitrofurantoin displayed a resistance rate of 17.4%, and co-trimoxazole exhibited a rate of 14.5%, indicating moderate levels of resistance. Furthermore, the data revealed noteworthy resistance to key antibiotics like ciprofloxacin (11.1%), imipenem (13.7%), and ceftriaxone (11.87%), which are crucial for treating severe bacterial infections. Conversely, some antibiotics exhibited lower resistance rates, including chloramphenicol (0.9%) and cefepime (1.7%).

Cefotaxime, a third-generation cephalosporin antibiotic, has traditionally been employed in the treatment of various *Salmonella* infections, as indicated by the existing literature. Successful applications of cefotaxime include treating diverse *Salmonella* infections such as typhoid fever, paratyphoid fever, osteomyelitis, and meningitis (Dolan et al., 1987; Soe and Overturf, 1987; Ekinci et al., 2002). However, there have been documented instances of *Salmonella enterica* strains demonstrating resistance to cefotaxime (Sriyapai et al., 2022; Yang et al., 2023; Sallam et al., 2023; Sasaki et al., 2023). In the present study, a detailed analysis of cefotaxime-resistant NTS isolates revealed that human sources contributed the highest percentage, with 162 out of 242 instances, constituting nearly 67%. Additionally, 17.8% of these resistant strains originated from food items (43 out of 242) (Fig. 5.15). Notably, cephalosporins, specifically the fourth-generation drug (cefepime), demonstrated significantly higher efficacy, with a minimal number of NTS strains exhibiting resistance to this particular antimicrobial drug.

Beta-lactam antibiotics, encompassing penicillins, cephalosporins, and carbapenems, have traditionally served as a primary therapeutic approach for *Salmonella* infections (Pandey and Cascella, 2023). Despite their status as the preferred treatment for salmonellosis, a considerable number of clinically resistant isolates have emerged (Diab et al., 2023; Yousefi-Mashouf and Moshtaghi, 2023; Jeamsripong et al., 2023). The widespread use of beta-lactam antibiotics has further contributed to the rise of *Salmonella* strains resistant to these antibiotics, thereby diminishing their effectiveness (Cebrián et al., 2006; Zeng and Lin, 2013). Consistent with these observations, the current study delineates a parallel trend. The documented resistance

profiles of Non-Typhoidal *Salmonella* (NTS) to Piperacillin/tazobactam (27.3%), Ampicillin (27.1%), Amoxicillin/clavulanic acid (25.9% resistance), Imipenem (13.7%), and Meropenem (10.2%) pose significant challenges in the realm of antimicrobial therapy (Fig. 5.15). These percentages underscore a substantial subset of NTS strains exhibiting reduced susceptibility to these antibiotics. Furthermore, an in-depth analysis of resistance patterns in NTS from diverse sources reveals that isolates from human origins exhibit the highest resistance, followed by those from food and animal sources (Fig. 5.15). This highlights varying degrees of resistance in NTS across different reservoirs, underscoring the importance of understanding and addressing antibiotic resistance in both clinical and non-clinical settings. Potential contributors to this resistance include the misuse or overuse of these agents, selective pressures favouring the propagation of resistant strains, and intrinsic bacterial mechanisms conferring resistance.

Shifting the focus to the next pivotal category of broad-spectrum antimicrobial drugs—fluoroquinolones and quinolones—these agents are commonly employed in treating infections caused by NTS and various other pathogens (Eguale et al., 2017). Fluoroquinolones, particularly ciprofloxacin, and ofloxacin, are effective drugs for treating both Typhoidal and non-Typhoidal salmonellosis (Shane et al., 2017; Shaheen et al., 2021). Quinolones and fluoroquinolones are a class of antibacterial drugs that work by inhibiting the activity of two essential bacterial enzymes viz. DNA gyrase and topoisomerase IV (Topo IV) (Fàbrega et al., 2009; Correia et al., 2017). Although quinolones and fluoroquinolones are known for their efficacy, they have been linked to the development of bacterial resistance, potentially undermining their ability to effectively treat a range of bacterial infections (Hooper and Jacoby, 2016).

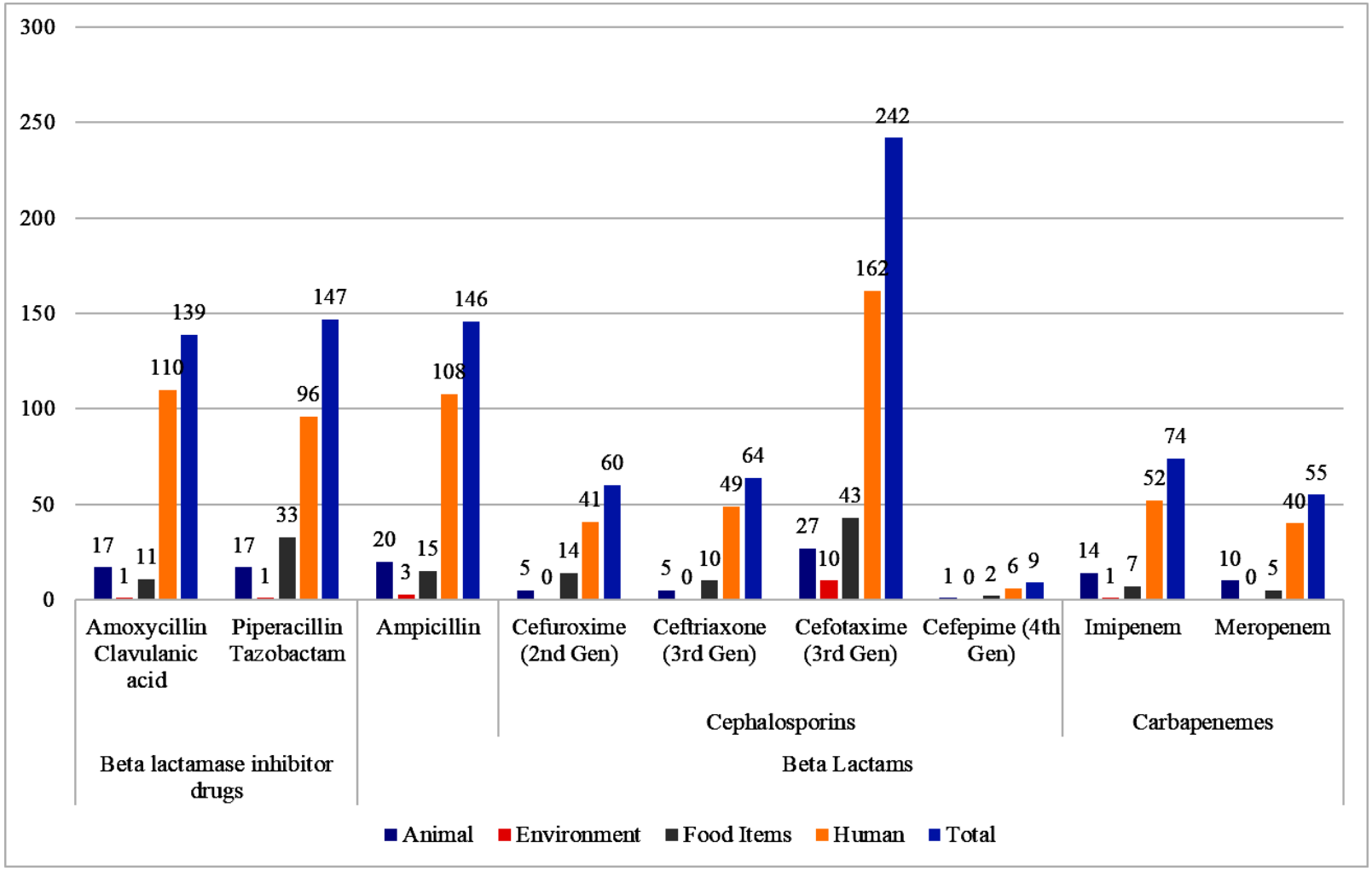


Fig. 5.15: Resistance profile of NTS from different sources against Beta Lactams & Beta-lactamase inhibitors drugs.

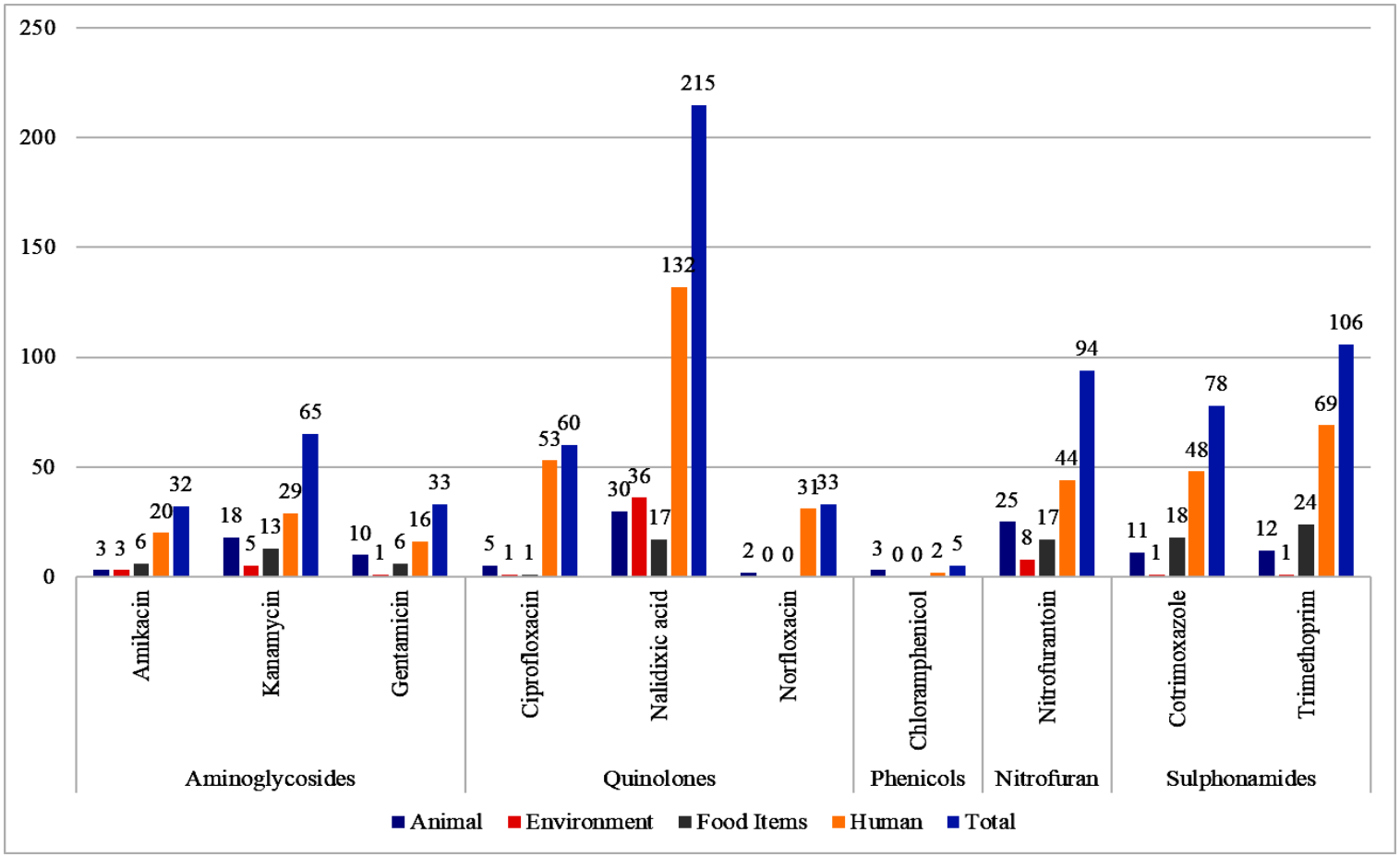


Fig. 5.16: Resistance profile of NTS from different sources against Aminoglycosides and Quinolones/Fluoroquinolones, Phenicols, Nitrofurans, and Sulphonamide.

In the present research, a noteworthy level of nalidixic acid resistant NTS isolates has been identified, securing the second position in the context of this study, as detailed in Table 5.6. Among the 215 NTS isolates exhibiting resistance to nalidixic acid, a predominant fraction—132 NTS isolates—originated from human sources, as depicted in Fig. 5.16. This emphasizes a concerning trend of nalidixic acid resistance among NTS strains prevalent in human populations. Moreover, the NTS isolates from environmental (36/215) and animal (30/215) sources also demonstrated a significant resistance profile to nalidixic acid, indicating the potential dissemination of resistant strains in non-human reservoirs. Notably, a smaller subset, comprising 17 out of 215 isolates from food sources, also exhibited resistance to nalidixic acid, shedding light on the broader spectrum of reservoirs contributing to resistance development. Furthermore, a substantial proportion of NTS isolates were found resistant to key fluoroquinolone antibiotics, with 53 out of 60 isolates displaying resistance to ciprofloxacin and 31 out of 33 isolates showing resistance to norfloxacin. These findings underscore a concerning trend of reduced susceptibility to fluoroquinolones among the studied isolates. The observed resistance to ciprofloxacin and norfloxacin, which are regularly used drugs in clinical settings, highlights the potential challenges in treating infections caused by these resistant strains.

Furthermore, an in-depth analysis of the resistance pattern exhibited by Non-Typhoidal *Salmonella* (NTS) towards 19 antimicrobial drugs reveals a notable lack of effectiveness, towards sulphonamides and nitrofurans, and to a lesser extent, aminoglycosides. Specifically, within the sulphonamides class, the drugs cotrimoxazole and trimethoprim demonstrated significant levels of resistance. Cotrimoxazole resistance was observed in a total of ***78 (14.***5***%)*** isolates, with 106 (19.7%) isolates exhibiting resistance to trimethoprim (Table 5.6). This resistance trend is further emphasized within the sulphonamides category, with a majority of the resistant isolates originating from the human category, as illustrated in Fig. 5.16.

Resistance to nitrofurantoin was observed in a total of 94 isolates, accounting for 17.4% of the total NTS tested. Notably, within this subset, 46.8% (44 out of 94) of the

resistant isolates originated from human sources, as depicted in Fig. 5.16, while 26.6% (25 out of 94) were traced back to animal sources. This resistance profile suggests a significant presence of nitrofurantoin-resistant Non-Typhoidal *Salmonella* (NTS) strains, emphasizing the need for targeted strategies to address resistance in both human and animal reservoirs.

These findings underscore the diminished efficacy of certain antimicrobial drugs against NTS strains and highlight the urgency of understanding and addressing resistance patterns, especially in the context of human infections, to guide effective therapeutic strategies and mitigate the broader challenge of antimicrobial resistance.

The global occurrence of drug resistance in non-Typhoidal *Salmonella* (NTS) presents a mounting concern with substantial implications for public health (Tack et al., 2020; Yousefi-Mashouf and Moshtaghi, 2023; Tiew et al., 2022; Kong-Ngoen et al., 2022; Yin et al., 2022). The widespread emergence of antibiotic resistance among NTS isolates has created a challenging scenario, significantly restricting the array of antibiotics available for effective therapeutic interventions. This limitation poses a serious threat to the successful treatment of severe NTS infections and necessitates urgent attention and strategic measures to address the escalating issue of antimicrobial resistance.

5.4 Multidrug resistance among the NTS isolates:

The escalating prevalence of multidrug-resistant (MDR) non-Typhoidal *Salmonella* (NTS) poses a substantial and rising threat to public health, carrying significant implications for the effectiveness of treatment strategies. Studies have unveiled alarming rates of resistance, exemplified by a notable 25% resistance rate to ceftriaxone in specific regions (Hengkrawit and Tangjade, 2022). This resistance trend underscores the formidable challenge of effectively addressing MDR-NTS infections. The issue of multidrug resistance extends beyond human populations, especially within animal hosts. The context of animal reservoirs becomes particularly significant, as it adds a layer of complexity to the dynamics of resistance spread (An et al., 2017). The interconnectedness between human and animal health emphasizes the need for a holistic approach to tackling

multidrug resistance, considering the potential interplay and transmission of resistant strains across different ecosystems.

The present study provides a comprehensive view of the drug resistance profiles (both multiple drug resistance and extensive drug resistance) exhibited by NTS strains, providing insights into their resistance patterns against a combination of antibiotics. Table 5.7 categorizes these strains based on their regional origin and the number of drugs to which they demonstrated resistance. Notably, the southern part of the country stands out, displaying the highest percentage of resistance across all drug groups, which may be linked to the larger number of NTS isolates obtained from this region compared to others.

The data underscores a distinct regional variation, with the Southern and Northern regions showing a notably higher prevalence of MDR and elevated levels of drug resistance in NTS. This observation is further emphasized by the remarkably high resistance profiles in both regions, where nearly twenty NTS isolates demonstrated resistance against 10 or more drugs. Conversely, the contributions from other regions appear comparatively lower.

Table 5.7: Region-wise resistance profile of different NTS against a combination of drugs.

No. of resistant drugs	Indian Regions					Total
	Central N(%)	East N(%)	North N(%)	South N(%)	West N(%)	
0- Drug	1(50.0)	6(16.2)	32(32.7)	60(19.2)	15(16.9)	**114(21.2)**
1-Drug	0	5(13.5)	16(16.3)	56(17.9)	31(34.8)	**108(20.0)**
2-Drugs	1(50.0)	4(10.8)	13(13.3)	46(14.7)	19(21.4)	**83(15.4)**
3-Drugs	0	3(8.1)	9(9.2)	24(7.7)	8(9.0)	**44(8.2)**
4-Drugs	0	0	8(8.2)	22(7.0)	5(5.6)	**35(6.5)**
5-Drugs	0	2(5.4)	10(10.2)	20(6.4)	4(4.5)	**36(6.7)**
6-Drugs	0	2(5.4)	2(2.0)	34(10.8)	3(3.4)	**41(7.6)**
7-Drugs	0	6(16.2)	4(4.1)	20(6.4)	1(1.1)	**31(5.8)**
8-Drugs	0	6(16.2)	0	8(2.6)	2(2.3)	**16(3.0)**
9-Drugs	0	1(2.7)	1(1.0)	6(1.9)	1(1.1)	**9(1.7)**
10-Drugs	0	0	0	6(1.9)	0	**6(1.1)**
11-Drugs	0	2(5.4)	0	5(1.6)	0	**7(1.3)**
12-Drugs	0	0	0	3(1.0)	0	**3(0.6)**
13-Drugs	0	0	1(1.0)	0	0	**1(0.2)**
14-Drugs	0	0	1(1.0)	1(0.3)	0	**2(0.4)**
15-Drugs	0	0	1(1.0)	2(0.6)	0	**3(0.6)**
Total	**2**	**37**	**98**	**313**	**89**	**539**

Additionally, the study investigates the distribution of Multi-Drug Resistance (MDR) and Extensively Drug-Resistant (XDR) patterns in NTS, categorized by their respective origins. Within the pool of 539 NTS isolates tested against 19 different antimicrobial agents, a substantial majority showcased resistance to one or more of these agents. Table 5.8 illustrates these patterns, revealing a significant prevalence of heightened drug resistance levels among NTS isolates from both human and animal sources. Specifically, 34 out of 83 isolates (41%) from animal sources and 158 out of 319 isolates (49.5%) from human sources exhibit MDR and XDR behaviours.

Table 5.8: Source-wise resistance profile of different NTS against a combination of drugs.

No. of resistant Drugs	Source categories				Total
	Animal N(%)	Environment N(%)	Food item N(%)	Human N(%)	
0- Drug	24(28.9)	1(2.6)	28(28.3)	61(19.1)	114(21.2)
1-Drug	15(18.1)	20(52.6)	20(20.2)	53(16.6)	108(20.0)
2-Drugs	10(12.1)	12(31.6)	14(14.1)	47(14.7)	83(15.4)
3-Drugs	10(12.1)	1(2.6)	10(10.1)	23(7.2)	44(8.2)
4-Drugs	4(4.8)	2(5.2)	6(6.1)	23(7.2)	35(6.5)
5-Drugs	8(9.6)	0	5(5.1)	23(7.2)	36(6.7)
6-Drugs	2(2.4)	1(2.6)	4(4.0)	34(10.7)	41(7.6)
7-Drugs	1(1.2)	0	6(6.1)	24(7.5)	31(5.8)
8-Drugs	3(3.6)	0	5(5.1)	8(2.5)	16(3.0)
9-Drugs	2(2.4)	0	1(1.0)	6(1.9)	9(1.7)
10-Drugs	0	0	0	6(1.9)	6(1.1)
11-Drugs	1(1.2)	1(2.6)	0	5(1.6)	7(1.3)
12-Drugs	0	0	0	3(0.9)	3(0.6)
13-Drugs	1(1.2)	0	0	0	1(0.2)
14-Drugs	1(1.2)	0	0	1(0.3)	2(0.4)
15-Drugs	1(1.2)	0	0	2(0.6)	3(0.6)
Total	**83**	**38**	**99**	**319**	**539**

The diversity of multidrug-resistant isolates was notably pronounced, as depicted in Table 5.8. A substantial number of these isolates displayed resistance against 1 to 7 antimicrobial agents, illustrating the intricate and varied patterns of resistance within the NTS population.

A significant observation from this study is the identification of NTS isolates displaying resistance to more than eight antimicrobial drugs. This particular discovery carries substantial clinical implications, indicating the ineffectiveness of a significant portion of commonly utilized antibiotics in addressing NTS infections. The resistance to multiple antibiotics underlines the complexity associated with managing NTS infections and underscores the necessity for alternative therapeutic approaches, as conventional antibiotic treatments may be inadequate in effectively addressing these cases. These findings highlight the prevalence of multidrug resistance in NTS strains across various sources.

These findings stress the critical need for comprehensive strategies aimed at combating the emergence and dissemination of multidrug resistance. The incorporation of surveillance, antimicrobial stewardship, and research endeavours is essential in understanding the underlying mechanisms of resistance and devising targeted interventions. Given that multidrug-resistant NTS remains a substantial public health challenge, collaborative efforts spanning human and veterinary medicine are imperative to mitigate its impact and safeguard the effectiveness of antibiotics for both human and animal populations.

5.5 Results of Extended Spectrum β –Lactamase (ESBL) production:

The Centre for Disease Control and Prevention (CDC) has categorized ESBL-producing *Enterobacteriaceae* as a "serious threat" in its report on antibiotic resistance (CDC, 2019). ESBL refers to any acquired beta-lactamase, not intrinsic to a species, rendering antibiotics ineffective, particularly beta-lactam antibiotics like 3rd & 4th - Generation cephalosporins, along with monobactams, and carbapenems (Pfaller and Segreti, 2006; Bali et al., 2010; Fashae, 2010; ECDC, 2012).

In the *Enterobacteriaceae* family, cephalosporins resistance is primarily caused due to the production of broad-spectrum beta-lactamases, including ESBLs and AmpC beta-lactamases (de Jong et al., 2014). ESBL production has been identified in various *Salmonella* serovars, leading to multidrug resistance and presenting a substantial public health risk. The rise and dissemination of distinct ESBL-producing *Salmonella enterica* serovars have been documented. Comprehensive information on the prevalence of ESBL-producing *Salmonella* is essential in preventing the proliferation of multidrug-resistant strains and ensure public health safety (Ziech et al., 2016; Dor et al., 2020).

ESBLs are associated with heightened morbidity and mortality rates, increased healthcare expenses, the potential for foodborne transmission, and asymptomatic carriage (De Kraker et al., 2011; Leverstein et al., 2011; Kluytmans et al., 2013; Woerther et al., 2013). These bacteria commonly reside in the intestines of humans and animals, posing challenges in terms of control and eradication (Carattoli, 2008; Seiffert et al., 2013). Evidence indicates that plasmid-mediated transfer of ESBL genes can occur between different strains, species, and genera in *Enterobacteriaceae* family (Carattoli, 2009). Moreover, ESBL-producing organisms often exhibit simultaneous resistance to other antibiotic groups, further constraining effective treatment options (Carattoli, 2013). The implications of ESBL production underscore the necessity for testing to detect ESBL production in bacterial isolates and assess its impact on public health. Timely detection of ESBL-producing strains is crucial to ensure proper treatment thereby minimizing the risk of transmission to other patients (Garrec et al., 2011). The prevalence of ESBL-producing

bacteria in healthy populations is on the rise, highlighting the increasing importance of effective surveillance methods (Woerther et al., 2013).

With this objective in mind, an initiative was taken to investigate the presence of ESBL-producing isolates among the current pool of NTS isolates. All the 539 NTS isolates underwent testing for ESBL production through a phenotypic confirmatory test using the disk diffusion method. The NTS isolates were tested against four antimicrobial drugs: cefotaxime (CTX) and ceftazidime (CAZ), as well as their combinations with clavulanic acid, known as CAC and CEC (Photograph 2).

Photograph 2: *Testing of ESBL by phenotypic confirmatory test.*

To determine ESBL positivity, the isolates were evaluated based on their phenotypic response. Specifically, a positive ESBL result was confirmed when the inhibition zone around any combination of antimicrobial agents, i.e., CAC and CEC, showed an increase of ≥ 5mm compared to the inhibition zone produced around the single antimicrobial agent, i.e., CAZ and CTX, respectively. This approach aimed to identify and characterize ESBL-producing isolates within the NTS population to gain a complete understanding of antibiotic resistance patterns.

Among the 539 isolates, only 24 NTS isolates (4.45%) were identified to exhibit Extended-Spectrum Beta-Lactamase (ESBL) activity, as indicated in Fig. 5.17. Among these 24 ESBL-positive isolates, 11 NTS isolates demonstrated ESBL detection solely through ceftazidime and its combination with clavulanic acid, while 9 NTS isolates showed

ESBL detection through cefotaxime and cefotaxime-clavulanic acid out of the two drugs tested. In contrast, 4 NTS isolates exhibited ESBL positivity detected by both tested drugs.

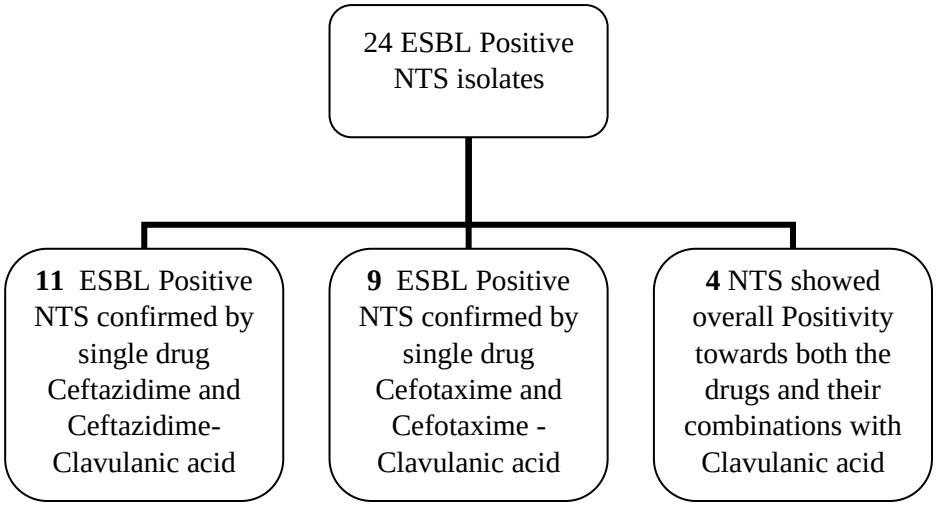


Fig. 5.17: Detection of ESBL producer NTS.

Further in-depth analysis of the source of isolation of the 24 ESBL positive NTS revealed that 16 isolates were of human origin while 4, 3 & 1 isolates were obtained from environment sources, animal sources, and food items respectively. Table 5.9 provides further information about the number of different NTS serovars obtained from sources such as human feces, cerebrospinal fluid (CSF), and tissue samples. Of the 16 ESBL-producing NTS serovars of human origin, the majority i.e. 14, were obtained from human feces while one NTS each from CSF and tissue samples was found to produce ESBL.

S. enterica serovar Typhimurium dominated in human fecal samples followed by serovars Lindenburg (n=3), Weltevreden (n=2), Choleraesuis (n=1), and Enteritidis (n=1). While *S. enterica* serovar Typhimurium (n=1) from CSF and *S. enterica* serovar Weltevreden (n=1) from tissue sample was identified.

Table 5.9: ESBL producing NTS serovars

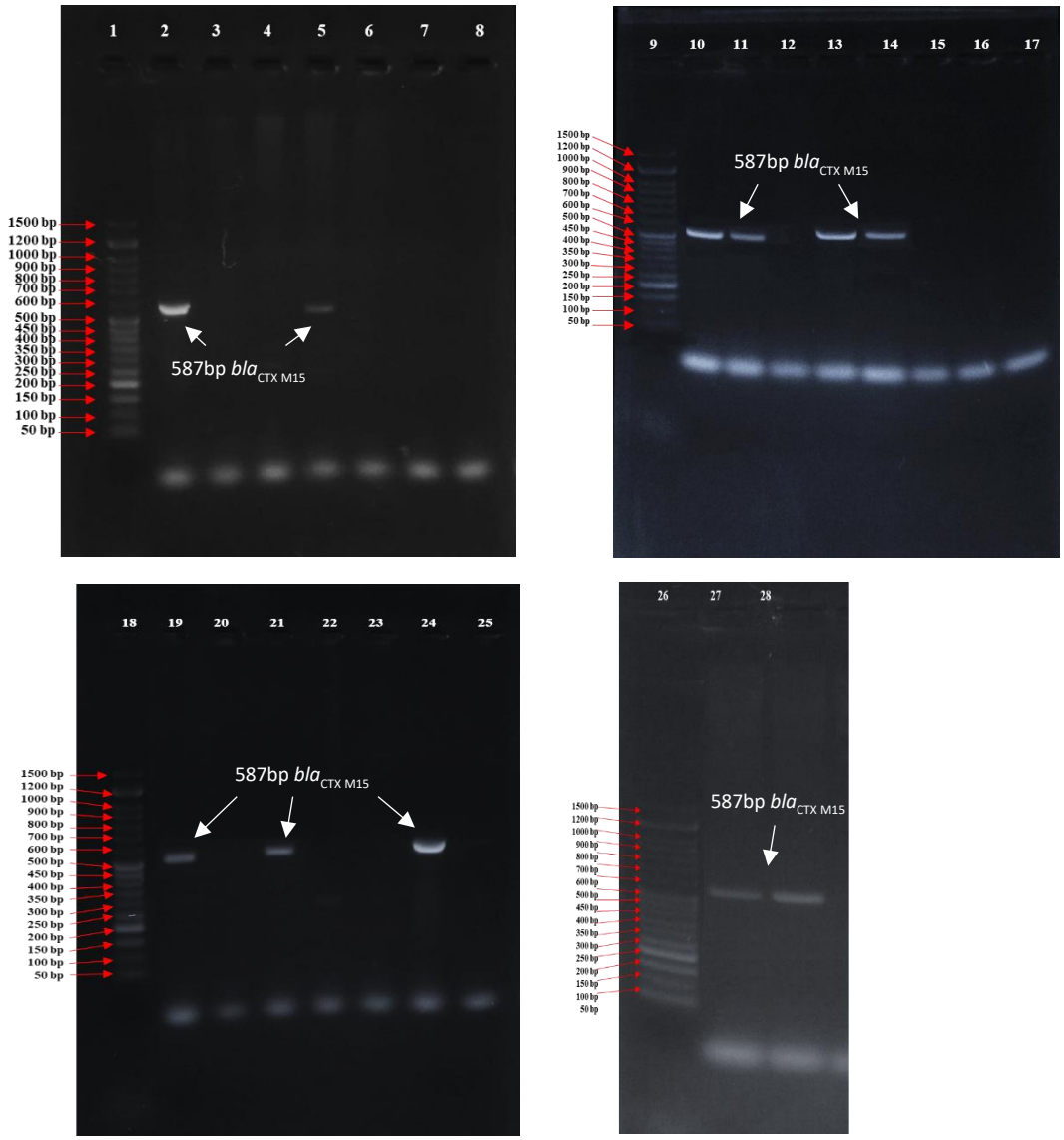
Source	Code #	NTS serovar	Source type	ESBL (*bla*-) genes			
				CTX-M15	**CMY-2**	**SHV**	**TEM**
Human (n=16)	E1-S238	*S. enterica* serovar Weltevreden	Tissue	+ve	+ve	-ve	-ve
	E2-S330	*S. enterica* serovar Typhimurium	Unknown	-ve	+ve	-ve	-ve
	E3-S434	*S. enterica* serovar Weltevreden	Faeces	-ve	-ve	-ve	-ve
	E4-S444	*S. enterica* serovar Lindenburg	Faeces	+ve	-ve	-ve	+ve
	E6-S211	*S. enterica* serovar Choleraesuis	Faeces	-ve	-ve	-ve	-ve
	E12-S430	*S. enterica* serovar Typhimurium	Faeces	+ve	+ve	-ve	-ve
	E13-S442	*S. enterica* serovar Typhimurium	Faeces	-ve	+ve	-ve	-ve
	E14-S529	*S. enterica* serovar Typhimurium	Faeces	-ve	+ve	-ve	-ve
	E16-S230	*S. enterica* serovar Lindenburg	Faeces	+ve	+ve	-ve	+ve
	E17-S232	*S. enterica* serovar Typhimurium	Faeces	-ve	+ve	-ve	-ve
	E18-S234	*S. enterica* serovar Lindenburg	Faeces	+ve	+ve	-ve	+ve
	E19-S243	*S. enterica* serovar Typhimurium	Faeces	-ve	+ve	-ve	-ve
	E20-S256	*S. enterica* serovar Typhimurium	Faeces	-ve	+ve	-ve	-ve
	E21-S264	*S. enterica* serovar Weltevreden	Faeces	+ve	+ve	-ve	+ve
	E22-S347	*S. enterica* serovar Typhimurium	CSF	-ve	+ve	-ve	-ve
	E24-S239	*S. enterica* serovar Enteritidis	Faeces	+ve	-ve	-ve	-ve
Environment (n=4)	E5-S26	*S. enterica* serovar Hissar	Poultry environment	-ve	-ve	-ve	-ve
	E9-S284	*S. enterica* serovar Mathura	Animal fodder storage	+ve	-ve	-ve	-ve
	E10-S290	*S. enterica* serovar Mathura	Animal fodder storage	-ve	-ve	-ve	-ve

Source	Code #	NTS serovar	Source type	ESBL (*bla*-) genes			
				CTX-M15	**CMY-2**	**SHV**	**TEM**
	E23-S286	*S. enterica* serovar Mathura	Animal fodder storage	+ve	-ve	-ve	-ve
Animal (n=3)	E7-S239	*S. enterica* serovar Typhimurium	Faeces	-ve	-ve	-ve	-ve
	E8-S246	*S. enterica* serovar Typhimurium	Faeces	+ve	-ve	-ve	-ve
	E11-S337	*S. enterica* serovar Anatum	Fetal stomach content	+ve	+ve	-ve	+ve
Food (n=1)	E15-S556	*S. enterica* serovar Senftenberg	Poultry meat	-ve	+ve	-ve	-ve

Furthermore, samples from environmental; animals; and food sources were found to harbour ESBL-producing NTS serovars such as Mathura (n=3) and Hissar (n=1); Typhimurium (n=1), Anatum (n=1); and Senftenberg (n=1) respectively. Detection of ESBL-producing *S. enterica* serovar Typhimurium is a crucial due to its prevalence in food items (Nadimpalli et al., 2019) indicating that these strains are present in the food supply and have the potential to cause human infections. These strains are capable of conferring resistance to cephalosporins commonly used to treat severe salmonellosis in paediatric patients (Nadimpalli et al., 2019), harbouring a self-transferable plasmid, which facilitates the spread of ESBL genes among different *Salmonella* strains (González-López et al., 2014). *S. enterica* serovar Typhimurium ST131, which produces $bla_{\text{CTX-M15}}$ ESBL, is one of the highly prevalent among *S. enterica* serovar Typhimurium and has been detected in South America, Asia, and Africa (González-López et al., 2014). The detection of *S. enterica* serovar Typhimurium in cerebrospinal fluid (CSF) in the present study is a rare but serious occurrence, as it can lead to meningitis and central nervous system infection. Research has shown that this serovar is capable of invading the brain and triggering meningitis in animal models, indicating its potential to cause similar infections in humans (Wickham et al., 2007; Chaudhuri et al., 2018). Studies have reported the presence of *S.* Typhimurium in the CSF of individuals with meningitis, highlighting the clinical

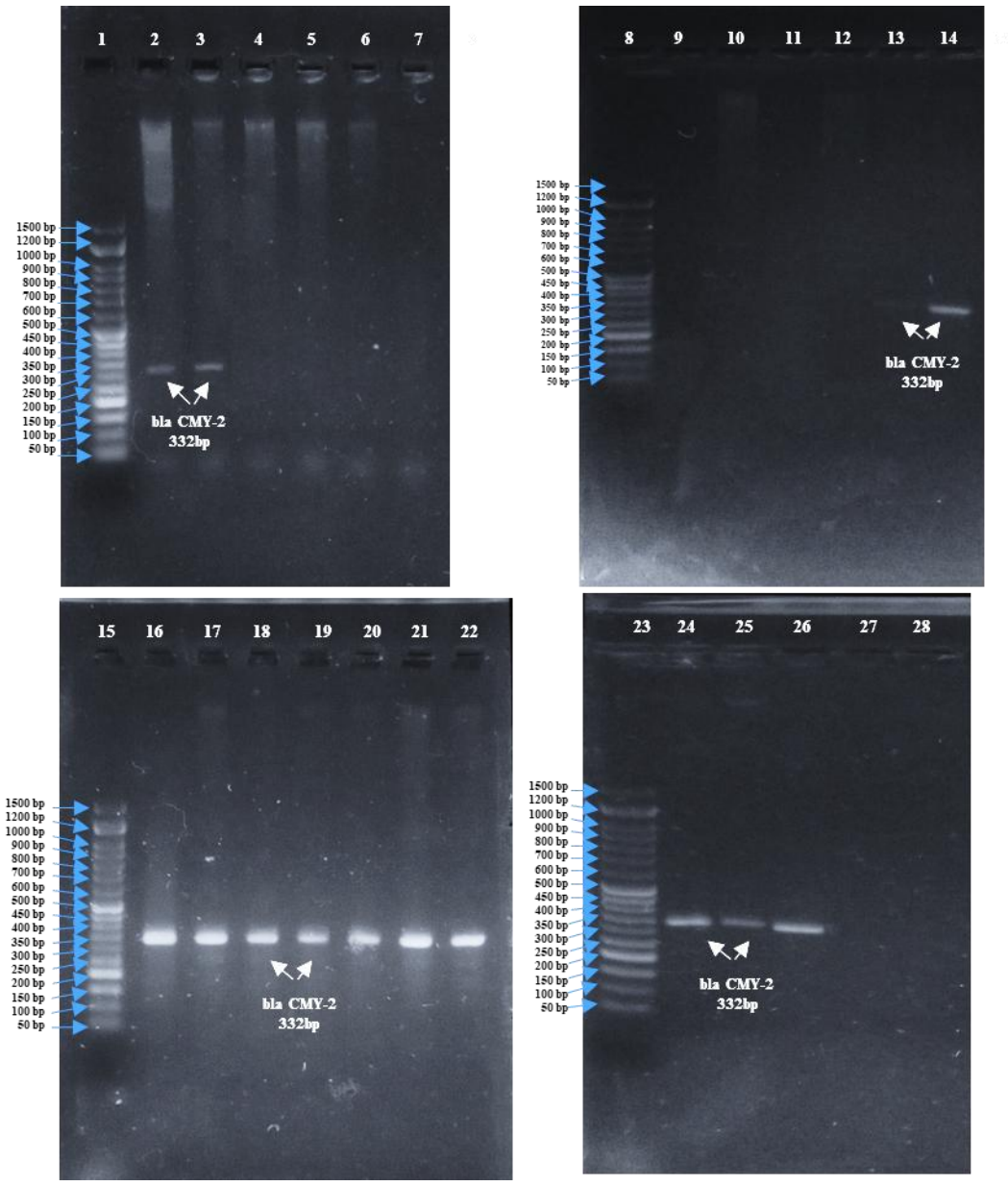
significance of this pathogen in causing invasive central nervous system infections (van Sorge et al., 2011).

In this study, a targeted investigation was undertaken on 24 phenotypically confirmed ESBL-producing NTS serovars. The primary aim was to discern and characterize the presence of ESBL genes, specifically *bla*$_{\text{CTX-M15}}$, *bla*$_{\text{CMY-2}}$, *bla*$_{\text{SHV}}$, and *bla*$_{\text{TEM,}}$ which commonly occur in NTS isolated in India (Ziech et al., 2016; Okpa et al., 2020; Sedrakyan et al., 2020; dos Santos et al., 2021; Verma et al., 2022). The findings revealed significant diversity in the distribution of these genes among the studied serovars, with some strains carrying one, two, or three of the specified ESBL genes, while others exhibited the absence of all the four gene types. 5 out of 24 NTS did not indicate presence of any of the four genes targeted. The prevalence of the two genes, namely *bla*$_{\text{CTX-M15}}$ and *bla*$_{\text{CMY-2}}$, was particularly striking, as a total of 11 and 14 isolates respectively, demonstrated the presence of these two genes underscoring their dominance in the majority of these NTS serovars. Notably, none of the 24 NTS serovars were found to harbour the *bla*$_{\text{SHV}}$ gene. The pictorial presentation of these genes is given in the Photographs 3-5.



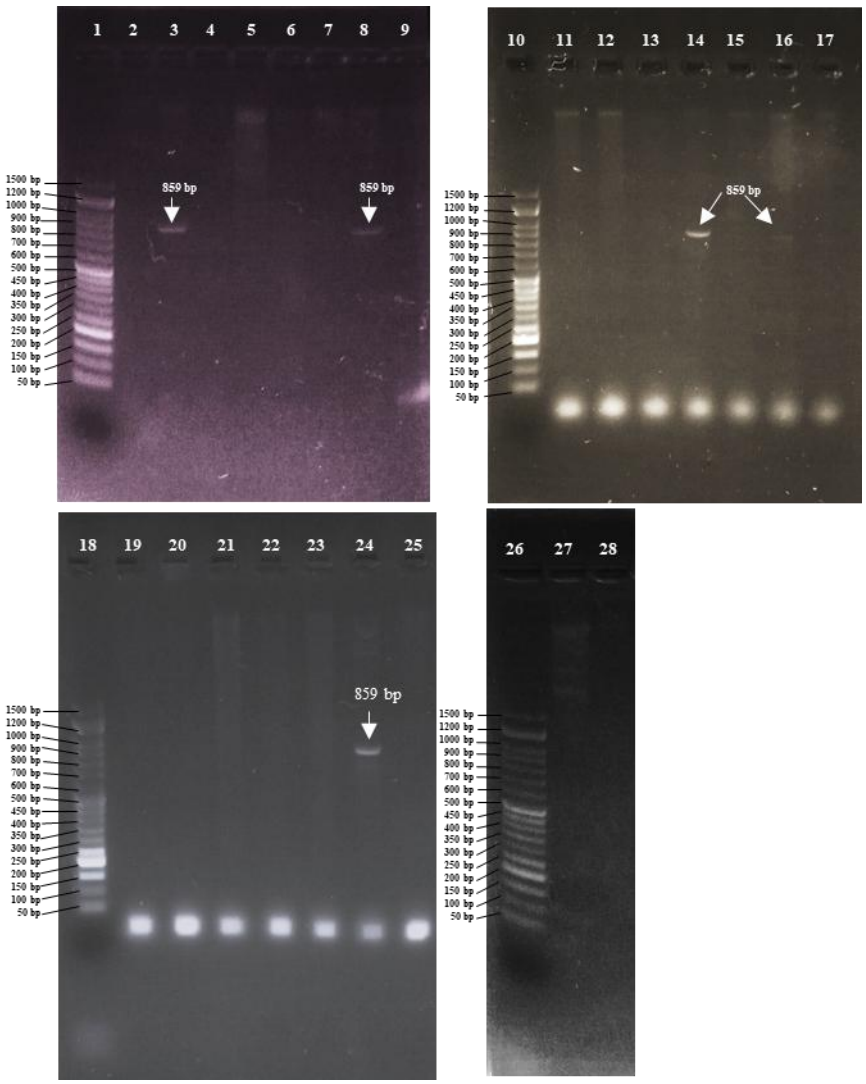
Photograph 3: Detection of *bla*$_{\text{CTX-M15}}$ (587bp) gene in NTS.

Lane 1: 50-1500bp Ladder
Lane 2: E1 -POSITIVE
Lane 3: E2 -NEGATIVE
Lane 4: E3 -NEGATIVE
Lane 5: E4 -POSITIVE
Lane 6: E5 -NEGATIVE
Lane 7: E6 -NEGATIVE
Lane 8: E7 -NEGATIVE
Lane 9: 50-1500bp Ladder
Lane 10: E8 -POSITIVE
Lane 11: E9 -POSITIVE
Lane 12: E10 -NEGATIVE
Lane 13: E11 -POSITIVE
Lane 14: E12 -POSITIVE
Lane 15: E13 -NEGATIVE
Lane 16: E14 -NEGATIVE
Lane 17: E15 -NEGATIVE
Lane 18: 50-1500bp Ladder
Lane 19: E16 -POSITIVE
Lane 20: E17 -NEGATIVE
Lane 21: E18 -POSITIVE
Lane 22: E19 -NEGATIVE
Lane 23: E20 -NEGATIVE
Lane 24: E21 -POSITIVE
Lane 25: E22 -NEGATIVE.
Lane 26: 50-1500bp Ladder
Lane 27: E23 -POSITIVE
Lane 28: E24 -POSITIVE



Photograph 4: Detection of *bla*$_{CMY-2}$ (332bp) gene in NTS.

Lane 1: 50-1500bp Ladder
Lane 2: E1 -POSITIVE
Lane 3: E2 -POSITIVE
Lane 4:E3 -NEGATIVE
Lane 5: E4 -NEGATIVE
Lane 6:E5 -NEGATIVE
Lane 7: E6 -NEGATIVE
Lane 8: 50-1500bp Ladder
Lane 9: E7 -NEGATIVE
Lane 10: E8 -NEGATIVE
Lane 11: E9 -NEGATIVE
Lane 12: E10 -NEGATIVE
Lane 13: E11 -POSITIVE
Lane 14: E12 -POSITIVE
Lane 15: 50-1500bp Ladder
Lane 16: E13 -POSITIVE
Lane 17: E14 -POSITIVE
Lane 18: E15 -POSITIVE
Lane 19: E16 -POSITIVE
Lane 20: E17 -POSITIVE
Lane 21: E18 -POSITIVE
Lane 22: E19 -POSITIVE.
Lane 23: 50-1500bp Ladder
Lane 24: E20 -POSITIVE
Lane 25: E21 -POSITIVE
Lane 26: E22 -POSITIVE
Lane 27: E23 -NEGATIVE
Lane 28: E24 -NEGATIVE.



Photograph 5: Detection of *bla*TEM (859bp) gene in NTS.

Lane 1: 50-1500bp Ladder
Lane 2: E20 -NEGATIVE
Lane 3: E21 -POSITIVE
Lane 4: E22 -NEGATIVE
Lane 5: E1 -NEGATIVE ;
Lane 6: E2 -NEGATIVE
Lane 7: E3 -NEGATIVE
Lane 8: E4 -POSITIVE
Lane 9: E5 -NEGATIVE
Lane 10: 50-1500bp Ladder
Lane 11: E13 -NEGATIVE
Lane 12: E14 -NEGATIVE
Lane 13: E15 -NEGATIVE
Lane 14: E16 -POSITIVE
Lane 15: E17 -NEGATIVE
Lane 16: E18 -POSITIVE
Lane 17: E19 -NEGATIVE
Lane 18: 50-1500bp Ladder
Lane 19: E6 -NEGATIVE
Lane 20: E7 -NEGATIVE
Lane 21: E8 -NEGATIVE
Lane 22: E9 -NEGATIVE
Lane 23: E10 -NEGATIVE
Lane 24: E11 -POSITIVE
Lane 25: E12 -NEGATIVE.
Lane 26: 50-1500bp Ladder
Lane 27: E23 -NEGATIVE
Lane 28: E24: -NEGATIVE.

Additionally, the analysis of the 16 isolates from human sources revealed a notable diversity in terms of the presence of ESBL genes. Among these isolates, 7 were found to carry the *bla*$_{\text{CTX-M15}}$ gene, 12 harboured the *bla*$_{\text{CMY-2}}$ gene, and 4 carried the *bla*$_{\text{TEM}}$ gene. Intriguingly, two isolates did not show any of the four specified ESBL genes. Two serovars, specifically Lindenburg (n=2; code # E16 & E18) and Weltevreden (n=1; code # E21), originating from human fecal samples, were identified as hosts for a maximum of three ESBL genes out of the four (excluding *bla*$_{\text{SHV}}$). Furthermore, a total of 4 isolates, comprising two instances of *S. enterica* serovar Weltevreden (E1) and two of *S. enterica* serovar Typhimurium (E12), were found to carry the *bla*$_{\text{CTX-M15}}$ with *bla*$_{\text{CMY-2}}$ gene combination. Additionally, one isolate, specifically *S. enterica* serovar Lindenburg (E4), was identified as carrying the *bla*$_{\text{CTX-M15}}$ with the *bla*$_{\text{TEM}}$ gene combination. This intricate pattern of ESBL gene combinations within these isolates underscores the genetic diversity of antibiotic resistance mechanisms in different serovars, providing valuable insights into the multifaceted nature of *Salmonella* infections in the human population.

Among the four environmental isolates, two isolates of *S. enterica* serovar Mathura showed the presence of *bla*$_{\text{CTX-M15}}$ genes only while other three genes were not detected. This specific genetic profile highlights the unique molecular characteristics within *S. enterica* serovar Mathura, showcasing a limited spectrum of ESBL genes in these particular environmental isolates. The remaining two isolates in this group did not show presence of any of the four genes.

Interestingly, one of the three NTS isolates obtained from animal sources—*S. enterica* serovar Anatum (E11)—was found to harbour three resistance genes: *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CMY-2}}$, and *bla*$_{\text{TEM}}$. The second isolate, *S. enterica* serovar Typhimurium, carried only the *bla*$_{\text{CTX-M-15}}$ gene, while no resistance genes were detected in the third isolate.

The single NTS isolate, *S. enterica* serovar Senftenberg, obtained from poultry meat, demonstrated the presence of only the *bla*$_{\text{CMY-2}}$ gene among the four gene types examined.

The observed differences in ESBL gene patterns across various serovars and samples emphasize the intricate nature of antibiotic resistance in *Salmonella* infections. This

focused genetic analysis provides valuable insights into the molecular profile of ESBL-producing *Salmonella* strains, shedding light on the nuanced variability in gene composition within this subset of NTS isolates.

The identification of ESBL genes in NTS is of significance due to its implications for public health and antimicrobial resistance. Strains of *Salmonella* producing ESBLs exhibit multidrug resistance, complicating the treatment of infections as detected in this study (Table 5.10).

In India, studies have described the detection of ESBL genes in NTS, indicating the potential for severe infections that pose challenges for effective management. These studies highlight the widespread prevalence of ESBL genes in various settings across India, encompassing clinical isolates, animals, and food production environments. A comprehensive systematic review and meta-analysis by Kuralayanapalya et al. (2019) described the prevalence of ESBL-producing pathogens derived from animals and animal samples in India. Additionally, a multi-centric study conducted in tertiary care hospitals identified $bla_{\text{OXA-1}}$ and bla_{CTXM15} as the most prevalent ESBL genes in *E. coli* and *K. pneumoniae* isolated during the study period (Gautam et al., 2019). Further investigation into the coexistence of ESBL genes along with carbapenemase, AmpC β-lactamase, and aminoglycoside resistance genes in India was carried out by Gajamer et al. (2020). Most importantly, the horizontal transfer of these genes to other foodborne pathogens and commensal bacteria in the environment poses a significant risk, intensifying the threat of antibiotic resistance (Garcia et al., 2019). Particularly concerning is the potential contamination of food products by non-Typhoidal *Salmonella* carrying ESBL genes on conjugative plasmids, leading to the proliferation of ESBL-producing strains. This highlights the critical importance of monitoring and controlling the spread of these genes within the food chain (Bae et al., 2015). The increase of ESBL-producing NTS strains in India poses a significant challenge, fuelled by the heightened demand for animal products and the widespread use of antibiotics. The discerned prevalence is indicative of a concerning trend. The extensive application of antibiotics in animal husbandry, encompassing non-therapeutic uses for prophylaxis and growth promotion, emerges as a

prominent contributor to the surge in antibiotic resistance within the region (Kuralayanapalya et al., 2019). Although the specific prevalence of ESBL-producing *Salmonella* in Indian food products wasn't explicitly provided in the results, the broader context implies that antibiotic resistance in food products remains a noteworthy concern in the country. This complex interplay of factors necessitates a robust and multifaceted approach to address the burgeoning issue of antibiotic resistance in both animal and food domains in India.

In conclusion, the presence of ESBL genes amongst NTS in India underscores the urgent need for robust surveillance and control measures to effectively address the growing challenge of antimicrobial resistance in the country.

Table 5.10: Relation between ESBL-producing NTS and antibiotic resistance.

Code #	NTS serovar	AMC	AMP	AK	C	CIP	COT	CPM	CTR	CTX	CXM	GEN	IPM	K	MRP	NA	NIT	NX	PIT	TR	No. of Drugs Resistant	EBSL gene detected
E1S238	*S. enterica* serovar Weltevreden																				15	$bla_{\text{CTX-M15}}$, $bla_{\text{CMY-2}}$
E2S330	*S. enterica* serovar Typhimurium																				0	$bla_{\text{CMY-2}}$
E3S434	*S. enterica* serovar Weltevreden																				0	-
E4S444	*S. enterica* serovar Lindenburg																				8	$bla_{\text{CTX-M15}}$, bla_{TEM}
E5S26	*S. enterica* serovar Hissar																				6	-
E6S211	*S. enterica* serovar Choleraesuis																				1	-
E7S239	*S. enterica* serovar Typhimurium																				5	-
E8S246	*S. enterica* serovar Typhimurium																				2	$bla_{\text{CTX-M15}}$
E9S284	*S. enterica* serovar Mathura																				2	$bla_{\text{CTX-M15}}$
E10S290	*S. enterica* serovar Mathura																				2	-
E11S337	*S. enterica* serovar Anatum																				13	$bla_{\text{CTX-M15}}$, $bla_{\text{CMY-2}}$, bla_{TEM}
E12S430	*S. enterica* serovar Typhimurium																				6	$bla_{\text{CTX-M15}}$,$bla_{\text{CMY-2}}$
E13S442	*S. enterica* serovar Typhimurium																				5	$bla_{\text{CMY-2}}$
E14S529	*S. enterica* serovar Typhimurium																				7	$bla_{\text{CMY-2}}$
E15S556	*S. enterica* serovar Senftenberg																				3	$bla_{\text{CMY-2}}$
E16S230	*S. enterica* serovar Lindenburg																				9	$bla_{\text{CTX-M15}}$, $bla_{\text{CMY-2}}$, bla_{TEM}
E17S232	*S. enterica* serovar Typhimurium																				8	$bla_{\text{CMY-2}}$
E18S234	*S. enterica* serovar Lindenburg																				9	$bla_{\text{CTX-M15}}$, $bla_{\text{CMY-2}}$, bla_{TEM}
E19S243	*S. enterica* serovar Typhimurium																				7	$bla_{\text{CMY-2}}$
E20S256	*S. enterica* serovar Typhimurium																				7	$bla_{\text{CMY-2}}$
E21S264	*S. enterica* serovar Weltevreden																				7	$bla_{\text{CTX-M15}}$, $bla_{\text{CMY-2}}$, bla_{TEM}
E22S347	*S. enterica* serovar Typhimurium																				7	$bla_{\text{CMY-2}}$
E23S286	*S. enterica* serovar Mathura																				2	$bla_{\text{CTX-M15}}$
E24S239	*S. enterica* serovar Enteritidis																				14	$bla_{\text{CTX-M15}}$

AMC- Amoxycillin/Clavulanic acid; AMP-Ampicillin; AK- Amikacin; C-Chloramphenicol; CIP-Ciprofloxacin; COT-Co-Trimoxazole; CPM-Cefepime, CTR-Ceftriaxone, CTX-Cefotaxime, CXM-Cefuroxime, GEN-Gentamicin, IPM-Imipenem; K-Kanamycin; MRP -Meropenem; NA-Nalidixic Acid; NIT-Nitrofurantoin, NX-Norfloxacin; PIT- Piperacillin/Tazobactam; TR-Trimethoprim.

Resistant Intermediate Sensitive

5.6 Detection of Carbapenemase enzyme activity:

Carbapenems serve as "last resort" antibiotics against severe infections caused by multidrug-resistant (MDR) Gram-negative bacteria, including certain NTS strains (Chang et al., 2020; Hsu et al., 2023). While carbapenem resistance in NTS is relatively uncommon, instances of such resistance have been detected in humans, companion animals, and food sources (Fernández et al., 2018; Hsu et al., 2023). Although carbapenems are not typically the primary choice for treating *Salmonella* infections, their significance lies in their reserved status as critical antibiotics for addressing MDR infections (Hsu et al., 2023). The emergence of carbapenem resistance in zoonotic pathogens like NTS presents a significant threat to public health, as these resistant strains can potentially be transmitted to humans via the food chain (Keintz and Marcelin, 2022; Hsu et al., 2023). Combination therapy using ceftriaxone and fluoroquinolone may be considered in severe infections until susceptibility testing results become available (Keintz and Marcelin, 2022).

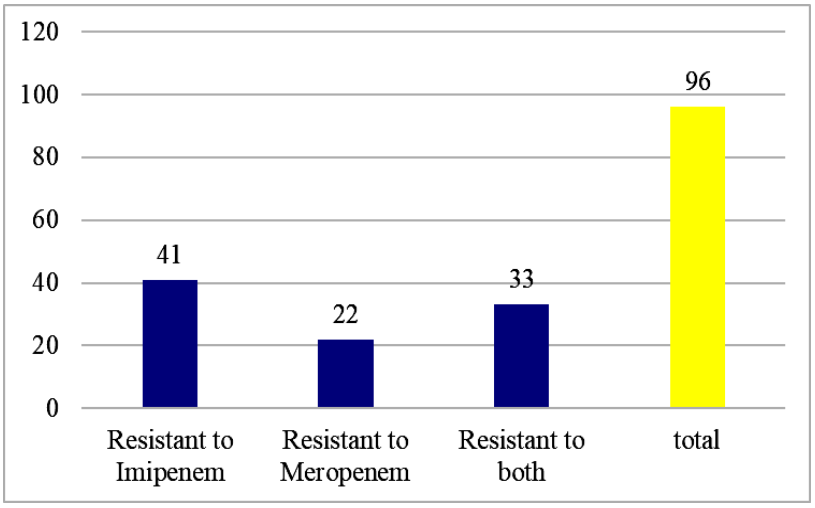


Fig. 5.18: NTS isolates tested for carbapenemase detection.

In the current investigation, an extensive antibiogram profiling analysis of 539 NTS isolates was conducted, highlighting a noteworthy observation. Among the studied isolates, a total of 96 NTS isolates exhibited resistance to carbapenem antibiotics, specifically meropenem and imipenem. The breakdown of resistance patterns revealed

that 41 isolates were resistant to imipenem, 22 displayed resistance to meropenem, and 33 isolates exhibited resistance to both drugs simultaneously. (Fig. 5.18).

Photograph 6: Testing of Carbapenemase activity

As carbapenem drugs are becoming ineffective mostly due to the emergence of Carbapenemase-producing strains (Fernández et al., 2018; Nielsen et al., 2021; Harding-Crooks et al., 2023), therefore these 96 NTS isolates were tested further for the presence of enzyme carbapenemase (Photograph 6). The Modified Hodge Test (MHT) serves as a phenotypic assay designed to identify the presence of carbapenemase enzymes. This methodology relies on the capacity of carbapenemase-producing strains, including the test isolate, to deactivate carbapenem antibiotics. In the test, a carbapenem-susceptible indicator strain, typically *E. coli* ATCC 25922, is streaked alongside the test strain and a carbapenem-containing disc. A positive result manifests as the indicator strain's growth towards the disc in a cloverleaf-like pattern. This phenomenon occurs because the carbapenemase produced by the test microorganism deactivates the carbapenem diffusing from the disc, allowing the carbapenem-susceptible *E. coli* ATCC 25922 to grow towards it.

Fig. 5.19 represents the source-wise distribution of the 96 carbapenem-resistant NTS isolates. This indicates the majority of these resistance isolates originated from human samples (n=67) followed by animal (n=19), food (n=9), and environmental (n=1) sources. These isolates were tested for the presence of the carbapenemase enzyme. Notably, the findings of this study revealed that none of the isolates exhibited the presence of the carbapenemase enzyme (Photograph 6). The absence of

carbapenemase activity suggests that, as of now, these strains did not possess carbapenemase activity. However, it is important to acknowledge the potential for future occurrences of carbapenem-resistant NTS isolates. Despite the current absence of carbapenemase in the tested NTS isolates, vigilance and continued monitoring are crucial due to the evolving nature of bacterial resistance. The possibility of the occurrence of resistant strains in the future necessitates ongoing surveillance to promptly identify and address any changes in the resistance profile of NTS isolates.

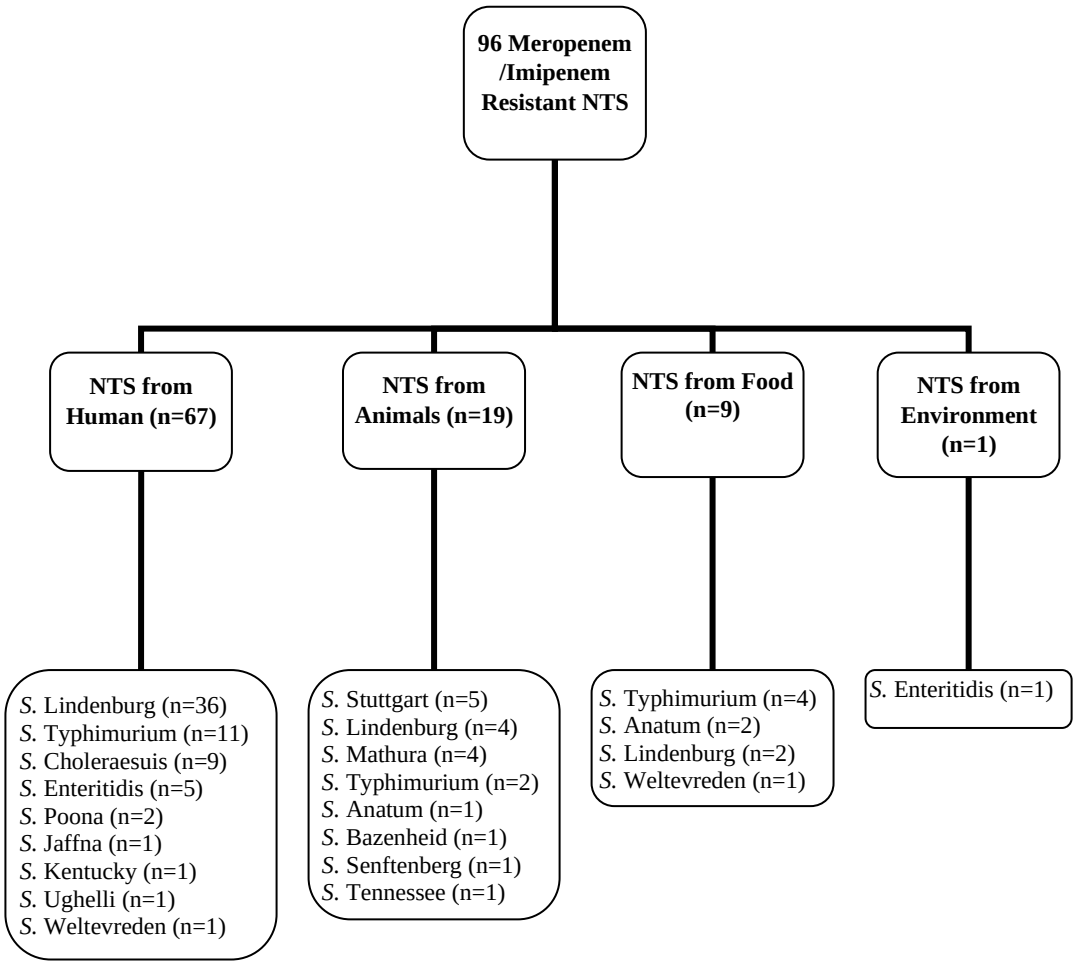


Fig. 5.19: Distribution of Carbapenem-resistant NTS across different sources.

As highlighted earlier, while occurrences of carbapenem resistance in NTS are rare, it remains imperative to implement proactive measures to prevent its emergence. Among the most crucial steps is the enhancement of antibiotic stewardship, aimed at reducing the utilization of broad-spectrum antimicrobials, including carbapenems, in both human and veterinary medicine. Implementing strict regulations on antibiotic

prescribing practices for the treatment of salmonellosis is essential to mitigate the potential development of carbapenem resistance in NTS strains (Chang et al., 2020).

Additionally, enhancing infection control measures, promoting personal hygiene, improving animal husbandry conditions, monitoring antimicrobial use in agriculture, educating healthcare professionals and the public, and supporting research and development are other measures that can be taken to prevent carbapenem resistance in NTS (Meletis, 2016).

5.7 Detection of Cefotaxime Resistance Gene:

The surge in antibiotic resistance among *Salmonella* species presents a significant and escalating concern (Su et al., 2004). Over time, there has been a substantial increase in the prevalence of antimicrobial resistance among *Salmonella* strains, with rates rising from 20%–30% in the early 1990s to the peak 70% in certain regions. According to the CDC (CDC, 2019), there has been a consistent upward trend in antibiotic-resistant NTS infections. Historically, antibiotics like ampicillin, chloramphenicol, and co-trimoxazole were regarded as primary treatment options for NTS infections. However, the widespread and indiscriminate use of antibiotics in both human medicine and agriculture, particularly within the poultry industry, has led to the emergence of multidrug-resistant *Salmonella* strains on a global scale (Lee et al., 2009; Meng et al., 2011; Vo et al., 2010; Wannaprasat et al., 2011; Chen et al., 2013, Crump et al., 2015; Adesiji et al., 2018).

The antibiotics traditionally employed for salmonellosis treatment include β-lactams, fluoroquinolones, macrolides, and trimethoprim-sulfamethoxazole (Hagel et al., 2015). However, a challenging scenario has emerged with the increased prevalence of MDR *S. enterica* strains in recent years (EFSA, 2015). This resistance trend extends to third-generation cephalosporins, as reported from food sources (including retail meat) and animals (Hasman et al., 2005; Seral and Weill, 2011; EFSA, 2011; Eller et al., 2013; Afema et al., 2015). This resistance presents a substantial challenge in the management of infections, as it restricts the range of available treatment options. Consequently, clinicians may resort to employing broader-spectrum antibiotics, a practice that not only carries a heightened risk of adverse effects but also contributes to

the ongoing development of additional antibiotic resistance. This emphasizes the urgent need for judicious antibiotic use, innovative therapeutic strategies, and robust surveillance to mitigate the escalating threat of antibiotic-resistant *Salmonella* and preserve the efficacy of available treatment options.

Resistance to 3rd generation of cephalosporins primarily due to the production of ESBLs e.g. CTX-M and AmpC β-lactamases e.g. CMY-2, that can spread resistance to a wide range of beta-lactam antibiotics (penicillins and cephalosporins) by hydrolyzing them (Miriagou et al., 2004). The recent emergence of plasmid-mediated ESBL gene, particularly the *bla*$_{\text{CTX-M}}$ variant, is a matter of significant concern, given its widespread presence in *Salmonella* spp. and its association with the hydrolysis of cefotaxime (Nair et al., 2018). The horizontal transfer of *bla*$_{\text{CTX-M}}$ ESBL genes through mechanisms such as conjugation plasmids and transposons represent the primary processes facilitating the acquisition of *bla*$_{\text{CTX-M}}$ ESBLs. This mode of genetic exchange raises alarm due to its potential to disseminate antibiotic resistance traits among *Salmonella* populations, further complicating the treatment landscape and emphasizing the need for enhanced surveillance.

In the current study, the analysis of drug antibiogram profiling conducted on 539 NTS isolates unveiled a noteworthy pattern of resistance, particularly concerning Cefotaxime, a third-generation cephalosporin. Specifically, a significant proportion, accounting for 242 isolates (44.9%), exhibited resistance to this antimicrobial drug—the highest resistance observed among all tested antibiotics (19 drugs) in the present study (Table 5.6). Among these 242 NTS, 19 isolates were already included in the pool of 24 ESBL-producing NTS isolates. Therefore, the same have been separated to avoid duplicity in the test and analysis. Therefore remaining 223 NTS out of 242 were tested further for the detection of the cefotaxime resistance gene (*bla*$_{\text{CTX-M15}}$).

Table 5.11 represents the distribution of the 223 Cefotaxime-resistant NTS isolates across various geographical locations in India, along with their association with different source categories. Notably, a substantial majority of these isolates, comprising 152 instances (68%), originated from the southern region of the country. While this elevated proportion may be attributed to the comparatively higher number of NTS

isolates received from this specific region, as detailed in Table 5.4, the prevalence of drug-resistant serovars in this area raises a significant concern.

Table 5.11: Distribution of Cefotaxime resistance across different regions.

S. No.	NTS serovar	Geographic Region in India				Total
		EAST	NORTH	SOUTH	WEST	
1	*S. enterica* serovar Anatum	0	2	0	0	2
2	*S. enterica* serovar Bazenheid	0	3	0	0	3
3	*S. enterica* serovar Choleraesuis	0	0	25	0	25
4	*S. enterica* serovar Enteritidis	0	0	15	0	15
5	*S. enterica* serovar Hissar	0	0	0	2	2
6	*S. enterica* serovar Kentucky	0	0	4	0	4
7	*S. enterica* serovar Lindenburg	0	7	**56**	8	71
8	*S. enterica* serovar Mathura	0	4	0	5	9
9	*S. enterica* serovar Poona	0	0	2	0	2
10	*S. enterica* serovar Senftenberg	0	1	0	0	1
11	*S. enterica* serovar Stuttgart	4	0	0	0	4
12	*S. enterica* serovar Tennessee	2	0	1	0	3
13	*S. enterica* serovar Typhimurium	4	8	**41**	17	70
14	*S. enterica* serovar Virchow	1	0	0	0	1
15	*S. enterica* serovar Weltevreden	0	0	8	3	11
	Total	**11**	**25**	**152**	**35**	**223**

While acknowledging the relatively lower count of cefotaxime-resistant NTS observed in other regions, it is crucial to recognize that these figures should not be considered insignificant. The proportion of samples received from these areas may not accurately reflect the true prevalence of NTS or its associated drug-resistant nature. Consequently, it is imperative to emphasize the necessity for comprehensive surveillance activities in these regions. Such initiatives are essential to obtain a more accurate and representative understanding of the current status of NTS prevalence and drug resistance, enabling informed public health strategies and interventions to address the multifaceted challenges posed by antibiotic-resistant NTS.

Among the 223 cefotaxime-resistant NTS isolates, 15 NTS serovars out of the total 17 identified serovars in this study exhibited cefotaxime resistance, with serovars

Lindenburg (71) and Typhimurium (70) standing out prominently, as detailed in Table 5.11. The distribution of these cefotaxime-resistant isolates showed a higher concentration in southern regions, followed by western and northern regions. Notably, the majority of these isolates originated from human sources (148/223), constituting nearly 66% of the total collection. This was followed by 18.8% (42/223) from food sources, 11.7% (26/223) from animals, and 3.1% (7/223) from environmental sources, as indicated in Table 5.12. Further, serovars Lindenburg and Typhimurium emerged as predominant contributors to cefotaxime-resistant NTS among isolates from human, animal, and food-related sources. Serovar Typhimurium has been already implicated in various studies to be the most prevalent NTS serovar all over the world occurring in diverse hosts (Scallan et al., 2011; DiMarzio et al., 2013; Wang et al., 2019; Bythwood et al., 2019).

Additionally, the source-specific prevalence of cefotaxime resistance was observed, with certain resistance serovars being exclusive to specific sources. For instance, serovars Choleraesuis (n=25), Enteritidis (n=15), Kentucky (n=4), and Poona (n=2) were exclusively found in human samples. Serovars Anatum (n=2) and Virchow (n=1) were observed solely in food samples, while Hissar (n=2) was identified in environmental isolates. Furthermore, serovars Bazenheid (n=3), Senftenberg (n=1), and Stuttgart (n=4) were exclusively present in animal isolates. The specific distribution of cefotaxime-resistant NTS serovars underscores the varied sources contributing to antimicrobial resistance. This highlights the importance of targeted interventions across various sectors to address the complex challenges posed by antimicrobial resistance effectively.

Table 5.12: Distribution of Cefotaxime resistance across different source categories.

S. No.	NTS serovars	Cefotaxime-resistant NTS from different sources				Total
		Animal	Environment	Food item	Human	
1	*S. enterica* serovar Anatum	0	0	2	0	**2**
2	*S. enterica* serovar Bazenheid	3	0	0	0	**3**
3	*S. enterica* serovar Choleraesuis	0	0	0	25	**25**
4	*S. enterica* serovar Enteritidis	0	0	0	15	**15**
5	*S. enterica* serovar Hissar	0	2	0	0	**2**
6	*S. enterica* serovar Kentucky	0	0	0	4	**4**
7	*S. enterica* serovar Lindenburg	**8**	0	**11**	**52**	**71**
8	*S. enterica* serovar Mathura	4	5	0	0	**9**
9	*S. enterica* serovar Poona	0	0	0	2	**2**
10	*S. enterica* serovar Senftenberg	1	0	0	0	**1**
11	*S. enterica* serovar Stuttgart	4	0	0	0	**4**
12	*S. enterica* serovar Tennessee	1	0	2	0	**3**
13	*S. enterica* serovar Typhimurium	**4**	0	**23**	**43**	**70**
14	*S. enterica* serovar Virchow	0	0	1	0	**1**
15	*S. enterica* serovar Weltevreden	1	0	3	7	**11**
	Total	**26**	**7**	**42**	**148**	**223**

Given the evolving drug resistance trends linked to the presence of $bla_{\text{CTX-M}}$ genes, as discussed earlier, a targeted investigation was initiated on this subset of 223 cefotaxime-resistant NTS isolates to assess the prevalence of the $bla_{\text{CTX-M15}}$ gene. This approach aligns with the methodology employed by Taneja et al. (2014) that revealed the prevalence of the $bla_{\text{CTX-M15}}$ gene in 11.6% of NTS isolated from gastroenteritis cases. Moreover, the study unveiled the $bla_{\text{CTX-M15}}$ gene in some newer NTS serovars, including Thompson, Infantis, and Newport, underscoring the expanding reach of this resistance determinant within the landscape of non-Typhoidal *Salmonella* in India.

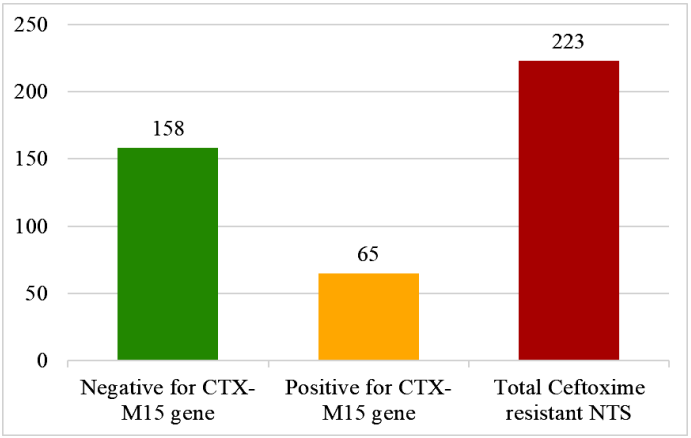
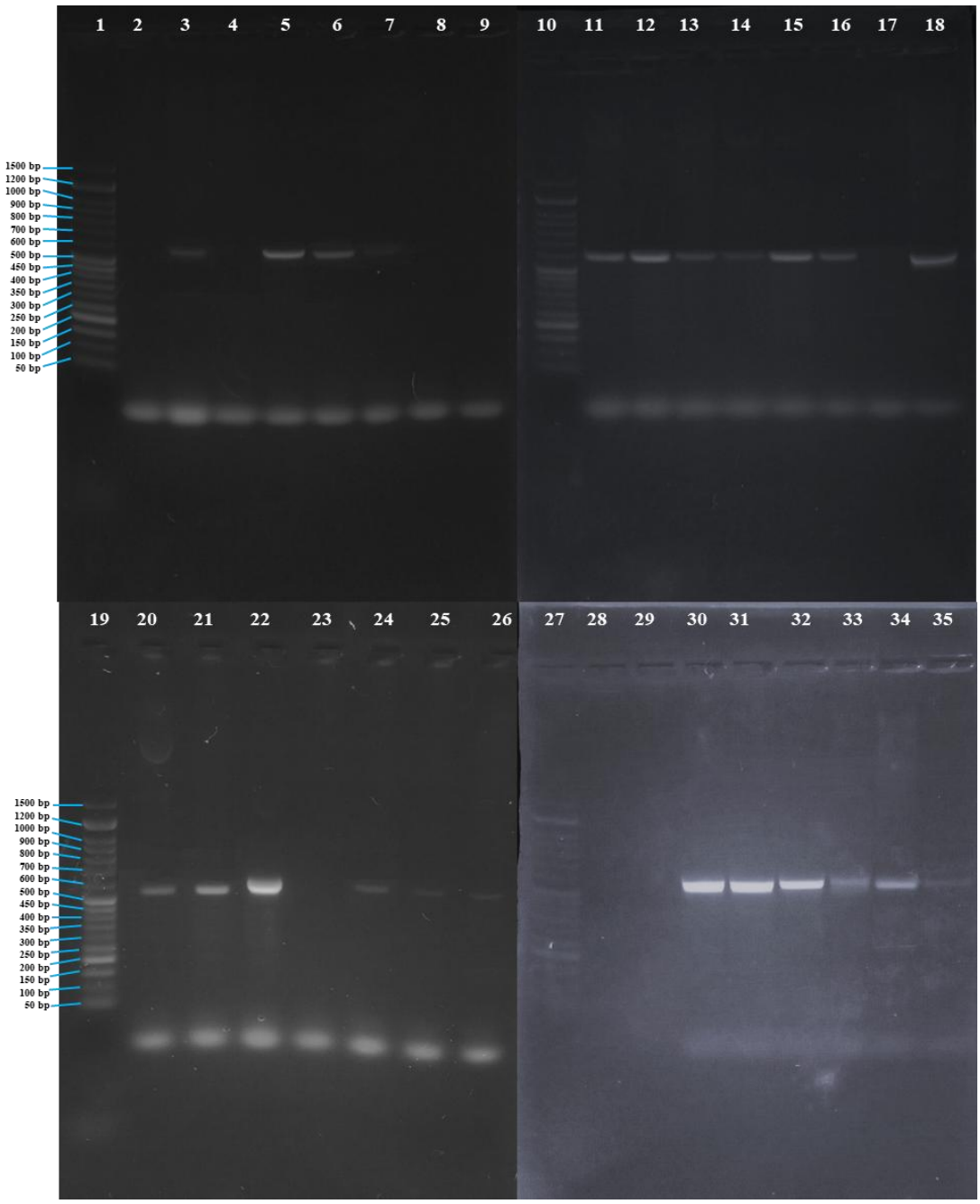


Fig. 5.20: Proportion of *bla*$_{\text{CTX-M15}}$ positive NTS.

In the current study, the investigation of the *bla*$_{\text{CTX-M15}}$ gene in the 223 cefotaxime-resistant isolates unveiled encouraging results with 65 isolates, constituting 29.1%, identified as harbouring the *bla*$_{\text{CTX-M15}}$ gene (Fig. 5.20). This finding indicates the prevalence of this specific *bla*$_{\text{CTX-M15}}$ variant within the subset of cefotaxime-resistant NTS isolates. While the remaining 158 isolates did not show the presence of this gene. Photograph 7 is a representation of the *bla*$_{\text{CTX-M15}}$ gene among these NTS.



Photograph 7: Representative photograph of *bla*$_{\text{CTX-M15}}$ gene positive cefotaxime resistant NTS.

Well No. 1,10,19, 27: 50-1500bp Ladder.

Well No. 3,5,6,7, 11, 12,13, 14, 15, 16, 18, 20, 21, 22, 23,24, 25, 30, 31, 32, 33, 34, 35 represent *bla*$_{\text{CTX-M15}}$ gene (587bp) positive.

Table 5.13. Regional distribution of *bla*$_{CTX-M15}$ gene-carrying NTS serovars

S. No.	*bla*$_{CTX-M15}$ gene-positive NTS	Regions of India				Total
		EAST	NORTH	SOUTH	WEST	
1	*S. enterica* serovar Anatum	0	1	0	0	**1**
2	*S. enterica* serovar Bazenheid	0	1	0	0	**1**
3	*S. enterica* serovar Choleraesuis	0	0	3	0	**3**
4	*S. enterica* serovar Enteritidis	0	0	9	0	**9**
5	*S. enterica* serovar Kentucky	0	0	1	0	**1**
6	*S. enterica* serovar Lindenburg	0	1	16	2	**19**
7	*S. enterica* serovar Mathura	0	1	0	2	**3**
8	*S. enterica* serovar Typhimurium	3	2	18	4	**27**
9	*S. enterica* serovar Weltevreden	0	0	1	0	**1**
Total		**3**	**6**	**48**	**8**	**65**

In the context of this study, Table 5.13 serves as a comprehensive representation of the geographical spread of *bla*$_{CTX-M15}$ gene-carrying Non-Typhoidal *Salmonella* (NTS) isolates across diverse regions of India. The data revealed noteworthy regional variations in the distribution of this resistance determinant, offering crucial insights into the intricate landscape of antimicrobial resistance in the country. The regional breakdown showcased distinct patterns, with the Southern region demonstrating the highest prevalence rate at 73.8% (48 out of 65). Serovars Lindenburg (16) and Typhimurium (18) were particularly prevalent in this region, collectively accounting for 70% of *bla*$_{CTX-M15}$ gene-positive isolates. A similar trend was observed in other regions, notably for the occurrence of *bla*$_{CTX-M15}$ gene-bearing S. Typhimurium. Furthermore, the Southern region exhibited the presence of other *bla*$_{CTX-M15}$ gene-positive serovars, including Enteritidis (9), Choleraesuis (3), Kentucky (1), and Weltevreden (1). Additionally, a lower occurrence of less common serovars such as Anatum (1), Bazenheid (1), and Mathura (1), was also observed in this study. These

findings underscore the dynamic regional distribution of the *bla*$_{\text{CTX-M15}}$ gene among cefotaxime-resistant NTS isolates, emphasizing the need for nuanced and region-specific approaches in the formulation of public health strategies to combat the escalating challenge of antibiotic resistance in NTS. Targeted surveillance and intervention efforts should consider not only the overall prevalence of cefotaxime resistance in NTS but also the specific distribution of resistance genes among different serovars and regions. Such insights are pivotal for devising effective and context-specific measures to address the escalating threat of antibiotic resistance in Non-Typhoidal *Salmonella*, safeguarding public health on a regional and national scale.

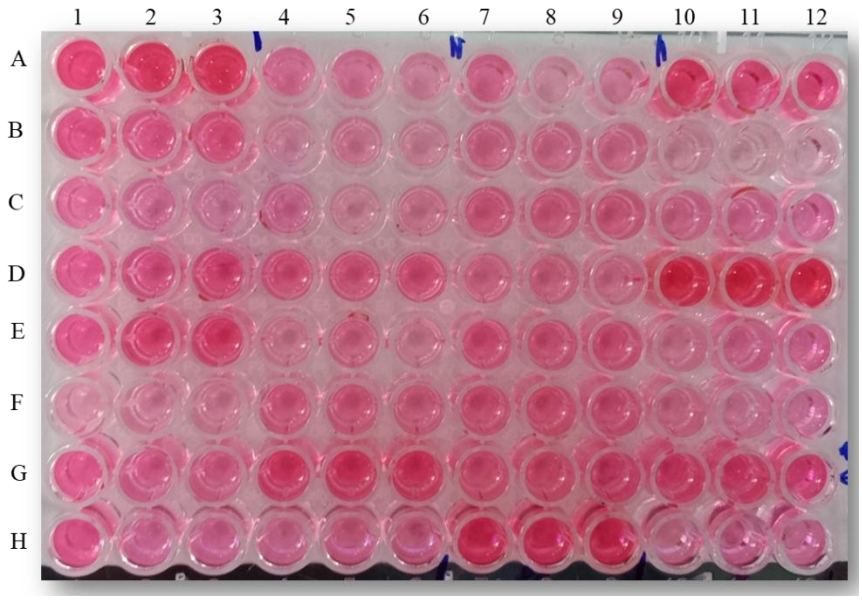
The current research aimed to assess the prevalence of NTS serovars across diverse geographical regions and sources within the country. It involved characterizing these serovars through various methods, including biotyping, serotyping, antibiogram profiling, and examining virulence markers such as biofilm and colicin production. Additionally, the study investigated the presence of enzymes like extended-spectrum beta-lactamase and carbapenemase, as well as the detection of genes responsible for ESBL characteristics and drug resistance in NTS. Given the importance of NTS serovars in human, animal, food, and environmental contexts, the research underscored the necessity for continued investigation to gain a better understanding of NTS epidemiology, as available data on its prevalence are limited or inconsistent. The study findings raise significant public health concerns and highlight the imperative to strengthen NTS surveillance in the country. Identifying and controlling virulent NTS serovars is crucial for enhancing knowledge and implementing effective control measures at both regional and national levels to mitigate infections caused by this group of pathogens.

5.8 Results of Biofilm production by NTS isolates:

Biofilms are intricate microbial communities, predominantly bacteria, characterized by their adherence to surfaces and envelopment in a viscous matrix consisting of extracellular polymeric substances (EPS). These substances encompass polysaccharides, nucleic acids, proteins, and other molecules synthesized by the microorganisms residing within the biofilm (Harrell et al., 2021). The elaborate cell network formed by biofilms serves as a predominant mode of bacterial growth in various environmental settings. Biofilm formation occurs as microorganisms attach themselves to surfaces, creating a defensive and adhesive matrix (Harrell et al., 2021). Within this biofilm matrix or microenvironment, bacteria gain a multifaceted protective shield, imparting resilience against environmental stressors such as UV exposure (Espeland and Wetzel, 2001), the action of disinfectants, immune responses from hosts (Jensen et al., 2010), and the effects of antibiotics (Stewart and Costerton, 2001; Mah and O'Toole, 2001; Mah, 2012), making them challenging to eradicate (Burmolle et al., 2010). This biofilm formation is frequently implicated in the development of various infections and outbreaks, contributing significantly to the resistance and persistence of bacteria on diverse biotic and abiotic surfaces (Steenackers et al., 2012). Non-Typhoidal *Salmonella* (NTS) has been found to exhibit biofilm-forming capabilities, allowing it to persistently colonize various environments and surfaces, both inside and outside the host (Abdallah et al., 2014; Khatoon et al., 2018; Vasicek and Gunn, 2023). Biofilm formation enhances bacterial survival and transmission, posing a significant public health challenge (Liu et al., 2023). To prevent biofilm formation by NTS, it is important to comprehend the factors that influence its formation.

In this study, biofilm production was assessed in all 539 NTS isolates using the tissue culture plate (TCP) method with slight modifications based on the protocol by Christensen et al. (1985). Widely recognized as the gold standard for detecting biofilm formation in bacteria, the TCP method is esteemed for its quantitative and dependable nature (Halim et al., 2018). It is a quantitative and reliable approach to assessing biofilm production. The TCP assay involves the growth of bacteria in a microtiter plate, allowing the adherence and formation of biofilms on the plate's surface. After

incubation, the biofilms are stained, and quantified, providing a measure of biofilm formation (Photograph 8). This method is more quantitative and reliable for biofilm detection (Mathur et al., 2006).



Photograph 8: Demonstration of Tissue Culture Plate (TCP) method for biofilm detection.

Well No. A1-H6: NTS in triplicate.
Well No. H7-H9: Positive control (*E.coli* K-12 culture).
Well No. H10-H12: Blank

Note: *Pink colour is due to elution of the safranin by 30% v/v Acetic acid. The intensity of colour corresponds to the amount of biofilm produced.*

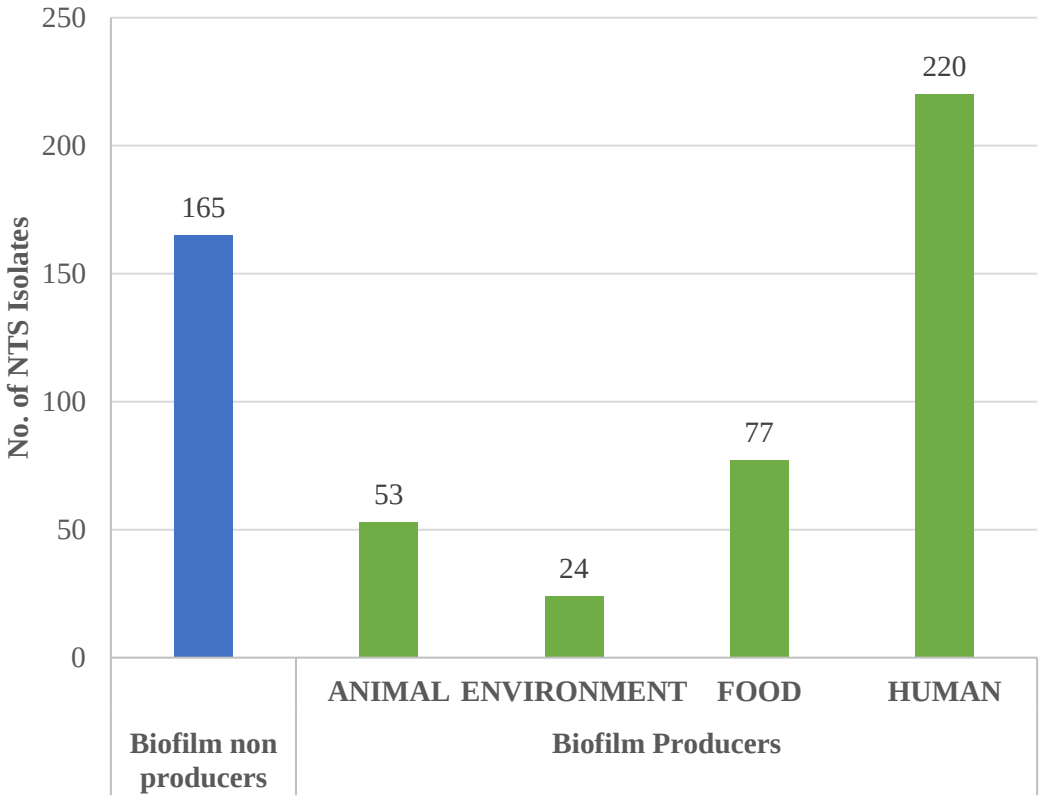


Fig. 5.21: Visualization of Biofilm Production: Producers vs. Non-Producer NTS.

Among the 539 NTS samples analysed, a substantial portion, specifically 374 isolates, constituting nearly 70% of the NTS pool, exhibited biofilm-producing capabilities (Fig. 5.21). This group of biofilm producers demonstrated varying degrees of biofilm formation. Upon closer examination of these 374 biofilm producers, it was revealed that the majority—220 NTS isolates, accounting for 58.8%—originated from human sources (Fig. 5.22). Additionally, 77 isolates (20.6%) were traced back to food, 53 (14.2%) to animals, and 24 (6.4%) to environmental sources. Further analysis, unveiled that approximately 6.4% (24 out of 374) were identified as strong biofilm producers, while 28.3% (106 out of 374) exhibited a moderate level of biofilm formation. The remaining 65.2% displayed a weak level of biofilm formation

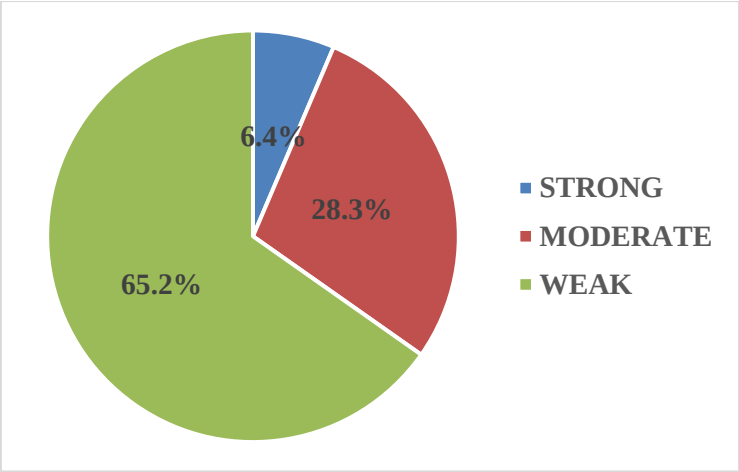


Fig. 5.22: Segmentation of Biofilm Producing NTS According to Varied Levels of Biofilm Formation.

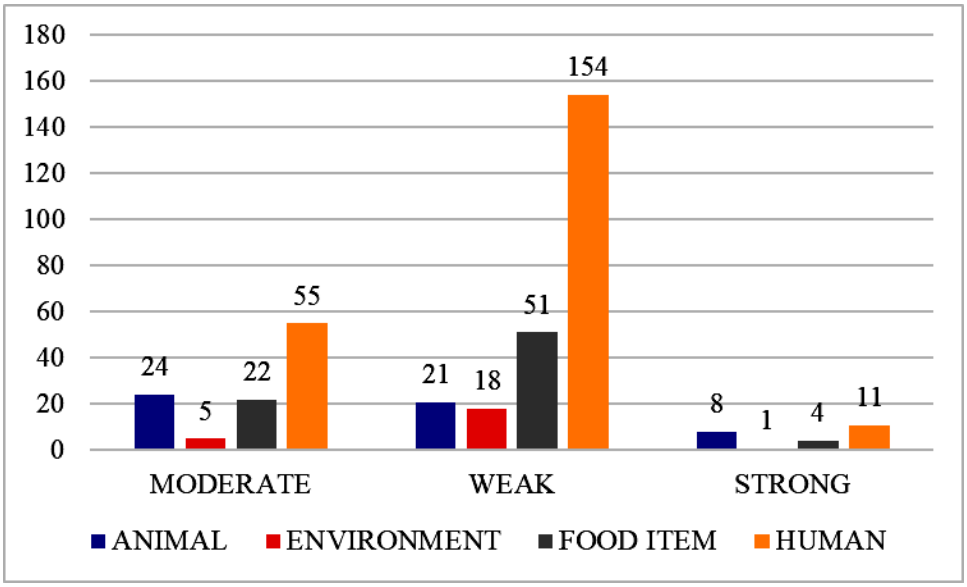


Fig. 5.23: Distribution of biofilm-producing NTS across different sources.

In this study, biofilm formation was predominantly seen amongst the NTS isolates obtained from human sources (Fig. 5.23). A greater positivity rate for biofilm production was seen amongst NTS obtained from human samples, against different grades of biofilm production, compared to other sources.

The dataset of NTS exhibiting strong biofilm production, offers a detailed exploration across diverse sources, presenting a subtle picture of serovar-specific

prevalence (Fig. 5.24). In animal sources, *S. enterica* serovar Lindenburg accounted for 4 isolates, while serovar Anatum and Mathura contributed 2 isolates each. The environmental source yielded 1 isolate of serovar Enteritidis showcasing strong biofilm production. Within food sources, *S. enterica* serovar Typhimurium exhibited prominence with 2 isolates, accompanied by serovar Anatum and Lindenburg, each contributing 1 isolate. Importantly, human sources displayed a varied distribution with *S. enterica* serovar Typhimurium taking the lead with 5 isolates, followed by Choleraesuis and Enteritidis with 2 isolates each, and serovar Lindenburg contributing 2 isolates. This comprehensive breakdown elucidates the serovar-specific prevalence of strong biofilm production within NTS across different sources.

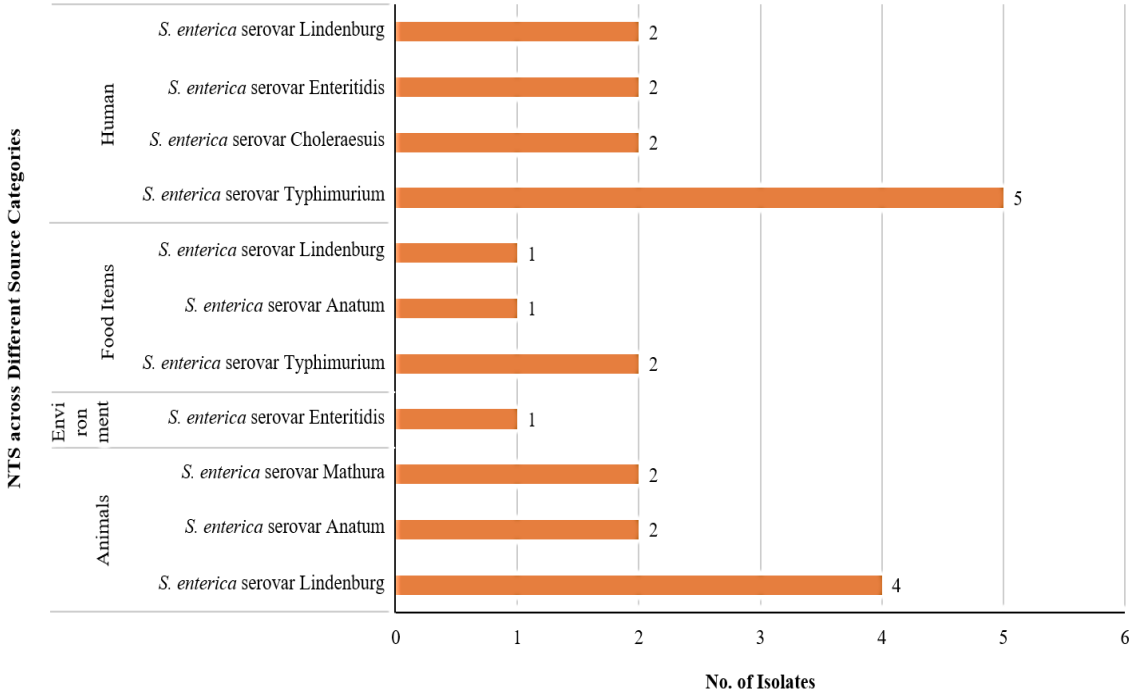


Fig. 5.24: Assessment of Strong Biofilm Producing NTS Across Diverse Sources.

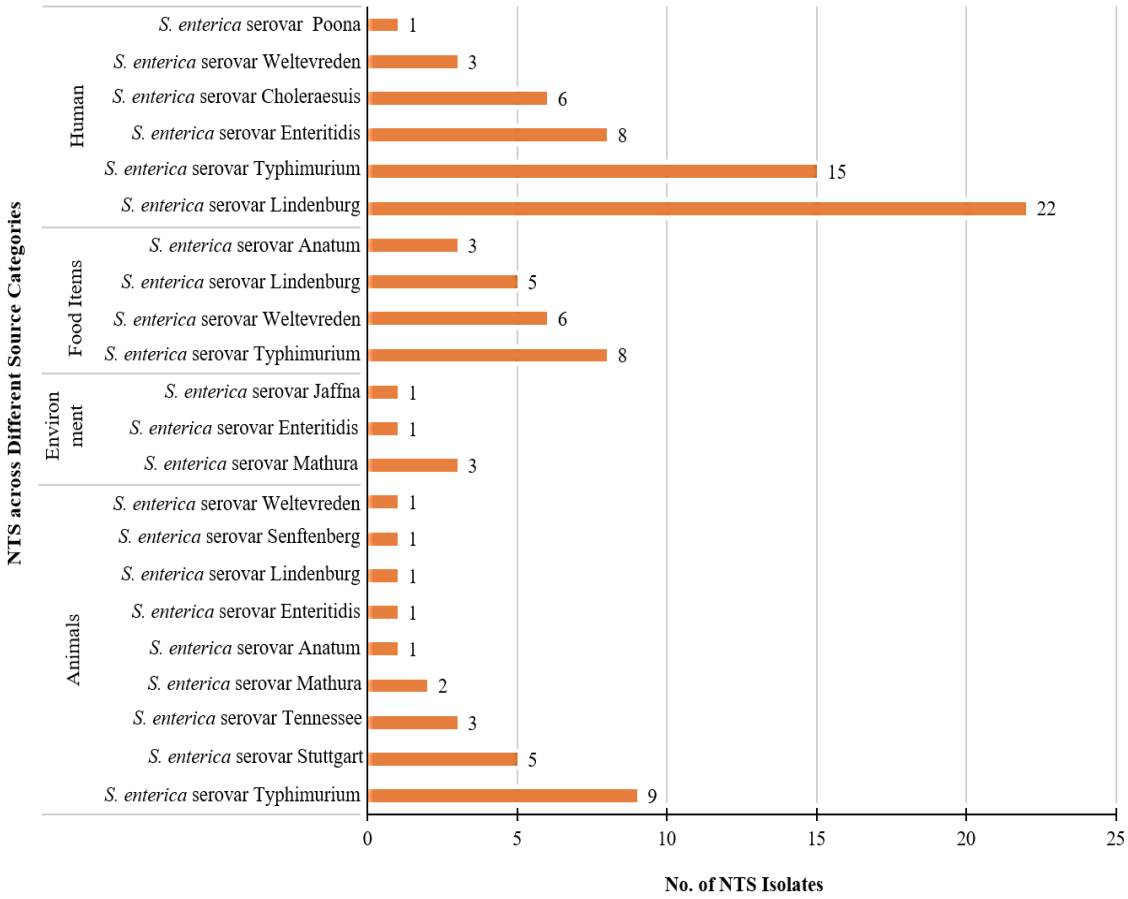


Fig. 5.25: Assessment of Moderate level biofilm producing NTS from different sources.

The investigation delved into the distribution of moderate biofilm-producing NTS isolates across distinct sources, shedding light on the prevalence and diversity of biofilm formation within different serovars (Fig. 5.25). Notably, among animal sources, *S. enterica* serovar Typhimurium exhibited prominence, contributing 9 isolates, followed by other serovars including Stuttgart (5), Tennessee (3), Mathura (2), Anatum (1), Enteritidis (1), Lindenburg (1), Senftenberg (1), and Weltevreden (1). Environmental sources revealed a distinctive profile, encompassing serovars Mathura (3), Enteritidis (1), and Jaffna (1). In food-derived isolates, serovars Typhimurium (8), Weltevreden (6), Lindenburg (5), and Anatum (3) predominated. Remarkably, human sources exhibited a diverse array, with *S. enterica* serovar Lindenburg leading with 22 isolates, followed by Typhimurium (15), Enteritidis (8), Choleraesuis (6), Weltevreden (3), and Poona (1).

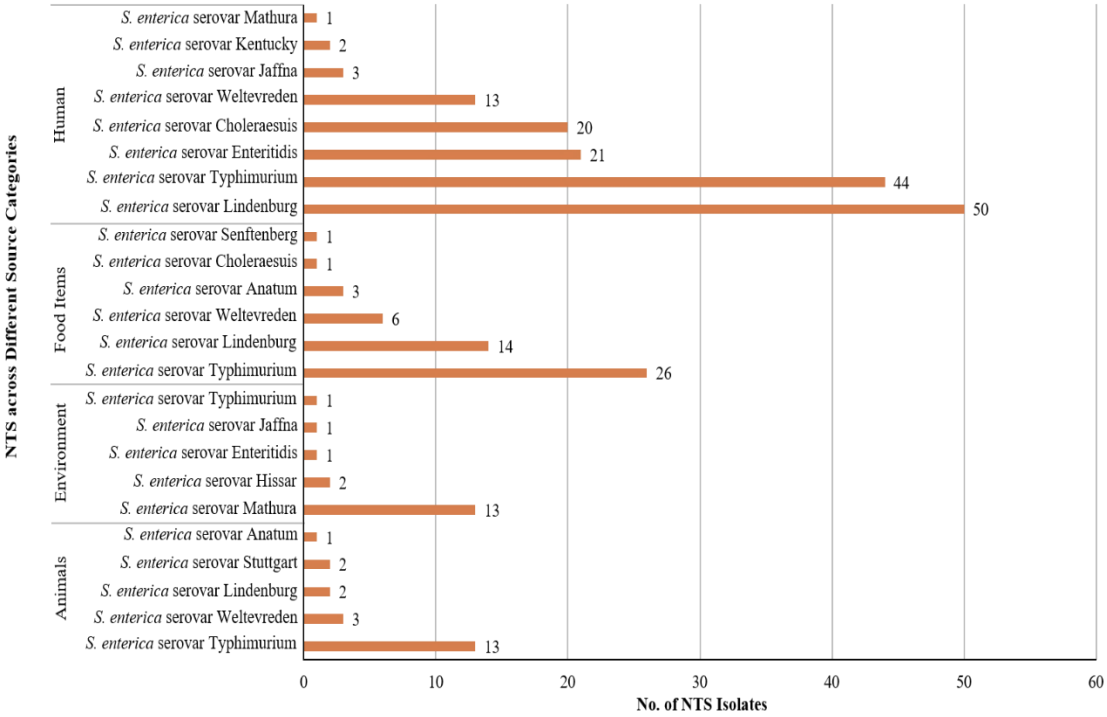


Fig. 5.26: Assessment of Weak-level biofilm producing NTS from different sources.

Fig. 5.26 illuminates the prevalence and serovar-specific associations of weak biofilm-producing NTS isolates, with a focus on diverse sources. In human sources, *S. enterica* serovar Lindenburg takes centre stage with 50 isolates, closely followed by Typhimurium (44), Enteritidis (21), Choleraesuis (20), Weltevreden (13), Jaffna (3), Kentucky (2), and a single isolate of Mathura. Food-derived isolates showcase a significant presence, particularly of Typhimurium (26), Lindenburg (14), Weltevreden (6), Anatum (3), Choleraesuis (1), and Senftenberg (1). Environmental sources exhibit a strong dominance of Mathura with 13 isolates, alongside contributions from Hissar (2), Enteritidis (1), Jaffna (1), and an additional isolate of Typhimurium. In animal sources, Typhimurium again stands out with 13 isolates, followed by contributions from Weltevreden (3), Lindenburg (2), Stuttgart (2), and Anatum (1).

In this study, a comprehensive assessment was conducted on a collection of NTS isolates to investigate their biofilm formation capabilities and antibiotic resistance profiles (Table 5.14). The results revealed noteworthy findings, which are crucial for

understanding the epidemiology and management of NTS infections. Among the NTS isolates analysed, a substantial proportion, constituting 290 isolates (53.8%), exhibited biofilm-producing characteristics along with resistance to various antibiotics used in this study. Additionally, 135 isolates (25%) demonstrated biofilm non-producing traits while still exhibiting resistance to antibiotics. Conversely, 84 isolates (15.6%) were identified as biofilm producers but exhibited sensitivity to all the drugs tested. Interestingly, a smaller subset, comprising 30 isolates (5.5%), demonstrated neither biofilm-producing tendencies nor resistance to antibiotics. The observed association underscores the potential interdependence of these two microbial characteristics. This finding has significant implications for understanding the virulence factors of NTS and their resistance mechanisms, shedding light on the intricate dynamics between biofilm formation and MDR behaviour.

The connection between biofilm production and multidrug resistance (MDR) in NTS strains has been thoroughly examined. Several studies have emphasized a significant link between biofilm formation and antibiotic resistance in NTS strains. For instance, research focusing on biofilm-forming NTS isolates from poultry and poultry-products revealed a significant relationship between biofilm formation and antibiotic resistance (Siddique et al., 2021). Another study underscored that *Salmonella* biofilms display up to 1000 times higher resistance to antibiotics compared to planktonic cells, with biofilm formation significantly contributing to bacterial resistance across various antibiotic classes (Aleksandrowicz et al., 2023). Moreover, the presence of multidrug resistance and efflux pump activity has been linked to biofilm-forming NTS isolates (Aleksandrowicz et al., 2023). Additionally, biofilm formation has been associated with *Salmonella* persistence and virulence, enabling the bacteria to disrupt host functions, develop drug resistance, and enhance resistance to host defence mechanisms (Harrell et al., 2021).

These findings collectively underscore the critical role of biofilm production in the multidrug resistance of NTS strains, with important implications for the development of antibiofilm strategies and the management of NTS infections.

Table 5.14. Relationship between biofilm formation and multiple drug resistance in NTS.

No. of drugs found resistant	Biofilm Producers			Biofilm non-producer	Total
	Strong	Moderate	Weak		
0	3	29	52	30	**114**
1	2	22	58	26	**108**
2	4	8	48	23	**83**
3	3	6	21	14	**44**
4	3	8	12	12	**35**
5	0	10	13	13	**36**
6	3	8	12	18	**41**
7	2	5	11	13	**31**
8	0	4	3	9	**16**
9	0	0	7	2	**9**
10	0	0	4	2	**6**
11	1	3	2	1	**7**
12	0	1	1	1	**3**
13	1	0	0	0	**1**
14	1	0	0	1	**2**
15	1	2	0	0	**3**
Total	**24**	**106**	**244**	**165**	**539**

The role of biofilm formation in the pathogenicity of NTS is significant as it contributes to the persistence and potential for chronic or latent infections, as well as aiding in the survival of NTS in various environments (Harika et al., 2020; Bai et al., 2021; Harrell et al., 2021). Biofilms create a stable habitat for bacteria, promoting symbiotic interactions with various organisms, similar to the colonization of marine species (Harrell et al., 2021). Regarding food safety, the formation of biofilms by NTS can result in heightened resistance to antimicrobial agents and sanitizers, complicating efforts for efficient cleaning and disinfection in food processing settings (Bai et al., 2021). Biofilm-forming bacteria can exhibit increased antibiotic resistance, making them more difficult to treat and control (Harika et al., 2020). The ability to form

biofilms has been linked to the host specificity and virulence of NTS strains, with some strains being more virulent and able to form biofilms, while others are less virulent and unable to form biofilms (MacKenzie et al., 2017).

Controlling biofilm formation in non-Typhoidal *Salmonella* is essential for preventing chronic infections, antibiotic resistance, and environmental persistence. Several strategies can be employed to inhibit biofilm formation in NTS such as the use of antibiotics can help reduce biofilm formation and disrupt existing biofilms. However, antibiotic resistance is a growing concern, making it crucial to develop new strategies to combat biofilm formation (Bai et al., 2021). Creating novel anti-biofilm compounds tailored to target NTS biofilm formation holds promise in thwarting biofilm development and enhancing treatment efficacy (MacKenzie et al., 2019). Identifying and utilizing biofilm inhibitors can help prevent biofilm formation in NTS. These inhibitors can be natural compounds, synthetic chemicals, or even bacterial products that interfere with biofilm formation or disrupt existing biofilms (Bai et al., 2021). Physical methods, such as high-pressure water or steam sterilization, can be used to disrupt biofilms and remove them from surfaces (Harrell et al., 2021). Implementing good sanitation practices, such as regular cleaning and disinfection of food processing equipment and medical devices, can help prevent biofilm formation and reduce the risk of NTS infections (Bai et al., 2021). Exploring the molecular mechanisms that drive biofilm formation in NTS can offer valuable insights into identifying pivotal factors and potential targets for devising novel strategies to regulate and manage biofilm formation effectively (MacKenzie et al., 2017).

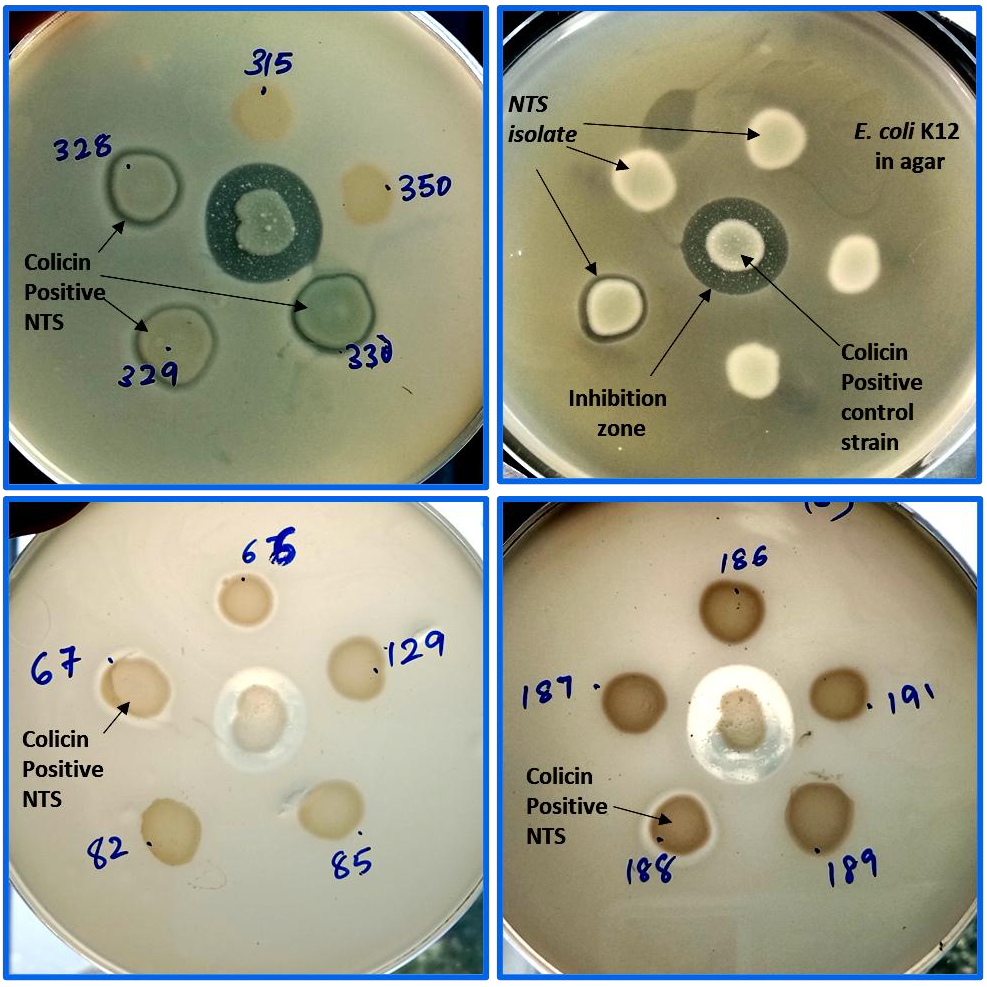
In summary, controlling biofilm formation in NTS can be achieved through various strategies, however, further research and development of new methods to control biofilm formation in NTS are essential for preventing chronic infections and reducing the risk of antibiotic resistance.

5.9 Results of Colicin production by the NTS isolates:

Bacteriocins are antimicrobial peptides or proteins produced by bacteria, both Gram-positive and Gram-negative, through ribosomal pathways (Jack et al., 1995; Simons et al., 2020; Chérier et al., 2021). These molecules exhibit unique characteristics, being immune to the effects of their production and contributing to various ecological functions. Playing a pivotal role in the environment, bacteriocins showcase antimicrobial activity and engage in nutrient competition (Chérier et al., 2021; Marković et al., 2022). Their antibacterial properties allow them to either kill or inhibit the growth of competing microorganisms. While not essential for bacterial growth, bacteriocins serve as valuable tools in outcompeting other microorganisms for limited nutrients within their environment (Sengkhui et al., 2023). Colicins possess promising applications in both biotechnology and medicine, serving as antimicrobial proteins effective for controlling strains of the same or related species (Hahn-Löbmann et al., 2019). Some of the potential applications of colicins include food preservation wherein, colicins are used to control pathogenic bacteria in fruits and vegetables as well as on processed or cut produce, and as an additive in ready-to-eat fruits and vegetables (Hahn-Löbmann et al., 2019, Marković et al., 2022); in medicine, colicins have been studied for anticancer activity (Marković et al., 2022); colicin as bacterio-control agents finds application in dairy products, meat processing equipment or other related apparatuses to inhibit microbial growth (Marković et al., 2022); and finally, an important application i.e. the antimicrobial potential of colicins has been utilized in the prevention of extraluminal contamination of urinary catheters by *E. coli* (Roy and Riley, 2019).

Several studies, such as the one by Kaldhone et al. (2019), have demonstrated the production of colicins by *Salmonella*. The role of IncI1 plasmids in bacteriocin production, specifically colicin production by *Salmonella*, has been evaluated in one study. Additionally, another study reports the carriage of incompatibility group (Inc) FIB plasmids by S. Typhimurium isolates responsible for colicin production (Aljahdali et al., 2020).

In alignment with these studies, an examination was carried out to evaluate colicin production among the current set of NTS isolates (539). The phenotypic soft agar overlay technique described by Parreira et al. (1998) was employed for this assessment. This technique, frequently utilized for colicin production testing, involves incorporating a colicin-sensitive strain into the top agar layer to identify colicins produced by the test organisms. The presence of a zone of inhibition in the top agar signifies colicin production. (Photograph 9).



Photograph 9: Demonstration of colicin activity by soft agar overlay technique.

The results from the colicin detection test showed a relatively low occurrence of colicin-producing NTS isolates, with only 4.63% (25 out of 539) exhibiting colicin activity (Table 5.15). Among these, the highest proportion of colicin-producing isolates came from human sources (14/25, 54.0%), followed by isolates from food items (6/25, 24.0%) and animals (5/25, 20.0%). Notably, no colicin activity was observed in any of the environmental isolates.

Table 5.15: Comparative Analysis: Source Categories vs. Colicin Production in NTS.

Source of NTS	Source sub-category	NTS serovar	No. of colicin-positive NTS
Human (n=14)	Faeces	*S. enterica* serovar Lindenburg	7
		S. enterica serovar Typhimurium	5
	Urine	*S. enterica* serovar Lindenburg	1
	Tissue	*S. enterica serovar* Weltevreden	1
Animal (n=5)	Faeces	*S. enterica* serovar Typhimurium	3
	Poultry	*S. enterica* serovar Mathura	2
Food items (n=6)	Poultry meat	*S. enterica* serovar Senftenberg	1
	Poultry feed	*S. enterica* serovar Typhimurium	1
	Raw Pork	*S. enterica* serovar Anatum	3
		S. enterica serovar Lindenburg	1
		Total	**25**

These findings (Table 5.15) further elaborate on the sources of NTS isolates and colicin production across various contexts. In human samples (n=14), most colicin-positive NTS isolates were from fecal samples, notably from *S. enterica* serovars Lindenburg and Typhimurium. Colicin activity was also present in NTS from urine and tissue samples, specifically from serovars Lindenburg and Weltevreden. In animal samples (n=5), colicin-producing strains of *S. enterica* serovar Typhimurium were found in feces, and poultry samples exhibited colicin activity mainly in serovar Mathura. Food items (n=6), including poultry meat, feed, and raw pork, also showed colicin-positive NTS isolates from various serovars.

This study highlights the diversity of colicin-producing NTS strains, underlining their roles as reservoirs in human, animal, and food sources. While serovar Typhimurium is known for colicin production (Barker, 1980; Aljahdali et al., 2020), we report novel findings of colicin activity in serovars such as Mathura, Anatum, and Senftenberg, previously undocumented in the literature, particularly in India. This work broadens our understanding of colicin production among NTS serovars, revealing unexplored aspects of their ecology and transmission pathways. Future research is needed to delve into the mechanisms, ecological roles, and potential public health applications of colicin production in these unique NTS strains, ultimately enhancing our understanding of NTS dynamics and informing potential strategies for infection control.

CHAPTER – 6

SUMMARY
&
CONCLUSION

SUMMARY AND CONCLUSION

6.1. SUMMARY :

For generations, infectious diseases have posed substantial challenges to global public health, carrying the potential for considerable morbidity and mortality and presenting threats to societal structures and healthcare infrastructures. Among the various causative microorganisms, the *Salmonella* genus emerges as a formidable contender, distinguished by its adaptability, resilience, and ability to infect a diverse array of hosts. This genus is classified into major groups viz. Typhoidal and non-Typhoidal *Salmonella* (NTS). While Typhoidal *Salmonella* strains, typified by *Salmonella* Typhi and *Salmonella* Paratyphi, cause the typhoid fever -a systemic disease; NTS serovars collectively exhibit a broad spectrum of clinical manifestations including bloodstream infections, and localized gastrointestinal diseases. NTS mostly spreads through contaminated food & drinks and drinking water supplies. NTS has gained global scientific attention due to its ability to cause a broad range of enteric diseases, presenting diagnostic and therapeutic challenge, particularly in the developing world. There is no denying that NTS is becoming more prevalent throughout Asia. The epidemiology of NTS in India remains relatively underdeveloped due to the absence of effective surveillance systems and the lack of suitable diagnostic tools. Consequently, data on clinical symptoms, the course of the illness, patient demographics, and the contaminating serovars, however, are either non-existent or scarce. Therefore, precise and comprehensive data regarding the epidemiology of NTS must be produced periodically to build surveillance systems that are both effective and rational for controlling NTS-related health issues. Consequently, the current study has been designed to examine the prevalence and other traits of non-Typhoidal *Salmonella* derived from various sources (humans, animals, food, and environment) across the nation. This study focuses on studying the prevalence and characteristics of 539 NTS isolates obtained from diverse sources spread over different geographic locations across the country. The key aspects included: biotyping and serotyping; antibiogram profiling, detection of extended-spectrum beta-lactamase (ESBL) and carbapenemase production; identification of genes responsible for ESBL and cefotaxime resistance; and evaluation of virulence factors (biofilm, colicin).

6.1.1. SAMPLE COLLECTION:

In this study, 999 bacterial isolates received at 'National *Salmonella* and *Escherichia* Centre' (NSEC) in CRI, Kasauli were used. The isolates were randomly received from a variety of locations including hospitals, research institutions (agricultural and veterinary), as well as fisheries and food laboratories spanning different regions throughout India. These isolates were then classified into four distinct groups based on their origin: human, food, environmental, and animal sources.

6.1.2. BIOTYPING:

The biotyping of the 999 bacterial isolates involved a series of morphological and biochemical tests. These isolates were then cultured on freshly prepared MacConkey Agar plates, with subsequent selection of non-lactose fermenting (NLF) colonies for streaking onto duplicate nutrient agar (NA) slants. Identification was carried out using morphological, biochemical, and serological assays. Based on the results of these tests, consistent with the Genus *Salmonella*, (Gram-negative, motile bacilli, approx. 2-3µm in length; Nitrate Reduction +ve; Catalase +ve; Oxidase -ve; IMViC Test : -ve, +ve, -ve, +ve; Urease +ve; Glucose A/G, TSI K/A, H_2S positive, with or without CO_2) were designated as confirmed *Salmonella* isolates. The results confirmed the presence of 699 out of 999 (70%) *Salmonellae* isolates whereas the remaining 300 (30%) isolates belonged to other genera and therefore were not used further in the study.

6.1.3. SEROTYPING:

After the initial biotyping, the study proceeded to confirm different serovars within the *Salmonella* genus. Slide agglutination tests were conducted on the 699 *Salmonella* isolates using polyvalent and monovalent sera targeting the somatic 'O' and flagellar 'H' antigens. Among these, 539 isolates were confirmed as Non-Typhoidal *Salmonella* (NTS). The main focus of the study shifted to this subset of 539 confirmed NTS. The breakdown of serovars revealed 17 different types, listed in decreasing order of occurrence (Data already published. **Kumar et al., 2022**):

1. *S. enterica* serovar Typhimurium (4,12:i:1,2), n=167
2. *S. enterica* serovar Lindenburg (6,8:i:1,2), n=135
3. *S. enterica* serovar Enteritidis (9,12:gm:-), n =56
4. *S. enterica* serovar Choleraesuis (6,7:c:1,5), n =41
5. *S. enterica* serovar Weltevreden (3,10 : r : Z_6), n=44
6. *S. enterica* serovar Mathura (9, 46:i:e,n,Z_{15}), n= 33
7. *S. enterica* serovar Anatum (3,10: e, h:1,6), n=12
8. *S. enterica* serovar Jaffna (9,12:d:Z_{35}), n=10
9. *S. enterica* serovar Tennessee (6,7:Z_{29}:-), n=9
10. *S. enterica* serovar Stuttgart (6,7:i:Z_6), n= 8
11. *S. enterica* serovar Kentucky (8, 20: i: Z_6), n=6
12. *S. enterica* serovar Bazenheid (8,20: Z_{10}:1,2), n = 4
13. *S. enterica* serovar Hissar (6,7:c:1,2), n =4
14. *S. enterica* serovar Virchow (6,7:r:1,2), n= 4
15. *S. enterica* serovar Poona (13, 22: z: 1,6), n=3
16. *S. enterica* serovar Senftenberg (1,3,19:g,t:-), n=2
17. *S. enterica* serovar Ughelli (3, 10: r : 1,5), n= 1

This detailed breakdown sheds light on the relative abundance of each serovar, enabling a deeper understanding of the epidemiological scenario of NTS in our study.

6.1.4. PREVALENCE OF NTS:

In our dataset, it is important to highlight the significant prevalence of *S. enterica* serovar Typhimurium and Lindenburg, constituting 30.98% and 25.05% of the isolates, respectively. Additionally, *S. enterica* serovar Enteritidis represented 10.39% of the total isolates. These findings underscore the noteworthy dominance of these specific serovars within the *Salmonella* population studied. The diversity observed reflects the multifaceted nature of NTS, with various serovars exhibiting substantial prevalence rates. The presence of these serovars in the dataset raises questions about their ecological niches, potential sources, and transmission dynamics. Despite their diversity, these serovars are crucial elements in NTS epidemiology, necessitating

thorough examination as they may provide insights into emerging patterns, host specificity, or regional variations in NTS distribution across India.

6.1.5. GEOGRAPHICAL DISTRIBUTION OF NTS:

The collection of NTS isolates covered 14 states and union territories (UTs) throughout India. This extensive geographic coverage was instrumental in capturing the diversity of NTS serovars prevalent in different regions of the country. Samples were systematically received from a variety of sources, including clinical specimens, animal reservoirs, and environmental samples, representing a broad spectrum of potential NTS hosts and transmission pathways. This extensive and diverse collection strategy aimed to provide a comprehensive understanding of the epidemiology of NTS in India, shedding light on regional variations, prevalent serovars, and potential sources of infection.

6.1.6. DISTRIBUTION OF NTS ACROSS DIFFERENT REGIONS:

The distribution pattern of NTS serovars indicates a notable concentration of isolates from the southern region of India, surpassing proportions in other regions. The non-uniform rate of NTS isolates submissions across the country suggests varying consistency among laboratories. The high number of NTS isolates from human sources (319/539, 59.18%) highlights the persistent public health challenge posed by NTS-related illnesses, necessitating continuous efforts in diagnosis, surveillance, and prevention. The prevalence of NTS in food items (99/539, 18.37%) underscores the importance of stringent food safety practices, monitoring supply chains, and public awareness to minimize foodborne transmission risks. The collection of NTS isolates from animal sources (83/539, 15.4%) emphasizes the zoonotic potential of NTS infections, requiring strategies to reduce prevalence in animals and enhance biosecurity in agriculture. Though NTS isolates from environmental sources were comparatively lower (38/539, 7.05%), it highlights the role of environmental factors in transmission dynamics, emphasizing their consideration in NTS epidemiology.

6.1.7. NTS DISTRIBUTION ACROSS HUMAN SAMPLES (N=319):

Among 319 isolates from human sources, fecal samples emerged as the dominant reservoir, comprising 63.95% (204 out of 319), while blood samples contributed substantially at 22.88% (73 out of 319). highlighting the clinical importance of NTS infections. Other sources (urine, cerebrospinal fluid, pus, tissue, and body fluids) collectively yielded < 15% (40/319) of total isolates, with cervical abscess and sputum samples presenting the lowest count. Among the total 17 NTS serovars identified, 10 were present in human-related samples, with Lindenburg (32.28%) and Typhimurium (27.89%) as dominant, collectively representing nearly 60% of the collection. Serovars Enteritidis (15.67%) and Choleraesuis (12.54%) had a comparatively lesser presence, while the remaining six serovars contributed just under 11.6%. Detailed analysis of source sub-categories revealed Lindenburg (36.76%) and Typhimurium (25.49%) dominance in fecal sources. Serovar Mathura was isolated from human blood, a crucial finding not previously documented in existing literature. While the isolation rate is low, it emphasizes the need for further investigations into the epidemiological role, transmission sources, and clinical implications of S. enterica serovar Mathura in human infections. Future studies should explore its potential sources and transmission dynamics.

6.1.8. NTS FROM ANIMAL SOURCES (N=83):

Among 83 isolates obtained from animal sources, 43 (51.8%) NTS isolates were from fecal samples, poultry feces and other poultry samples contributed respectively 11(13.3%) and 7(8.4%). Notably, one isolate was obtained from calf fetal stomach content, indicating NTS presence in young livestock. Four isolates from poultry diarrhoea samples highlight the importance of monitoring and addressing NTS infection in poultry populations. Sixteen isolates (19.3%) with an unknown site within the animal category underscore the need for precise sample tracking in epidemiological studies.

The analysis identified that 10 out of a total 17 NTS serovars were from animal sources, with serovar Typhimurium being highly prevalent (38.6%). This raises concerns due to its known zoonotic potential and association with severe human

infections. Serovar Lindenburg (13.3%), Weltevreden (10.8%), and Stuttgart (9.6%) were also notable. Other serovars, including Mathura, Anatum, Bazenheid, Tennessee, Enteritidis, and Senftenberg, collectively represented 27.7%. The majority were traced back to animal fecal samples. This comprehensive insight emphasizes the importance of surveillance and targeted measures to mitigate potential risks associated with zoonotic transmission.

6.1.9. NTS FROM FOOD (N=99):

The analysis of 99 isolates from different food items revealed distinctive distribution patterns. Raw pork had the highest number of isolates at 32 (32.3%); seafood contributed 12 (12.1%) isolates; meat (fish, pork, beef) encompassed 11 (11.1%) isolates; poultry meat samples yielded 6 (6.1%) isolates; one isolate was found in poultry feed. These findings emphasize the importance of safe food handling practices and suggest food materials as a potential route for NTS contamination. Raw cow or buffalo milk samples accounted for 13 (13.1%) isolates, underscoring the importance of pasteurization and hygiene. Animal feed contributed 12 (12.1%) isolates, highlighting the need for quality control in animal nutrition. Shrimp samples and eggs had 3 and 1 isolates, respectively, emphasizing safe handling and cooking practices.

In the analysis of NTS data from food samples, serovar Typhimurium emerged as the predominant serovar, representing 45.4% (45 out of 99 samples) of the total isolates. It displayed a widespread presence across diverse food items, including eggs, shrimps, animal feed items, pork, fish, beef, and various seafoods. This highlights the importance of thorough food safety measures to address and mitigate the impact of this serovar across diverse food categories. Serovar Lindenburg was the second most prevalent serovar, with an isolation rate of 21.2% (21/99). It was found in raw milk, poultry meat, and seafood. However, in five instances, the specific origin of serovar Lindenburg remained unknown, indicating a need for further investigation and detailed surveillance to determine potential reservoirs and transmission pathways.

6.1.10. NTS FROM ENVIRONMENT (N= 38):

In this study, 38 NTS isolates were derived from environmental sources, such as poultry environments, meat shop floors, and meat-cutting knives. The data underscores

the risk of cross-contamination during food preparation, emphasizing the need for stringent sanitation practices. Animal fodder environments yielded the highest number of NTS isolates, highlighting their significance as a reservoir for NTS and their potential impact on livestock health and food production chains. Among the different NTS serovars detected in environmental samples, serovar Mathura and Jaffna were prevalent in areas related to animal food storage, necessitating targeted interventions to uphold food safety standards in these environments. The presence of S. enterica serovar Hissar in poultry environments raised concerns about biosecurity measures and the potential transmission of *Salmonella* in poultry production chain, emphasizing the need to address factors contributing to contamination risks. Findings in meat processing and meat shop environments emphasized the importance of hygiene standards and targeted sanitation measures to eliminate the danger of *Salmonella* contamination. Overall, the study provides valuable insights for future research and intervention strategies aimed at diminishing *Salmonella* prevalence in the food supply chain.

6.1.11. ANTIBIOGRAM PROFILING OF NTS:

In a comprehensive analysis of antibiotic resistance patterns in NTS, cefotaxime exhibited the highest resistance, with a concerning prevalence of 44.90% (242 out of 539) among NTS strains. Following closely, nalidixic acid and piperacillin/tazobactam displayed resistance rates of 39.89% and 27.27%, respectively. Commonly prescribed antibiotics, such as ampicillin, showed a resistance rate of 27.09%, while amoxicillin/clavulanic acid and trimethoprim exhibited rates of 25.97% and 19.67%, respectively. Notably, nitrofurantoin displayed a resistance rate of 17.44%, and co-trimoxazole exhibited a rate of 14.47%. Imipenem, a broad-spectrum antibiotic, demonstrated a resistance rate of 13.73%. Furthermore, noteworthy resistance rates were observed for key antibiotics like ciprofloxacin (11.13%), imipenem (13.73%), and ceftriaxone (11.87%). In contrast, some antibiotics, including chloramphenicol (0.93%) and cefepime (1.67%), exhibited lower resistance rates.

A detailed analysis of cefotaxime-resistant NTS isolates revealed that 67% (162 out of 242) originated from human sources, while 17.8% (43 out of 242) were traced back to food items. Notably, among cephalosporins, the fourth-generation drug cefepime exhibited significantly higher efficacy, with minimal NTS strains showing

resistance. The resistance profiles of NTS to Piperacillin/tazobactam (27.27%), Ampicillin (27.09%), Amoxicillin/clavulanic acid (25.97%), Imipenem (13.73%), and Meropenem (10.20%) pose substantial challenges in antimicrobial therapy. This resistance is most prominent in NTS isolates from human origins, followed by those from food and animal sources.

The study identifies a notable level of resistance to nalidixic acid, particularly in human populations, with 132 out of 215 resistant Non-Typhoidal *Salmonella* (NTS) isolates originating from humans. Environmental (36/215) and animal (30/215) sources also exhibit significant resistance, suggesting the spread of resistant strains beyond humans. Additionally, 17 isolates from food sources show resistance to nalidixic acid, emphasizing diverse reservoirs contributing to resistance development. The study highlights a substantial proportion of isolates resistant to key fluoroquinolones, including ciprofloxacin (53/60) and norfloxacin (31/33), indicating reduced susceptibility to these commonly used antibiotics in clinical settings.

An in-depth analysis reveals notable ineffectiveness of NTS resistance to 19 antimicrobial drugs, especially sulphonamides and nitrofurans, with cotrimoxazole resistance in 106 isolates (19.67%) and trimethoprim resistance in 78 isolates (14.47%). This resistance trend is prominent in the human category, emphasizing challenges associated with sulphonamide resistance in human populations.

Resistance to nitrofurantoin was observed in 94 isolates, constituting 17.44% of the total NTS tested. Of these, 46.8% (44 out of 94) were from human sources, while 26.6% (25 out of 94) were traced to animal sources, emphasizing the significant presence of nitrofurantoin-resistant Non-Typhoidal *Salmonella* (NTS) strains. These findings highlight the urgent need for targeted strategies to address resistance in both human and animal reservoirs.

6.1.12. MULTIDRUG RESISTANCE AMONG THE NTS:

The present study provides a comprehensive view of the MDR and XDR profiles exhibited by NTS strains. Notably, the Southern part of the country displayed the highest level 48.2% (151/313) of MDR resistant NTS, followed by the Northern part 37.8% (37/98), Western part 26.9% (24/89). Further analysis of different sources

identified 53% of NTS from animal sources and 49.5% from human sources exhibit MDR and XDR behaviours. A crucial observation arising from this study is the detection of NTS isolates demonstrating resistance to more than eight antimicrobial drugs. This particular finding holds significant clinical implications, highlighting the ineffectiveness of a substantial portion of commonly used antibiotics in treating infections caused by non-Typhoidal *Salmonella*. These results underscore the immediate need for holistic approaches to combat the rise and dissemination of multidrug resistance. It is imperative to integrate surveillance, antimicrobial stewardship, and research endeavours to grasp the fundamental mechanisms of resistance and devise focused interventions.

6.1.13. EXTENDED SPECTRUM B –LACTAMASE (ESBL) PRODUCTION:

Out of the 539 Non-Typhoidal *Salmonella* (NTS) isolates subjected to ESBL production testing using cefotaxime (CTX) and ceftazidime (CAZ), along with their combinations with clavulanic acid (CAC and CEC), only 24 isolates (4.45%) were found to exhibit ESBL activity. Among these, 11 NTS demonstrated ESBL detection solely through ceftazidime and its combination with clavulanic acid, while 9 through cefotaxime and cefotaxime-clavulanic acid, and 4 NTS isolates exhibited ESBL positivity detected by both tested drugs. 16 ESBL-producing isolates were of human origin while 4, 3 & 1 isolates were obtained from environmental sources, animal sources, and food items respectively. The 24 isolates were tested for the presence of ESBL genes, (*bla*$_{\text{CTX-M15}}$, *bla*$_{\text{CMY-2}}$, *bla*$_{\text{SHV}}$, and *bla*$_{\text{TEM}}$). None of the NTS were found to harbor the *bla*$_{\text{SHV}}$ gene. The prevalence of two genes, namely *bla*$_{\text{CTX-M15}}$ and *bla*$_{\text{CMY-2}}$, was particularly notable, with a total of 11 and 14 isolates, respectively. Among the 16 isolates from human sources, 7 carried the *bla*$_{\text{CTX-M15}}$ gene, 12 harbored the *bla*$_{\text{CMY-2}}$ gene, and 4 exhibited the presence of the *bla*$_{\text{TEM}}$ gene. Two isolates from human fecal samples were identified as hosts for three genes (excluding *bla*$_{\text{SHV}}$). Four isolates carried the *bla*$_{\text{CTX-M15}}$ with the *bla*$_{\text{CMY-2}}$ gene combination; one isolate carried the *bla*$_{\text{CTX-M15}}$ with the *bla*$_{\text{TEM}}$ gene combination. Among the 4 environmental isolates, two isolates showed the presence of *bla*$_{\text{CTX-M15}}$ genes only. One isolate from an animal source was found to harbor three genes: *bla*$_{\text{CTX-M15}}$, *bla*$_{\text{CMY-2}}$, and *bla*$_{\text{TEM}}$.

6.1.14. DETECTION OF CARBAPENEMASE ENZYME ACTIVITY:

In the present study, the antibiogram profiling analysis of 539 NTS isolates revealed 96 NTS isolates resistant to carbapenem drugs (41 resistant to imipenem, 22 resistant to meropenem, and 33 both drugs). Therefore these 96 NTS isolates were tested further for the presence of enzyme carbapenemase using the Modified Hodge Test. The findings of this study revealed that none of the isolates exhibited the presence of the carbapenemase enzyme. Despite the current absence of carbapenemase in the tested NTS isolates, vigilance and continued monitoring are crucial due to the evolving nature of bacterial resistance. Ongoing surveillance is necessary to promptly detect and address any alterations in the resistance profile of NTS isolates.

6.1.15. DETECTION OF CEFOTAXIME DRUG RESISTANCE GENES:

In the current study, the antibiogram profiling of 539 NTS isolates revealed 242 NTS (44.9%), exhibiting resistance to Cefotaxime (CTX). Among these, 19 isolates were removed as they were already included in the pool of 24 ESBL-producing NTS isolates. Therefore remaining 223 NTS were tested further for the cefotaxime resistance gene (*bla*$_{\text{CTX-M15}}$). The study unveiled encouraging results with 65 (29.1%) isolates harbouring the *bla*$_{\text{CTX-M15}}$ gene encompassing 15 NTS serovars out of the total 17 identified in this study.

The data from the study unveiled significant regional variations in the distribution of the *bla*$_{\text{CTX-M15}}$ resistance gene. The Southern region stood out with the highest prevalence rate with 48 (73.8%) isolates carrying the *bla*$_{\text{CTX-M15}}$ gene. Serovars Lindenburg (n=16) and Typhimurium (n=18) were particularly dominant in this region, collectively constituting 70% of *bla*$_{\text{CTX-M15}}$ gene-positive isolates. Similar patterns were observed in other regions, especially concerning *S.* Typhimurium carrying the *bla*$_{\text{CTX-M15}}$ gene. Additionally, the Southern region exhibited the presence of other *bla*$_{\text{CTX-M15}}$ gene-positive serovars, including Enteritidis (n=9), Choleraesuis (n=3), Kentucky (n=1), and Weltevreden (n=1). The study also noted a lower occurrence of less common serovars such as Anatum (n=1), Bazenheid (n=1), and Mathura (n=1) in this region. These findings highlight the diverse distribution of the *bla*$_{\text{CTX-M15}}$ gene across different

serovars in the Southern region and emphasize the need for tailored interventions to address region-specific challenges in antimicrobial resistance.

6.1.16. BIOFILM PRODUCTION BY NTS ISOLATES:

In the present study, all 539 NTS isolates were tested for the production of biofilm. Among the 539 NTS samples analysed, 374 (70%) isolates, exhibited biofilm-producing capabilities. Among these, a majority (n=220) of the isolates sourced from human sources followed by 77 (20.6%) from food, 53 (14.2%) from animal sources, and 24 (6.4%) from environmental sources. Approximately 6.4% (24 out of 374) were identified as strong biofilm producers, while 28.3% (106 out of 374) exhibited a moderate level of biofilm formation. The remaining 65.2% displayed a weak level of biofilm formation. A greater positivity rate for biofilm production was seen amongst NTS obtained from human samples, against different grades of biofilm production, compared to other sources.

Further, the human sources exhibited a diverse array, with serovar Lindenburg leading with 22 isolates, followed by Typhimurium (n=15), Enteritidis (n=8), Choleraesuis (n=6), Weltevreden (n=3), and Poona (n=1). **A**mong animal sources serovar Typhimurium exhibited prominence, contributing 9 isolates, followed by Stuttgart (n=5), Tennessee (n=3), Mathura (n=2), Anatum (n=1), Enteritidis (n=1), Lindenburg (n=1), Senftenberg (n=1), and Weltevreden (n=1). Environmental sources revealed a distinctive profile, encompassing serovars Mathura (n=3), Enteritidis (n=1), and Jaffna (n=1). In food-derived isolates, serovars Typhimurium (n=8), Weltevreden (n=6), Lindenburg (n=5), and Anatum (n=3) dominated.

In this study, an association between the biofilm producing NTS isolates and multiple drug resistance behaviour has also been observed. The observed association underscores the potential interdependence of these two microbial characteristics. This finding has significant implications for understanding the virulence factors of NTS and their resistance mechanisms, shedding light on the intricate dynamics between biofilm formation and MDR.

6.1.17. COLICIN PRODUCTION BY THE NTS ISOLATES:

The results of the colicin detection test revealed that the existence of colicin activity among the NTS isolates was relatively limited, with 4.63% (25 out of 539) of the isolates demonstrating the ability to produce colicins. The colicin production rate was found to be higher among NTS isolates obtained from human sources (14/25, 54.0%), followed by those obtained from food items (6/25, 24.0%) and animals (5/25, 20.0%). Conversely, none of the environmental isolates showed colicin activity. Among human sources (n=13), the majority of colicin-positive NTS isolates were found in faecal samples, with *S. enterica* serovar Lindenburg and Typhimurium dominating the observed instances. Additionally, a noteworthy presence of colicin activity was detected in NTS from urine & tissue samples, specifically *S. enterica* serovar Lindenburg and *S. enterica* serovar Weltevreden respectively. In animal sources (n=5) among faecal samples, *S. enterica* serovar Typhimurium, exhibited colicin production. Poultry sources showed colicin activity primarily in *S. enterica* serovar Mathura. The data from food items (n=6) revealed colicin-positive NTS isolates in poultry meat, poultry feed, and raw pork, with various contributing serovars.

6.2. CONCLUSION:

Overall, the conclusion of this study emphasizes the pressing public health implications posed by non-typhoidal Salmonella (NTS) in India. The present study explores the prevalence of Non-Typhoidal *Salmonella* in diverse geographic locations across India with notable detection of NTS serovars from diverse sources including human, animal, food, and environmental sources. By systematically examining the prevalence and characterization of NTS serovars across diverse regions and sources of isolation, our research fills a critical gap in the current knowhow of NTS dynamics in India. The significance of this research lies in its ability to contribute to the existing body of knowledge surrounding NTS epidemiology, specifically by providing a comprehensive analysis of the distribution across diverse regions; diverse sources of isolation encompassing humans, animals, food and environmental sources; emerging drug resistance patterns of NTS; valuable insight of the molecular pathways involved such as detection of genes responsible for drug resistance in NTS; understanding the virulence of NTS serovars in terms of enzymes (lactamase, carbapenemase), biofilm and colicins production.

The findings of this study emphasize the growing challenge of antimicrobial resistance (AMR) in NTS, with isolates showing resistance to over eight commonly used antibiotics. This result highlights a critical concern for clinical treatment, as many traditional antibiotics are becoming less effective against NTS infections. The rise in resistance underscores the urgent need for the development of alternative treatment strategies, to address the increasing difficulty in managing these infections. This trend reflects broader global concerns about the threat posed by AMR, particularly within foodborne pathogens, and signals the necessity for revised therapeutic approaches and stronger antibiotic stewardship measures.

Furthermore, the identification of newer & colicin-producing NTS serovars, such as *S. enterica* serovars *Mathura*, *Anatum*, and *Senftenberg*, introduces a new dimension to understanding bacterial competition and resistance mechanisms. This novel finding expands our knowledge of the interactions between different *Salmonella* strains and the potential role of colicins in microbial ecology. Future research should delve deeper into the molecular mechanisms behind colicin production in NTS, exploring how it may influence not only resistance dynamics but also bacterial growth regulation. This discovery could lead to innovative approaches for managing bacterial populations and mitigating resistance development

The present work enriches our understanding of the challenges and opportunities in combating NTS. The identification and characterization of NTS strains in India have important implications for NTS disease prevention, surveillance, or intervention strategies, ultimately contributing to improved public health. It is important to acknowledge the limitations of this study, including limited resources and a lack of modern diagnostic tools & technologies, which may guide future research in expanding our understanding of NTS in the Indian context. In conclusion, this study not only highlights the ongoing challenges presented by NTS but also provides a foundation for future research efforts. It is anticipated that these insights will catalyse further exploration and innovation, ultimately enhancing NTS prevention and control strategies and contributing meaningfully to public health improvements.

BIBLIOGRAPHY

BIBLIOGRAPHY

Aarestrup FM, Hendriksen RS, Lockett J, Gay K, Teates K, McDermott PF, White DG, Hasman H, Sørensen G, Bangtrakulnonth A, Pornreongwong S. International spread of multidrug-resistant *Salmonella* Schwarzengrund in food products. Emerging Infectious Diseases. 2007 May;13(5):726.

Abdallah M, Benoliel C, Drider D, Dhulster P, Chihib NE. Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. Archives of microbiology. 2014 Jul;196(7):453-72.

Acheson D, Hohmann EL. Nontyphoidal salmonellosis. Clinical infectious diseases. 2001 Jan 15;32(2):263-9.

Adesiji YO, Shivakumaraswamy SK, Deekshit VK, Kallappa GS, Karunasagar I. Molecular characterization of antimicrobial multi-drug resistance in non-typhoidal *Salmonellae* from chicken and clam in Mangalore, India. Journal of Biomedical Research. 2018 Jun 6;32(3):237.

Afema JA, Mather AE, Sischo WM. Antimicrobial resistance profiles and diversity in S Almonella from humans and cattle, 2004–2011. Zoonoses and Public Health. 2015 Nov;62(7):506-17.

Agarwal L, Kumar Singh A, Agarwal A, Kumar Yadav R. Bacterial and Parasitic Intestinal Infections among Food Handlers in a Tertiary Care Hospital of Lucknow, Uttar Pradesh, India. Journal of Medical Microbiology and Infectious Diseases. 2022 Jun 10;10(2):54-7.

Akinyemi KO, Fakorede CO, Linde J, Methner U, Wareth G, Tomaso H, Neubauer H. Whole genome sequencing of Salmonella enterica serovars isolated from humans, animals, and the environment in Lagos, Nigeria. Bmc Microbiology. 2023 Jun 13;23(1):164.

Aktas Z, Day M, Kayacan CB, Diren S, Threlfall EJ. Molecular characterization of *Salmonella* Typhimurium and *Salmonella* Enteritidis by plasmid analysis and pulsed-field gel electrophoresis. International journal of antimicrobial agents. 2007 Dec 1;30(6):541-5. https://doi.org/10.1016/j.ijantimicag.2007.06.034

Al-Ansari MM, Aljubali MM, Somily AM, Albarrag AM, Masood A. Isolation and molecular characterization of multidrug-resistant *Salmonella* enterica serovars. Journal of Infection and Public Health. 2021 Dec 1;14(12):1767-76.

Aleksandrowicz A, Carolak E, Dutkiewicz A, Błachut A, Waszczuk W, Grzymajlo K. Better together–*Salmonella* biofilm-associated antibiotic resistance. Gut Microbes. 2023 Dec 31;15(1):2229937.

Aljahdali NH, Khajanchi BK, Weston K, Deck J, Cox J, Singh R, Gilbert J, Sanad YM, Han J, Nayak R, Foley SL. Genotypic and phenotypic characterization of incompatibility group fib positive *Salmonella* enterica serovar typhimurium isolates from food animal sources. Genes. 2020 Nov 4;11(11):1307.

Allen KJ, Poppe C. Occurrence and characterization of resistance to extended-spectrum cephalosporins mediated by β-lactamase CMY-2 in *Salmonella* isolated from food-producing animals in Canada, Can J Vet Res, 2002, vol. 66 (pg. 137-44)

Althouse C, Patterson S, Fedorka-Cray P, Isaacson RE. Type 1 fimbriae of *Salmonella* enterica serovar Typhimurium bind to enterocytes and contribute to colonization of swine in vivo. Infection and immunity. 2003 Nov;71(11):6446-52.

Alvarez-Martinez CE, Christie PJ. Biological diversity of prokaryotic type IV secretion systems. Microbiology and Molecular Biology Reviews. 2009 Dec;73(4):775-808.

An R, Alshalchi S, Breimhurst P, Munoz-Aguayo J, Flores-Figueroa C, Vidovic S. Strong influence of livestock environments on the emergence and dissemination of distinct multidrug-resistant phenotypes among the population of non-typhoidal *Salmonella*. PLoS One. 2017 Jun 7;12(6):e0179005.

Anderson CJ, Kendall MM. *Salmonella* enterica serovar Typhimurium strategies for host adaptation. Frontiers in microbiology. 2017 Oct 12;8:1983.

Andrews WH, Wang H, Jacobson A, Hammack T, Food and Drug Administration. Bacteriological analytical manual (BAM) chapter 5: Salmonella. Bacteriological Analytical Manual. 2018;110.

Angulo FJ, Collignon P, Powers JH, Chiller TM, Aidara-Kane A, Aarestrup FM. World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies for the use of antimicrobials in food production animals. Clinical infectious diseases. 2009 Jul 1;49(1):132-41.

Antony B. Non Typhoidal *Salmonella*e and its aetiological spectrum-An overview with Indian perspective. IP International Journal of Medical Microbiology and Tropical Diseases. 2023 Jan 18;8(1):3-9.

Aouf A, Messai Y, Salama MS, Aboushady HM, El-Anany MG, Alouache S, Bakour R. Resistance to β-lactams of human and veterinary *Salmonella* isolates in Egypt and Algeria. Afr J Microbiol Res. 2011 Apr 4;5(7):802-8.

Arcangioli MA, Leroy-Setrin S, Martel JL, Chaslus-Dancla E. Evolution of chloramphenicol resistance, with emergence of cross-resistance to florfenicol, in bovine *Salmonella* Typhimurium strains implicates definitive phage type (DT) 104. Journal of medical microbiology. 2000 Jan;49(1):103-10.

Arii J, Tanabe Y, Miyake M, Mukai T, Matsuzaki M, Niinomi N, Watanabe H, Yokota Y, Kohno Y, Noda M. Clinical and pathologic characteristics of nontyphoidal *Salmonella* encephalopathy. Neurology. 2002 Jun 11;58(11):1641-5.

Authority EF. European Food Safety Authority, and European Centre for Disease Prevention and Control. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA J. 2015;13:3991.

Authority EF. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2019–2020. EFSA Journal. 2022 Mar;20(3).

Aygan AS, Arikan BU. Mini review an overview on bacterial motility detection. Int. J. Agric. Biol. 2007;9:193-6.

Azriel S, Goren A, Shomer I, Aviv G, Rahav G, Gal-Mor O. The Typhi colonization factor (Tcf) is encoded by multiple non-typhoidal *Salmonella* serovars but exhibits a varying expression profile and interchanging contribution to intestinal colonization. Virulence. 2017 Nov 17;8(8):1791-807.

Backert S, Meyer TF. Type IV secretion systems and their effectors in bacterial pathogenesis. Current opinion in microbiology. 2006 Apr 1;9(2):207-17.

Bae D, Cheng CM, Khan AA. Characterization of extended-spectrum β-lactamase (ESBL) producing non-typhoidal *Salmonella* (NTS) from imported food products. International Journal of Food Microbiology. 2015 Dec 2;214:12-7.

Bai X, Nakatsu CH, Bhunia AK. Bacterial biofilms and their implications in pathogenesis and food safety. Foods. 2021 Sep 8;10(9):2117.

Baird-Parker AC. Foodborne salmonellosis. Lancet. 1990;336(Nov. 17):1231-5.

Bakowski MA, Braun V, Brumell JH. *Salmonella*-containing vacuoles: directing traffic and nesting to grow. Traffic. 2008 Dec;9(12):2022-31.

Bali EB, Acik L, Sultan N. Phenotypic and Molecular Characterization of SHV, Spectrum Beta-Lactamase (CTX-M-3 Like) from India and Gene Association with Insertion Sequence ISEcp1. Microbiology Letters. 2010, 2, 237-241.

Ballal M, Devadas SM, Shetty V, Bangera SR, Ramamurthy T, Sarkar A. Emergence and serovar profiling of non-typhoidal *Salmonellae* (NTS) isolated from gastroenteritis cases–A study from South India. Infectious Diseases. 2016 Dec 1;48(11-12):847-51.

Bangera SR, Umakanth S, Chowdhury G, Saha RN, Mukhopadhyay AK, Ballal M. Poultry: A receptacle for non-typhoidal *Salmonellae* and antimicrobial resistance. Iranian Journal of Microbiology. 2019 Feb;11(1):31.

Bao H, Wang S, Zhao JH, Liu SL. *Salmonella* secretion systems: Differential roles in pathogen-host interactions. Microbiological Research. 2020 Dec 1;241:126591.

Bao H, Zhao JH, Zhu S, Wang S, Zhang J, Wang XY, Hua B, Liu C, Liu H, Liu SL. Genetic diversity and evolutionary features of type VI secretion systems in *Salmonella*. Future microbiology. 2019 Jan;14(02):139-54.

Barak JD, Jahn CE, Gibson DL, Charkowski AO. The role of cellulose and O-antigen capsule in the colonization of plants by *Salmonella* enterica. Molecular Plant-Microbe Interactions. 2007 Sep;20(9):1083-91.

Barker J, Bloomfield SF. Survival of *Salmonella* in bathrooms and toilets in domestic homes following salmonellosis. Journal of applied Microbiology. 2000 Jul 1;89(1):137-44.

Barker RM. Colicinogeny in *Salmonella* typhimurium. Microbiology. 1980 Sep;120(1):21-6.

Barlag B, Hensel M. The giant adhesin SiiE of *Salmonella* enterica. Molecules. 2015 Jan 12;20(1):1134-50.

Barlow RS, DeBess EE, Winthrop KL, Lapidus JA, Vega R, Cieslak PR. Travel-associated Antimicrobial Drug–Resistant Nontyphoidal *Salmonellae*, 2004–2009. Emerging infectious diseases. 2014 Apr;20(4):603.

Barton-Behravesh C, Jones TF, Vugia DJ, Long C, Marcus R, Smith K, Thomas S, Zansky S, Fullerton KE, Henao OL, Scallan E. Deaths associated with bacterial pathogens transmitted commonly through food: foodborne diseases active surveillance network (FoodNet), 1996–2005. Journal of Infectious Diseases. 2011 Jul 15;204(2):263-7.

Basler M, Ho BT, Mekalanos JJ. Tit-for-tat: type VI secretion system counter attack during bacterial cell-cell interactions. Cell. 2013 Feb 14;152(4):884-94.

Baumler AJ, Tsolis RM, Heffron F. Fimbrial adhesins of *Salmonella* typhimurium: Role in bacterial interactions with epithelial cells. Mechanisms in the pathogenesis of enteric diseases. 1997:149-58. https://doi.org/10.1007/978-1-4899-1828-4_23/COVER

Bhan MK, Bahl R, Bhatnagar S. Typhoid and paratyphoid fever. The Lancet. 2005 Aug 27;366(9487):749-62.

Bhunia AK. Foodborne microbial pathogens: mechanisms and pathogenesis. Springer; 2018 May 21.

Blondel CJ, Jiménez JC, Contreras I, Santiviago CA. Comparative genomic analysis uncovers 3 novel loci encoding type six secretion systems differentially distributed in *Salmonella* serotypes. BMC genomics. 2009 Dec;10:1-7.

Blondel CJ, Yang HJ, Castro B, Chiang S, Toro CS, Zaldívar M, Contreras I, Andrews-Polymenis HL, Santiviago CA. Contribution of the type VI secretion system encoded in SPI-19 to chicken colonization by *Salmonella* enterica serotypes Gallinarum and Enteritidis. PLoS One. 2010 Jul 22;5(7):e11724.

Boddicker JD, Ledeboer NA, Jagnow J, Jones BD, Clegg S. Differential binding to and biofilm formation on, HEp-2 cells by *Salmonella* enterica serovar Typhimurium is dependent upon allelic variation in the fimH gene of the fim gene cluster. Molecular microbiology. 2002 Sep;45(5):1255-65.

Boyer F, Fichant G, Berthod J, Vandenbrouck Y, Attree I. Dissecting the bacterial type VI secretion system by a genome wide in silico analysis: what can be learned from available microbial genomic resources? BMC genomics. 2009 Dec;10(1):1-4.

Brink B. Urease test protocol. American society for microbiology. 2010 Nov 11:1-7.

Brummer B, Smith AM, Modise M, Thomas J, Mdose H, Mokoena R, Baleni D. Whole-Genome Sequencing assisted outbreak investigation of Salmonella Enteritidis, at a hospital in South Africa, September 2022. Access Microbiology. 2024 May 1:000835-v1.

Brunet YR, Khodr A, Logger L, Aussel L, Mignot T, Rimsky S, Cascales E. H-NS silencing of the *Salmonella* pathogenicity island 6-encoded type VI secretion system limits *Salmonella* enterica serovar Typhimurium interbacterial killing. Infection and immunity. 2015 Jul;83(7):2738-50.

Buchwald DS, Blaser MJ. A review of human salmonellosis: II. Duration of excretion following infection with nontyphi *Salmonella*. Reviews of infectious diseases. 1984 May 1;6(3):345-56.

Burmolle M, Thomsen TR, Fazli M, Dige I, Christensen L, Homøe P, Tvede M, Nyvad B, Tolker-Nielsen T, Givskov M, Moser C. Biofilms in chronic infections–a matter of opportunity–monospecies biofilms in multispecies infections. FEMS Immunology & Medical Microbiology. 2010 Aug 1;59(3):324-36.

Buxton R. Nitrate and nitrite reduction test protocols. American Society for Microbiology. 2011 Nov 1:1-20. Cited from website: https://asm.org/ASM/media/Protocol-Images/Nitrate-and-Nitrite-Reduction-Test-Protocols.pdf?ext=.pdf

Bythwood TN, Soni V, Lyons K, Hurley-Bacon A, Lee MD, Hofacre C, Sanchez S, Maurer JJ. Antimicrobial resistant *Salmonella* enterica Typhimurium colonizing chickens: the impact of plasmids, genotype, bacterial communities, and antibiotic administration on resistance. Frontiers in Sustainable Food Systems. 2019 Apr 16;3:20.

Campbell JV, Mohle-Boetani J, Reporter R, Abbott S, Farrar J, Brandl M, Mandrell R, Werner SB. An outbreak of *Salmonella* serotype Thompson associated with fresh cilantro. The Journal of infectious diseases. 2001 Mar 15;183(6):984-7.

Carattoli A. Animal reservoirs for extended spectrum β-lactamase producers. Clinical Microbiology and Infection. 2008 Jan;14:117-23.

Carattoli A. Plasmids and the spread of resistance. International journal of medical microbiology. 2013 Aug 1;303(6-7):298-304.

Carattoli A. Resistance plasmid families in Enterobacteriaceae. Antimicrobial agents and chemotherapy. 2009 Jun;53(6):2227-38.

Cascales E, Buchanan SK, Duché D, Kleanthous C, Lloubes R, Postle K, Riley M, Slatin S, Cavard D. Colicin biology. Microbiology and molecular biology reviews. 2007 Mar;71(1):158-229.

Cascales E, Cambillau C. Structural biology of type VI secretion systems. Philosophical Transactions of the Royal Society B: Biological Sciences. 2012 Apr 19;367(1592):1102-11.

CDC (2019). Antibiotic resistance threats in the United States 2019. Available at: https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf

CDC Yellow Book 2024, Salmonellosis, Nontyphoidal. Available from: https://wwwnc.cdc.gov/travel/yellowbook/2024/infections-diseases/salmonellosis-nontyphoidal

Cebrián L, Rodríguez JC, Escribano I, Royo G. In vitro generation of *Salmonella* spp. mutants following repeated exposure to β-lactam antibiotics. Journal of infection and chemotherapy. 2006 Jan 1;12(2):80-2.

Centers for Disease Control and Prevention (CDC). (2022) *Salmonella*. Available from: https://www.cdc.gov/salmonella/index.html. Retrieved on 18-05-2022.

Centers for Disease Control and Prevention. (2010). Multistate Outbreak of Human *Salmonella* Montevideo Infections. https://www.cdc.gov/salmonella/2010/montevideo-5-4-2010.html

Chang YJ, Chen MC, Feng Y, Su LH, Li HC, Yang HP, Yu MJ, Chen CL, Chiu CH. Highly antimicrobial-resistant Nontyphoidal *Salmonella* from retail meats and clinical impact in children, Taiwan. Pediatrics & Neonatology. 2020 Aug 1;61(4):432-8.

Chaudhuri D, Roy Chowdhury A, Biswas B, Chakravortty D. *Salmonella* typhimurium infection leads to colonization of the mouse brain and is not completely cured with antibiotics. Frontiers in microbiology. 2018 Jul 18;9:1632.

Chen CL, Wang CY, Chu C, Su LH, Chiu CH. Functional and molecular characterization of pSE34 encoding a type IV secretion system in *Salmonella* enterica serotype Enteritidis phage type 34. FEMS Immunology & Medical Microbiology. 2009 Dec 1;57(3):274-83.

Chen H, Qiu H, Zhong H, Cheng F, Wu Z, Shi T. Non-Typhoidal *Salmonella* Infections Among Children in Fuzhou, Fujian, China: A 10-Year Retrospective Review from 2012 to 2021. Infection and Drug Resistance. 2023 Dec 31:2737-49.

Chen HM, Wang Y, Su LH, Chiu CH. Nontyphoid *Salmonella* infection: microbiology, clinical features, and antimicrobial therapy. Pediatr Neonatol. 2013;54:147–52

Chen K, Gao Y, Li L, Zhang W, Li J, Zhou Z, He H, Chen Z, Liao M, Zhang J. Increased Drug Resistance and Biofilm Formation Ability in ST34-Type *Salmonella* Typhimurium Exhibiting Multicellular Behavior in China. Frontiers in Microbiology. 2022 Mar 18;13:876500.

Chen PL, Li CY, Hsieh TH, Chang CM, Lee HC, Lee NY, Wu CJ, Lee CC, Shih HI, Ko WC. Epidemiology, disease spectrum and economic burden of non-typhoidal

Salmonella infections in Taiwan, 2006–2008. Epidemiology & Infection. 2012 Dec;140(12):2256-63.

Chérier D, Patin D, Blanot D, Touzé T, Barreteau H. The biology of colicin M and its orthologs. Antibiotics. 2021 Sep 14;10(9):1109.

Chiu CH, Wu TL, Su LH, Chu C, Chia JH, Kuo AJ, Chien MS, Lin TY. The emergence in Taiwan of fluoroquinolone resistance in *Salmonella* enterica serotype Choleraesuis. New England Journal of Medicine. 2002 Feb 7;346(6):413-9.

Chiu CH, Wu TL, Su LH, Liu JW, Chu C. Fluoroquinolone resistance in *Salmonella* enterica serotype Choleraesuis, Taiwan, 2000–2003. Emerging infectious diseases. 2004 Sep;10(9):1674.

Cho S, Jackson CR, Frye JG. The prevalence and antimicrobial resistance phenotypes of *Salmonella*, Escherichia coli and Enterococcus sp. in surface water. Letters in Applied Microbiology. 2020 Jul 1;71(1):3-25.

Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, Beachey EH. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. Journal of clinical microbiology. 1985 Dec;22(6):996-1006.

Christie PJ. The mosaic type IV secretion systems. EcoSal Plus. 2016 Aug 4;7(1):10-128.

Chu J, Shin J, Kang S, Shin S, Chung YJ. Rapid and sensitive detection of Salmonella species targeting the hilA gene using a loop-mediated isothermal amplification assay. Genomics & Informatics. 2021 Sep;19(3).

Clinical and Laboratory Standards Institute (CLSI) (2014) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI Document M100-S24, Wayne, 34(1).

CLSI. 2022. Performance Standards for Antimicrobial Susceptibility Testing, M100 32nd Edition. Clinical and Laboratory Standards Institute, Wayne, PA.

Correia S, Poeta P, Hébraud M, Capelo JL, Igrejas G. Mechanisms of quinolone action and resistance: where do we stand?. Journal of medical microbiology. 2017 May;66(5):551-9.

Crawford RW, Gibson DL, Kay WW, Gunn JS. Identification of a bile-induced exopolysaccharide required for *Salmonella* biofilm formation on gallstone surfaces. Infection and immunity. 2008 Nov;76(11):5341-9.

Crawford RW, Reeve KE, Gunn JS. Flagellated but Not Hyper fimbriated *Salmonella* enterica Serovar Typhimurium Attaches to and Forms Biofilms on Cholesterol-Coated Surfaces: New Perspectives at the Heart of Surface-Associated Microbial Communities. Journal of bacteriology. 2010;192(12):2981-90.

Crosa JH, Brenner DJ, Ewing WH, Falkow S. Molecular relationships among the Salmonelleae. Journal of bacteriology. 1973 Jul;115(1):307-15.

Crump JA, Heyderman RS. A perspective on invasive *Salmonella* disease in Africa. Clinical Infectious Diseases. 2015 Nov 1;61(suppl_4):S235-40.

Crump JA, Kretsinger K, Gay K, Hoekstra RM, Vugia DJ, Hurd S, Segler SD, Megginson M, Luedeman LJ, Shiferaw B, Hanna SS. Clinical response and outcome of infection with *Salmonella* enterica serotype Typhi with decreased susceptibility to fluoroquinolones: a United States foodnet multicenter retrospective cohort study. Antimicrobial agents and chemotherapy. 2008 Apr;52(4):1278-84.

Crump JA, Ramadhani HO, Morrissey AB, Saganda W, Mwako MS, Yang LY, Chow SC, Morpeth SC, Reyburn H, Njau BN, Shaw AV. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected adults and adolescents in northern Tanzania. Clinical infectious diseases. 2011 Feb 1;52(3):341-8.

Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. Clinical microbiology reviews. 2015 Oct;28(4):901-37.

Dai W, Zhang Y, Zhang J, Xue C, Yan J, Li X, Zheng X, Dong R, Bai J, Su Y, Xie P. Analysis of antibiotic-induced drug resistance of *Salmonella* enteritidis and its biofilm formation mechanism. Bioengineered. 2021 Dec 20;12(2):10254-63.

D'Aoust JY. Psychrotrophy and foodborne *Salmonella* . International journal of food microbiology. 1991 Jul 1;13(3):207-15.

Darby J, Sheorey H. Searching for *Salmonella* . Australian Journal of General Practice. 2008 Oct 1;37(10):806.

Davies D. Understanding biofilm resistance to antibacterial agents. Nature reviews Drug discovery. 2003 Feb 1;2(2):114-22.

De Carvalho CC. Marine biofilms: a successful microbial strategy with economic implications. Frontiers in marine science. 2018 Apr 11;5:126.

de Jong A, Smet A, Ludwig C, Stephan B, De Graef E, Vanrobaeys M, Haesebrouck F. Antimicrobial susceptibility of *Salmonella* isolates from healthy pigs and chickens (2008–2011). Veterinary microbiology. 2014 Jul 16;171(3-4):298-306.

De Kraker ME, Davey PG, Grundmann H, BURDEN Study Group. Mortality and hospital stay associated with resistant Staphylococcus aureus and Escherichia coli bacteremia: estimating the burden of antibiotic resistance in Europe. PLoS medicine. 2011 Oct 11;8(10):e1001104.

Deblais L, Lorentz B, Scaria J, Nagaraja KV, Nisar M, Lauer D, Voss S, Rajashekara G. Comparative genomic studies of *Salmonella* Heidelberg isolated from chicken-and turkey-associated farm environmental samples. Frontiers in Microbiology. 2018 Aug 10;9:1841.

Dekker N, Tommassen J, Verheij HM. Bacteriocin release protein triggers dimerization of outer membrane phospholipase A in vivo. Journal of bacteriology. 1999 May 15;181(10):3281-3.

Delaloye J, Merlani G, Petignat C, Wenger A, Zaman K, Monnerat C, Matzinger O, Beck Popovic M, Vuichard P, Ketterer N, Tarr PE. Nosocomial nontyphoidal salmonellosis after antineoplastic chemotherapy: reactivation of asymptomatic colonization?. European Journal of Clinical Microbiology and Infectious Diseases. 2004 Oct;23:751-8.

Delgado-Suárez EJ, Palós-Guitérrez T, Ruíz-López FA, Hernández Pérez CF, Ballesteros-Nova NE, Soberanis-Ramos O, Méndez-Medina RD, Allard MW, Rubio-Lozano MS. Genomic surveillance of antimicrobial resistance shows cattle and poultry are a moderate source of multi-drug resistant non-typhoidal *Salmonella* in Mexico. PLoS One. 2021 May 5;16(5):e0243681.

Delgado-Suárez EJ, Selem-Mojica N, Ortiz-López R, Gebreyes WA, Allard MW, Barona-Gómez F, Rubio-Lozano MS. Whole genome sequencing reveals widespread distribution of typhoidal toxin genes and VirB/D4 plasmids in bovine-associated nontyphoidal *Salmonella* . Scientific Reports. 2018 Jun 29;8(1):9864. Li

Deng X, den Bakker HC, Hendriksen RS. Genomic epidemiology: whole-genome-sequencing–powered surveillance and outbreak investigation of foodborne bacterial pathogens. Annual review of food science and technology. 2016 Feb 28;7(1):353-74.

Devleesschauwer B, Haagsma JA, Mangen MJ, Lake RJ, Havelaar AH. The global burden of foodborne disease. Food Safety Economics: Incentives for a Safer Food Supply. 2018:107-22.

Diab MS, Thabet AS, Elsalam MA, Ewida RM, Sotohy SA. Detection of Virulence and β-lactamase resistance genes of non-typhoidal *Salmonella* isolates from human and animal origin in Egypt. Gut Pathogens. 2023 Dec;15(1):1-9.

Dieltjens L, Appermans K, Lissens M, Lories B, Kim W, Van der Eycken EV, Foster KR, Steenackers HP. Inhibiting bacterial cooperation is an evolutionarily robust anti-biofilm strategy. Nature Communications. 2020 Jan 9;11(1):107.

Diep B, Barretto C, Portmann AC, Fournier C, Karczmarek A, Voets G, Li S, Deng X, Klijn A. Salmonella serotyping; comparison of the traditional method to a microarray-based method and an in-silico platform using whole genome sequencing data. Frontiers in microbiology. 2019 Nov 11;10:2554.

DiMarzio M, Shariat N, Kariyawasam S, Barrangou R, Dudley EG. Antibiotic resistance in *Salmonella* enterica serovar Typhimurium associates with CRISPR sequence type. Antimicrobial agents and chemotherapy. 2013 Sep;57(9):4282-9.

Dione MM, Ikumapayi UN, Saha D, Mohammed NI, Geerts S, Ieven M, Adegbola RA, Antonio M. Clonal differences between non-typhoidal *Salmonella* (NTS) recovered from children and animals living in close contact in the Gambia. PLoS neglected tropical diseases. 2011 May 31;5(5):e1148.

Dolan SA, Everett ED, Harper MC. *Salmonella* vertebral osteomyelitis treated with cefotaxime. Archives of internal medicine. 1987 Sep 1;147(9):1667-8.

Dor Z, Shnaiderman-Torban A, Kondratyeva K, Davidovich-Cohen M, Rokney A, Steinman A, Navon-Venezia S. Emergence and spread of different ESBL-producing *Salmonella* enterica serovars in hospitalized horses sharing a highly transferable IncM2 CTX-M-3-encoding plasmid. Frontiers in Microbiology. 2020 Dec 17;11:616032.

dos Santos Bersot L, Carbonera NR, Valcanaia CD, Viana C, Nero LA. Multidrug-Resistant and Extended-Spectrum β-Lactamase–Producing *Salmonella* enterica Serotype Heidelberg Is Widespread in a Poultry Processing Facility in Southern Brazil. Journal of Food Protection. 2021 Dec 1;84(12):2053-8.

Dudhane RA, Bankar NJ, Shelke YP, Badge AK, Badge A. The Rise of Non-typhoidal *Salmonella* Infections in India: Causes, Symptoms, and Prevention. Cureus. 2023 Oct 8;15(10).

Dufresne K, Daigle F. *Salmonella* fimbriae: What is the clue to their hairdo. Current Topics In *Salmonella* and Salmonellosis. 2017 Apr 5:59-79.

Dufresne K, Saulnier-Bellemare J, Daigle F. Functional analysis of the chaperone-usher fimbrial gene clusters of *Salmonella* enterica serovar Typhi. Frontiers in Cellular and Infection Microbiology. 2018 Feb 8;8:26.

Duguid JP, Anderson ES, Alfredsson GA, Barker R, Old DC. A new biotyping scheme for *Salmonella* Typhimurium and its phylogenetic significance. Journal of Medical Microbiology. 1975 Feb;8(1):149-66.

Duguid JP, Anderson ES, Campbell I. Fimbriae and adhesive properties in *Salmonellae*. The Journal of pathology and bacteriology. 1966 Jul;92(1):107-37.

Duguid JP, Gillies RR. Fimbriae and haemagglutinating activity in *Salmonella* , Klebsiella, Proteus and Chromobacterium. In: Journal of Pathology and Bacteriology 1958 Jan 1 Vol. 75, No. 2, pp. 519-520.

Duguid JP, Old DC. Adhesive properties of Enterobacteriaceae. In Bacterial adherence 1980 Jun 30 (pp. 185-217). Dordrecht: Springer Netherlands.

Ebina Y, Nakazawa A. Cyclic AMP-dependent initiation and rho-dependent termination of colicin E1 gene transcription. Journal of Biological Chemistry. 1983 Jun 10;258(11):7072-8.

ECDC (European Centre for Disease Prevention and Control): Antimicrobial resistance surveillance in Europe 2012. In *Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net).* Stockholm: 2013.

Edel W, Kampelmacher EH. Comparative studies on *Salmonella* -isolation in eight European laboratories. Bulletin of the World Health Organization. 1968;39(3):487.

Edwards PR, Ewing WH. Edwards and Ewing's Identification of Enterobacteriaceae, 1986, 4th ed. Elseviers Science Publishing Co., Inc., New York, U.S.A.

Edwards RA, Puente JL. Fimbrial expression in enteric bacteria: a critical step in intestinal pathogenesis. Trends in microbiology. 1998 Jul 1;6(7):282-7.

Edwards RA, Schifferli DM, Maloy SR. A role for *Salmonella* fimbriae in intraperitoneal infections. Proceedings of the National Academy of Sciences. 2000 Feb 1;97(3):1258-62.

EFSA Panel on Biological Hazards (BIOHAZ). Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals. EFSA Journal. 2011 Aug;9(8):2322.

Eguale T, Birungi J, Asrat D, Njahira MN, Njuguna J, Gebreyes WA, Gunn JS, Djikeng A, Engidawork E. Genetic markers associated with resistance to beta-lactam and quinolone antimicrobials in non-typhoidal *Salmonella* isolates from humans and animals in central Ethiopia. Antimicrobial Resistance & Infection Control. 2017 Dec;6:1-0.

Ehuwa O, Jaiswal AK, Jaiswal S. *Salmonella* , food safety and food handling practices. Foods. 2021 Apr 21;10(5):907.

Ekinci Bİ, Coban AY, Birinci A, Durupinar B, Erturk M. In vitro effects of cefotaxime and ceftriaxone on *Salmonella* typhi within human monocyte-derived macrophages. Clinical microbiology and infection. 2002 Dec 1;8(12):810-3.

Eller C, Simon S, Miller T, Frick JS, Prager R, Rabsch W, Guerra B, Werner G, Pfeifer Y. Presence of β-lactamases in extended-spectrum-cephalosporin-resistant *Salmonella* enterica of 30 different serovars in Germany 2005–11. Journal of Antimicrobial Chemotherapy. 2013 Sep 1;68(9):1978-81.

Eltahawy AT, Khalaf RM. Urinary tract infection at a University Hospital in Saudi Arabia: incidence, microbiology, and antimicrobial susceptibility. Annals of Saudi Medicine. 1988 Jul;8(4):261-6.

Eng SK, Pusparajah P, Ab Mutalib NS, Ser HL, Chan KG, Lee LH. *Salmonella* : a review on pathogenesis, epidemiology and antibiotic resistance. Frontiers in Life Science. 2015 Jul 3;8(3):284-93.

Espeland EM, Wetzel RG. Complexation, stabilization, and UV photolysis of extracellular and surface-bound glucosidase and alkaline phosphatase: implications for biofilm microbiota. Microbial ecology. 2001 Dec;42:572-85.

Euzéby JP. Revised *Salmonella* nomenclature: designation of *Salmonella* enterica (ex Kauffmann and Edwards 1952) Le Minor and Popoff 1987 sp. nov. nom. rev. as the neotype species of the genus *Salmonella* Lignieres 1900 (Approv Lists 1980), rejection of the name *Salmonella* choleraesuis (Smith 1894) Weldin 1927 (Approved Lists 1980), and conservation of the name *Salmonella* typhi (Schroeter 1886) Warren and Scott 1930 (Approved Lists 1980). Request for an

Opinion. International Journal of Systematic and Evolutionary Microbiology. 1999 Apr;49(2):927-30.

Fàbrega A, Madurga S, Giralt E, Vila J. Mechanism of action of and resistance to quinolones. Microbial biotechnology. 2009 Jan;2(1):40-61.

Fagbamila IO, Ramon E, Lettini AA, Muhammad M, Longo A, Antonello K, Aworh MK, Kwaga JK, Abdu PA, Umoh JU, Kabir JA. Assessing the mechanisms of multi-drug resistant non-typhoidal *Salmonella* (NTS) serovars isolated from layer chicken farms in Nigeria. Plos one. 2023 Sep 7;18(9):e0290754.

Farmer J. J. III. Enterobacteriaceae: introduction and identification. In: Manual of clinical microbiology 6th ed. Murray P. R., Baron E. J., Pfaller M. A., Tenover F. C., and Yolken R. H. 1995 438 -449 ASM Press Washington, D.C

Fashae K, Ogunsola F, Aarestrup FM, Hendriksen RS. Antimicrobial Susceptibility and Serovars of *Salmonella* from Chickens and Humans in Ibadan, Nigeria. Journal of Infectious Disease in Developing Countries, 2010, 4, 484-494. https://doi.org/10.3855/jidc.909

Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon MA. Invasive non-typhoidal *Salmonella* disease: an emerging and neglected tropical disease in Africa. The Lancet. 2012 Jun 30;379(9835):2489-99.

Feasey NA, Masesa C, Jassi C, Faragher EB, Mallewa J, Mallewa M, MacLennan CA, Msefula C, Heyderman RS, Gordon MA. Three epidemics of invasive multidrug-resistant *Salmonella* bloodstream infection in Blantyre, Malawi, 1998–2014. Clinical Infectious Diseases. 2015 Nov 1;61(suppl_4):S363-71.

Fernandes SA, Ghilardi ÂC, Tavechio AT, Machado AM, Pignatari AC. Phenotypic and molecular characterization of *Salmonella* Enteritidis strains isolated in São Paulo, Brazil. Revista do Instituto de Medicina Tropical de São Paulo. 2003;45:59-63.

Fernández J, Guerra B, Rodicio MR. Resistance to carbapenems in non-typhoidal *S. enterica* serovars from humans, animals and food. Veterinary sciences. 2018 Apr 8;5(2):40.

Ferrari RG, Rosario DK, Cunha-Neto A, Mano SB, Figueiredo EE, Conte-Junior CA. Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a meta-analysis. Applied and environmental microbiology. 2019 Jul 15;85(14):e00591-19. Soliani

Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology. 2016 Sep;14(9):563-75.

Fookes M, Schroeder GN, Langridge GC, Blondel CJ, Mammina C, Connor TR, Seth-Smith H, Vernikos GS, Robinson KS, Sanders M, Petty NK. *Salmonella* bongori provides insights into the evolution of the *Salmonellae*. PLoS pathogens. 2011 Aug 18;7(8):e1002191.

Foster JW. Beyond pH homeostasis: The acid tolerance response of *Salmonellae*. Pummelled with protons, the bacterium launches a strategic acid defense initiative. ASM American Society for Microbiology News. 1992;58(5):266-70.

Fourat E, Kapadia S, Shah U, Zararia V, Bricas N. Understanding transition in animal based food consumption: a case study in the city of Vadodara in Gujarat (India). Review of Agricultural, Food and Environmental Studies. 2018 Sep;99:189-205.

Franco Melendez K, Crenshaw K, Barrila J, Yang J, Gangaraju S, Davis RR, Forsyth RJ, Ott CM, Kader R, Curtiss III R, Roland K. Role of RpoS in Regulating Stationary Phase *Salmonella* Typhimurium Pathogenesis-Related Stress Responses under Physiological Low Fluid Shear Force Conditions. Msphere. 2022 Aug 31;7(4):e00210-22.

Fronzes R, Remaut H, Waksman G. Architectures and biogenesis of non-flagellar protein appendages in Gram-negative bacteria. The EMBO journal. 2008 Sep 3;27(17):2271-80.

Gajamer VR, Bhattacharjee A, Paul D, Ingti B, Sarkar A, Kapil J, Singh AK, Pradhan N, Tiwari HK. High prevalence of carbapenemase, AmpC β-lactamase and aminoglycoside resistance genes in extended-spectrum β-lactamase-positive uropathogens from Northern India. Journal of global antimicrobial resistance. 2020 Mar 1;20:197-203.

Galanakis E, Bitsori M, Maraki S, Giannakopoulou C, Samonis G, Tselentis Y. Invasive non-typhoidal salmonellosis in immunocompetent infants and children. International journal of infectious diseases. 2007 Jan 1;11(1):36-9.

Galanis E, Wong DM, Patrick ME, Binsztein N, Cieslik A, Chalermchaikit T, Aidara-Kane A, Ellis A, Angulo FJ, Wegener HC. Web-based surveillance and global *Salmonella* distribution, 2000–2002. Emerging infectious diseases. 2006 Mar;12(3):381.

Galie S, García-Gutiérrez C, Miguélez EM, Villar CJ, Lombó F. Biofilms in the food industry: health aspects and control methods. Frontiers in microbiology. 2018 May 7;9:898.

Garcia C, Hinostroza N, Astocondor L, Ochoa T, Jacobs J. Characterization of ESBL-producing *Salmonella* enterica serovar infantis infection in humans, Lima, Peru. The American Journal of Tropical Medicine and Hygiene. 2019 Oct;101(4):746.

Garrec H, Drieux-Rouzet L, Golmard JL, Jarlier V, Robert J. Comparison of nine phenotypic methods for detection of extended-spectrum β-lactamase production by Enterobacteriaceae. Journal of clinical microbiology. 2011 Mar;49(3):1048-57.

Gast RK (2003) Paratyphoid infections. In: Saif M, Barnes HJ, Fadly AM, Glisson JR, McDougald LR, Swayne DE (eds) Poult, 11th edn. Iowa State University Press, Ames, pp 583–613

Gast RK, Guraya R, Guard J. *Salmonella* Enteritidis deposition in eggs after experimental infection of laying hens with different oral doses. Journal of food protection. 2013 Jan 1;76(1):108-13.

Gast RK, Guraya R, Jones DR, Guard J, Anderson KE, Karcher DM. Colonization of internal organs by *Salmonella* serovars Heidelberg and Typhimurium in experimentally infected laying hens housed in enriched colony cages at different stocking densities. Poultry Science. 2017 May 1;96(5):1402-9.

Gautam V, Thakur A, Sharma M, Singh A, Bansal S, Sharma A, Kapil A, Das BK, Sistla S, Parija SC, Veeraraghavan B. Molecular characterization of extended-spectrum β-lactamases among clinical isolates of Escherichia coli & Klebsiella pneumoniae: a multi-centric study from tertiary care hospitals in India. The Indian journal of medical research. 2019 Feb;149(2):208.

Gebeyehu A, Taye M, Abebe R. Isolation, molecular detection and antimicrobial susceptibility profile of *Salmonella* from raw cow milk collected from dairy farms and households in southern Ethiopia. BMC Microbiology. 2022 Mar 31;22(1). Available from: http://dx.doi.org/10.1186/s12866-022-02504-2

Giaouris ED, Nychas GJ. The adherence of *Salmonella* Enteritidis PT4 to stainless steel: The importance of the air–liquid interface and nutrient availability. Food microbiology. 2006 Dec 1;23(8):747-52.

Girlich D, Poirel L, Nordmann P. Value of the modified Hodge test for detection of emerging carbapenemases in Enterobacteriaceae. Journal of clinical microbiology. 2012 Feb;50(2):477-9.

Gollan BC, Luo L, Li Y, Clark-Corrigall JL, Qadri BM, Alshuwaier AA, Hinton JC, Khan CA. Colicin Receptor CirA Enhances Salmonella Typhimuriums Resistance to Colicin Ib in the Absence of the Cognate Immunity Protein. bioRxiv. 2024:2024-06.

Gong B, Li H, Feng Y, Zeng S, Zhuo Z, Luo J, Chen X, Li X. Prevalence, serotype distribution and antimicrobial resistance of non-typhoidal *Salmonella* in hospitalized patients in Conghua District of Guangzhou, China. Frontiers in Cellular and Infection Microbiology. 2022 Feb 2;12:54.

González-López JJ, Piedra-Carrasco N, Salvador F, Rodriguez V, Sanchez-Montalva A, Planes AM, Molina I, Larrosa MN. ESBL-producing *Salmonella* enterica serovar Typhi in traveler returning from Guatemala to Spain. Emerging infectious diseases. 2014 Nov;20(11):1918.

Gordon DM, O'Brien CL. Bacteriocin diversity and the frequency of multiple bacteriocin production in Escherichia coli. Microbiology. 2006 Nov;152(11):3239-44.

Gordon MA, Graham SM, Walsh AL, Wilson L, Phiri A, Molyneux E, Zijlstra EE, Heyderman RS, Hart CA, Molyneux ME. Epidemics of invasive *Salmonella* enterica serovar enteritidis and S. enterica Serovar typhimurium infection

associated with multidrug resistance among adults and children in Malawi. Clinical Infectious Diseases. 2008 Apr 1;46(7):963-9.

Gordon MA. Invasive non-typhoidal *Salmonella* disease–epidemiology, pathogenesis and diagnosis. Current opinion in infectious diseases. 2011 Oct;24(5):484. https://doi.org/10.1097/QCO.0b013e32834a9980

Grassl GA, Finlay BB. Pathogenesis of enteric *Salmonella* infections. Current opinion in gastroenterology. 2008 Jan 1;24(1):22-6.

Green ER, Mecsas J. Bacterial secretion systems: an overview. Virulence mechanisms of bacterial pathogens. 2016 Jun 22:213-39.

Greene SK, Stuart AM, Medalla FM, Whichard JM, Hoekstra RM, Chiller TM. Distribution of multidrug-resistant human isolates of MDR-ACSSuT *Salmonella* Typhimurium and MDR-AmpC *Salmonella* Newport in the United States, 2003–2005. Foodborne pathogens and disease. 2008 Oct 1;5(5):669-80.

Grimont PA, Weill FX. Antigenic formulae of the *Salmonella* serovars. WHO collaborating centre for reference and research on *Salmonella* . 2007 2;9:1-166.

Guerra B, Soto S, Helmuth R, Mendoza MC. Characterization of a self-transferable plasmid from *Salmonella* enterica serotype Typhimurium clinical isolates carrying two integron-borne gene cassettes together with virulence and drug resistance genes, Antimicrob Agents Chemother, 2002, vol. 46 (pg. 2977-81)

Guerra B, Soto SM, Argüelles JM, Mendoza MC. Multidrug resistance is mediated by large plasmids carrying a class 1 integron in the emergent *Salmonella* enterica serotype [4,5,12:i:-], Antimicrob Agents Chemother, 2001, vol. 45 (pg. 1305-8).

Guibourdenche M, Roggentin P, Mikoleit M, Fields PI, Bockemühl J, Grimont PA, Weill FX. Supplement 2003–2007 (No. 47) to the white-Kauffmann-Le minor scheme. Research in microbiology. 2010 Jan 1;161(1):26-9.

Guo X, Chen J, Brackett RE, Beuchat LR. Survival of *Salmonellae* on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. Applied and environmental microbiology. 2001 Oct 1;67(10):4760-4.

Hagel S, Epple HJ, Feurle GE, Kern WV, Jansen PL, Malfertheiner P, Marth T, Meyer E, Mielke M, Moos V, von Müller L. S2k-guideline gastrointestinal infectious diseases and Whipple's disease. Zeitschrift fur Gastroenterologie. 2015 May;53(5):418-59.

Halim RM, Kassem NN, Mahmoud BS. Detection of biofilm producing staphylococci among different clinical isolates and its relation to methicillin susceptibility. Open access Macedonian journal of medical sciences. 2018 Aug 8;6(8):1335.

Hall-Stoodley L, Stoodley P. Evolving concepts in biofilm infections. Cellular microbiology. 2009 Jul;11(7):1034-43.

Haneda T, Ishii Y, Danbara H, Okada N. Genome-wide identification of novel genomic islands that contribute to *Salmonella* virulence in mouse systemic infection. FEMS microbiology letters. 2009 Aug 1;297(2):241-9.

Hansen-Wester I, Stecher B, Hensel M. Type III secretion of *Salmonella* enterica serovar Typhimurium translocated effectors and SseFG. Infection and immunity. 2002 Mar;70(3):1403-9.

Harb A, O'dea M, Hanan ZK, Abraham S, Habib I. Prevalence, risk factors and antimicrobial resistance of *Salmonella* diarrhoeal infection among children in Thi-Qar Governorate, Iraq. Epidemiology & Infection. 2017 Dec;145(16):3486-96.

Harding-Crooks R, Smith D, Fanning S, Fox EM. Dissemination of carbapenemase-producing Enterobacteriaceae and associated resistance determinants through global food systems. Comprehensive Reviews in Food Science and Food Safety. 2023 Apr 21.

Harika K, Shenoy VP, Narasimhaswamy N, Chawla K. Detection of biofilm production and its impact on antibiotic resistance profile of bacterial isolates from chronic wound infections. Journal of global infectious diseases. 2020 Jul;12(3):129.

Harrell JE, Hahn MM, D'Souza SJ, Vasicek EM, Sandala JL, Gunn JS, McLachlan JB. *Salmonella* biofilm formation, chronic infection, and immunity within the intestine and hepatobiliary tract. Frontiers in cellular and infection microbiology. 2021 Feb 2;10:624622.

Harris JB, Baresch-Bernal A, Rollins SM, Alam A, LaRocque RC, Bikowski M, Peppercorn AF, Handfield M, Hillman JD, Qadri F, Calderwood SB. Identification of in vivo-induced bacterial protein antigens during human infection with *Salmonella* enterica serovar Typhi. Infection and immunity. 2006 Sep;74(9):5161-8.

Hasan R, Zafar A, Abbas Z, Mahraj V, Malik F, Zaidi A. Antibiotic resistance among *Salmonella* enterica serovars Typhi and Paratyphi A in Pakistan (2001-2006). The Jouochirnal of Infection in Developing Countries. 2008 Aug 1;2(04):289-94.

Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM. β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. Journal of Antimicrobial Chemotherapy. 2005 Jul 1;56(1):115-21.

Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, Lake RJ, Praet N, Bellinger DC, De Silva NR, Gargouri N, Speybroeck N. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. PLoS medicine. 2015 Dec 3;12(12):e1001923.

Helms M, Ethelberg S, Mølbak K, DT104 Study Group. International *Salmonella* typhimurium DT104 infections, 1992–2001. Emerging infectious diseases. 2005 Jun;11(6):859.

Hendriksen RS, Bangtrakulnonth A, Pulsrikarn C, Pornreongwong S, Hasman H, Song SW, Aarestrup FM. Antimicrobial resistance and molecular epidemiology of *Salmonella* Rissen from animals, food products, and patients in Thailand and Denmark. Foodborne Pathogens and Disease. 2008 Oct 1;5(5):605-19.

Hendriksen RS, Hyytia-Trees E, Pulsrikarn C, Pornruangwong S, Chaichana P, Svendsen CA, Ahmed R, Mikoleit M. Characterization of *Salmonella* enterica serovar Enteritidis isolates recovered from blood and stool specimens in Thailand. BMC microbiology. 2012 Dec;12:1-8.

Hendriksen RS, Larsen JN (2004). Laboratory protocols: Serotyping of *Salmonella* enterica O and H antigens. 6th ed. Global Salm-Surv: WHO. www.antimicrobialresistance.dk/data/images/salmonella4_pdf.pdf

Hendriksen RS, Vieira AR, Karlsmose S, Lo Fo Wong DM, Jensen AB, Wegener HC, Aarestrup FM. Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. Foodborne pathogens and disease. 2011 Aug 1;8(8):887-900.

Hengkrawit K, Tangjade C. Prevalence and trends in antimicrobial susceptibility patterns of multi-drug-resistance non-typhoidal *Salmonella* in Central Thailand, 2012–2019. Infection and Drug Resistance. 2022 Jan 1:1305-15.

Hensel M, Shea JE, Waterman SR, Mundy R, Nikolaus T, Banks G, Vazquez-Torres A, Gleeson C, Fang FC, Holden DW. Genes encoding putative effector proteins of the type III secretion system of *Salmonella* pathogenicity island 2 are required for bacterial virulence and proliferation in macrophages. Molecular microbiology. 1998 Oct;30(1):163-74.

Herschman HR, Helinski DR. Comparative study of the events associated with colicin induction. Journal of bacteriology. 1967 Sep;94(3):691-9.

Ho BT, Dong TG, Mekalanos JJ. A view to a kill: the bacterial type VI secretion system. Cell host & microbe. 2014 Jan 15;15(1):9-21.

Holmes (1998). The Enteriobactericeae: General characters. In: Topley and Wilson's Microbiology and Microbial Infections (pp 919-934.), Vol.2, 9th Edition, Arnold press.

Hooper DC, Jacoby GA. Topoisomerase inhibitors: fluoroquinolone mechanisms of action and resistance. Cold Spring Harbor perspectives in medicine. 2016 Sep;6(9).

Hopkins KL, Davies RH, Threlfall EJ. Mechanisms of quinolone resistance in Escherichia coli and *Salmonella* : recent developments. International journal of antimicrobial agents. 2005 May 1;25(5):358-73.

Hsu PC, Wang YW, Chen BH, Hong YP, Teng RH, Liu PY, Chiou CS. Carbapenem resistance in extensively drug-resistant *Salmonella* enterica serovar Agona and AmpC β-lactamase-producing S. Infantis. Microbiology Spectrum. 2023 Dec 12;11(6):e02922-23.

Hummers-Pradier E, Ohse AM, Koch M, Heizmann WR, Kochen MM. Management of urinary tract infections in female general practice patients. Family practice. 2005 Feb 1;22(1):71-7.

Humphries AD, Raffatellu M, Winter S, Weening EH, Kingsley RA, Droleskey R, Zhang S, Figueiredo J, Khare S, Nunes J, Adams LG. The use of flow cytometry to detect expression of subunits encoded by 11 *Salmonella* enterica serotype Typhimurium fimbrial operons. Molecular microbiology. 2003 Jun;48(5):1357-76.

Huong LQ, Reinhard F, Padungtod P, Hanh TT, Kyule MN, Baumann MP, Zessin KH. Prevalence of *Salmonella* in retail chicken meat in Hanoi, Vietnam. Annals of the New York Academy of Sciences. 2006 Oct;1081(1):257-61.

Hurley D, McCusker MP, Fanning S, Martins M. *Salmonella* –host interactions–modulation of the host innate immune system. Frontiers in immunology. 2014 Oct 7;5:481.

Hutson, Anne (2008). "Oxidative-Fermentative Test Protocol". American Society for Microbiology. Cited from website: https://asm.org/ASM/media/Protocol-Images/Oxidative-Fermentative-Test-Protocol.pdf?ext=.pdf

Ibrahim GM, Morin PM. Salmonella serotyping using whole genome sequencing. Frontiers in microbiology. 2018 Dec 13;9:2993.

Inthavong P, Srikitjakarn L, Kyule M, Zessin K, Baumann M, Douangngeun B, Fries R. Microbial contamination of pig carcasses at a slaughterhouse in Vientiane capital, Lao PDR. Southeast Asian journal of tropical medicine and public health. 2006 Nov 1;37(6):1237-41.

Issenhuth-Jeanjean S, Roggentin P, Mikoleit M, Guibourdenche M, De Pinna E, Nair S, Fields PI, Weill FX. Supplement 2008–2010 (no. 48) to the white–Kauffmann–Le minor scheme. Research in microbiology. 2014 Sep 1;165(7):526-30.

Iyer P, Barreto SG, Sahoo B, Chandrani P, Ramadwar MR, Shrikhande SV, Dutt A. Non-typhoidal *Salmonella* DNA traces in gallbladder cancer. Infectious agents and cancer. 2016 Dec;11:1-4.

Jack RW, Tagg JR, Ray B. Bacteriocins of gram-positive bacteria. Microbiological reviews. 1995 Jun;59(2):171-200.

Jacob JJ, Solaimalai D, Muthuirulandi Sethuvel DP, Rachel T, Jeslin P, Anandan S, Veeraraghavan B. A nineteen-year report of serotype and antimicrobial susceptibility of enteric non-typhoidal *Salmonella* from humans in Southern India: changing facades of taxonomy and resistance trend. Gut Pathogens. 2020 Dec;12(1):1-9.

Jacques M. Role of lipo-oligosaccharides and lipopolysaccharides in bacterial adherence. Trends in microbiology. 1996 Oct 1;4(10):408-10.

Jahan F, Chinni SV, Samuggam S, Reddy LV, Solayappan M, Su Yin L. The complex mechanism of the *Salmonella* typhi biofilm formation that facilitates

pathogenicity: a review. International Journal of Molecular Sciences. 2022 Jun 9;23(12):6462.

Jain P, Chowdhury G, Samajpati S, Basak S, Ganai A, Samanta S, Okamoto K, Mukhopadhyay AK, Dutta S. Characterization of non-typhoidal *Salmonella* isolates from children with acute gastroenteritis, Kolkata, India, during 2000–2016. Brazilian Journal of Microbiology. 2020 Jun;51:613-27.

James R, Penfold CN, Moore GR, Kleanthous C. Killing of E. coli cells by E group nuclease colicins. Biochimie. 2002 May 1;84(5-6):381-9.

Jeamsripong S, Kuldee M, Thaotumpitak V, Chuanchuen R. Antimicrobial resistance, Extended-Spectrum β-Lactamase production and virulence genes in *Salmonella* enterica and Escherichia coli isolates from estuarine environment. Plos one. 2023 Apr 28;18(4):e0283359.

Jensen PØ, Givskov M, Bjarnsholt T, Moser C. The immune system vs. Pseudomonas aeruginosa biofilms. FEMS Immunology & Medical Microbiology. 2010 Aug 1;59(3):292-305.

Jiang B, Xu H, Zhou Z. Acute multifocal osteomyelitis with septic arthritis caused by nontyphoidal *Salmonella* in an immunocompetent young adult: a case report. Journal of International Medical Research. 2023 Sep;51(9):03000605231198382.

Jones BD, Ghori N, Falkow S. *Salmonella* typhimurium initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. The Journal of experimental medicine. 1994 Jul 1;180(1):15-23.

Jones SL, Nguyen VK, Nguyen TM, Athan E. Prevalence of multiresistant Gram-negative organisms in a surgical hospital in Ho Chi Minh Cit, Vietnam. Tropical Medicine & International Health. 2006 Nov;11(11):1725-30.

Jones TF, Ingram LA, Cieslak PR, Vugia DJ, Tobin-D'Angelo M, Hurd S, Medus C, Cronquist A, Angulo FJ. Salmonellosis outcomes differ substantially by serotype. The Journal of infectious diseases. 2008 Jul 1;198(1):109-14.

Kaldhone PR, Carlton A, Aljahdali N, Khajanchi BK, Sanad YM, Han J, Deck J, Ricke SC, Foley SL. Evaluation of incompatibility group I1 (IncI1) plasmid-containing *Salmonella* enterica and assessment of the plasmids in bacteriocin production and biofilm development. Frontiers in veterinary science. 2019 Sep 6;6:298.

Kang HP, Dunne WM. Stability of repetitive-sequence PCR patterns with respect to culture age and subculture frequency. Journal of clinical microbiology. 2003 Jun;41(6):2694-6.

Kariuki S, Onsare RS. Epidemiology and genomics of invasive nontyphoidal *Salmonella* infections in Kenya. Clinical Infectious Diseases. 2015 Nov 1;61(suppl_4):S317-24.

Karthikeyan K, Thirunarayan M, Krishnan P. CTX-M15 type ESBL producing Salmonella from a paediatric patient in Chennai, India. Indian Journal of Medical Research. 2011 Oct 1;134(4):487-9.

Kasturi KN, Drgon T. Real-time PCR method for detection of Salmonella spp. in environmental samples. Applied and environmental microbiology. 2017 Jul 15;83(14):e00644-17.

Kaur A, Kapil A, Elangovan R, Jha S, Kalyanasundaram D. Highly-sensitive detection of Salmonella typhi in clinical blood samples by magnetic nanoparticle-based enrichment and in-situ measurement of isothermal amplification of nucleic acids. PLoS One. 2018 Mar 28;13(3):e0194817.

Keintz M, Marcelin J. Invasive Nontyphoidal Salmonellosis: An Antimicrobial-Resistant Foe. 2022 Retrieved January 12, 2024, from https://www.contagionlive.com/view/invasive-nontyphoidal-salmonellosis-an-antimicrobial-resistant-foe

Khan MI, Ochiai RL, Von Seidlein L, Dong B, Bhattacharya SK, Agtini MD, Bhutta ZA, Do GC, Ali M, Kim DR, Favorov M. Non-typhoidal *Salmonella* rates in febrile children at sites in five Asian countries. Tropical Medicine & International Health. 2010 Aug;15(8):960-3.

Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon. 2018 Dec 1;4(12).

Khuri-Bulos NA, Khalaf MA, Shehabi A, Shami K. Foodhandler-associated *Salmonella* outbreak in a university hospital despite routine surveillance cultures of kitchen employees. Infection control & hospital epidemiology. 1994 May;15(5):311-4.

Kim MS, Lim TH, Jang JH, et al. Prevalence and antimicrobial resistance of *Salmonella* species isolated from chicken meats produced by different integrated broiler operations in Korea. Poult Sci J. 2012;91:2370–5.

Kim SH, Wei CI. Biofilm formation by multidrug-resistant *Salmonella* enterica serotype Typhimurium phage type DT104 and other pathogens. Journal of food protection. 2007 Jan 1;70(1):22-9.

Kim SH, Wei CI. Molecular characterization of biofilm formation and attachment of *Salmonella* enterica serovar Typhimurium DT104 on food contact surfaces. Journal of food protection. 2009 Sep 1;72(9):1841-7.

King S, Metzger WI. A new plating medium for the isolation of enteric pathogens: II. Comparison of Hektoen Enteric Agar with SS and EMB Agar. Applied Microbiology. 1968 Apr;16(4):579-81.

Kiratisin P. Bacteraemia due to non-typhoidal *Salmonella* in Thailand: clinical and microbiological analysis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008 Apr 1;102(4):384-8.

Kirchweger P, Weiler S, Egerer-Sieber C, Blasl AT, Hoffmann S, Schmidt C, Sander N, Merker D, Gerlach RG, Hensel M, Muller YA. Structural and functional characterization of SiiA, an auxiliary protein from the SPI4-encoded type 1 secretion system from *Salmonella* enterica. Molecular Microbiology. 2019 Nov;112(5):1403-22.

Klerks MM, Franz E, van Gent-Pelzer M, Zijlstra C, Van Bruggen AH. Differential interaction of *Salmonella* enterica serovars with lettuce cultivars and plant-microbe factors influencing the colonization efficiency. The ISME journal. 2007 Nov;1(7):620-31.

Kluytmans JA, Overdevest IT, Willemsen I, Kluytmans-Van Den Bergh MF, Van Der Zwaluw K, Heck M, Rijnsburger M, Vandenbroucke-Grauls CM, Savelkoul PH, Johnston BD, Gordon D. Extended-spectrum β-lactamase–producing Escherichia coli from retail chicken meat and humans: comparison of strains, plasmids, resistance genes, and virulence factors. Clinical infectious diseases. 2013 Feb 15;56(4):478-87.

Kong-Ngoen T, Santajit S, Tunyong W, Pumirat P, Sookrung N, Chaicumpa W, Indrawattana N. Antimicrobial resistance and virulence of non-typhoidal *Salmonella* from retail foods marketed in Bangkok, Thailand. Foods. 2022 Feb 24;11(5):661.

Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, De Cesare A, Herman L, Hilbert F, Lindqvist R. Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Dewulf J, Hald T, Michel V, Niskanen T, Ricci A, Snary E, Boelaert F, Messens W and Davies R. *Salmonella* control in poultry flocks and its public health impact. EFSA Journal. 2019 Feb;17(2):5596-5751.

Krone WJ, De Vries P, Koningstein G, De Jonge AJ, De Graaf FK, Oudega B. Uptake of cloacin DF13 by susceptible cells: removal of immunity protein and fragmentation of cloacin molecules. Journal of bacteriology. 1986 Apr;166(1):260-8.

Krzyżewska-Dudek E, Kotimaa J, Kapczyńska K, Rybka J, Meri S. Lipopolysaccharides and outer membrane proteins as main structures involved in complement evasion strategies of non-typhoidal *Salmonella* strains. Molecular Immunology. 2022 Oct 1;150:67-77.

Ku WY, Liu YW, Hsu YC, Liao CC, Liang PH, Yuan HS, Chak KF. The zinc ion in the HNH motif of the endonuclease domain of colicin E7 is not required for DNA binding but is essential for DNA hydrolysis. Nucleic acids research. 2002 Apr 1;30(7):1670-8.

Kulwichit W, Chatsuwan T, Unhasuta C, Pulsrikarn C, Bangtrakulnonth A, Chongthaleong A. Drug-resistant nontyphoidal *Salmonella* bacteremia, Thailand. Emerging infectious diseases. 2007 Mar;13(3):501-502.

Kumar S, Kumar Y, Kumar G, Kumar G, Tahlan AK. Non-typhoidal *Salmonella* infections across India: emergence of a neglected group of enteric pathogens. Journal of Taibah University Medical Sciences. 2022 Oct 1;17(5):747-54.

Kuo HC, Lauderdale TL, Lo DY, Chen CL, Chen PC, Liang SY, Kuo JC, Liao YS, Liao CH, Tsao CS, Chiou CS. An association of genotypes and antimicrobial resistance patterns among *Salmonella* isolates from pigs and humans in Taiwan. PloS one. 2014 Apr 23;9(4):e95772.

Kuralayanapalya SP, Patil SS, Hamsapriya S, Shinduja R, Roy P, Amachawadi RG. Prevalence of extended-spectrum beta-lactamase producing bacteria from animal origin: A systematic review and meta-analysis report from India. PLoS One. 2019 Sep 4;14(9):e0221771.

Kurtz JR, Goggins JA, McLachlan JB. *Salmonella* infection: Interplay between the bacteria and host immune system. Immunology letters. 2017 Oct 1;190:42-50.

Kuvandik C, Karaoglan I, Namiduru M, Baydar I. Predictive value of clinical and laboratory findings in the diagnosis of enteric fever. The New Microbiologica. 2009 Jan 1;32(1):25-30.

Lai YH, Chung YA, Wu YC, Fang CT, Chen PJ. Disease burden from foodborne illnesses in Taiwan, 2012–2015. Journal of the Formosan Medical Association. 2020 Sep 1;119(9):1372-81.

Lakshminarayanan S, Jayalakshmy R. Diarrheal diseases among children in India: Current scenario and future perspectives. Journal of natural science, biology, and medicine. 2015 Jan;6(1):24-28.

Lal A, Cheeptham N. Decarboxylase Broth Protocol. American Society for Microbiology. 2015 Sep 2:10pp. https://asm.org/ASM/media/Protocol-Images/Decarboxylase-Broth-Protocol.pdf

Landini P, Zehnder AJ. The global regulatory hns gene negatively affects adhesion to solid surfaces by anaerobically grown Escherichia coli by modulating expression of flagellar genes and lipopolysaccharide production. Journal of bacteriology. 2002 Mar 15;184(6):1522-9.

Lapidot A, Romling U, Yaron S. Biofilm formation and the survival of *Salmonella* Typhimurium on parsley. International journal of food microbiology. 2006 Jun 15;109(3):229-33.

Latasa C, Roux A, Toledo-Arana A, Ghigo JM, Gamazo C, Penadés JR, Lasa I. BapA, a large secreted protein required for biofilm formation and host colonization of *Salmonella* enterica serovar Enteritidis. Molecular microbiology. 2005 Dec;58(5):1322-39.

Lauderdale TL, Aarestrup FM, Chen PC, Lai JF, Wang HY, Shiau YR, Huang IW, Hung CL. Multidrug resistance among different serotypes of clinical *Salmonella* isolates in Taiwan. Diagnostic microbiology and infectious disease. 2006 Jun 1;55(2):149-55.

Lay KS, Vuthy Y, Song P, Phol K, Sarthou JL. Prevalence, numbers and antimicrobial susceptibilities of *Salmonella* serovars and Campylobacter spp. in retail poultry in Phnom Penh, Cambodia. Journal of Veterinary Medical Science. 2011;73(3):325-9.

Le Minor, L. (1981). The Genus *Salmonella* . In: M. P. Starr, H. Stolp, H. G. Trüper, A. Balows and H. G. Schlegel. Eds. The Prokaryotes (pp. 1148 – 1159).Springer-Verlag Berlin Heidelberg, New York.

Ledeboer NA, Frye JG, McClelland M, Jones BD. *Salmonella* enterica serovar Typhimurium requires the Lpf, Pef, and Tafi fimbriae for biofilm formation on HEp-2 tissue culture cells and chicken intestinal epithelium. Infection and immunity. 2006 Jun;74(6):3156-69.

Ledeboer NA, Jones BD. Exopolysaccharide sugars contribute to biofilm formation by *Salmonella* enterica serovar typhimurium on HEp-2 cells and chicken intestinal epithelium. Journal of bacteriology. 2005 May 1;187(9):3214-26.

Lee HY, Su LH, Tsai MH, Kim SW, Chang HH, Jung SI, Park KH, Perera J, Carlos C, Tan BH, Kumarasinghe G. High rate of reduced susceptibility to ciprofloxacin and ceftriaxone among nontyphoid *Salmonella* clinical isolates in Asia. Antimicrobial Agents and Chemotherapy. 2009 Jun;53(6):2696-9.

Leifson E. New Culture Media Based on Sodium Desoxycholate for the Isolation of Intestinal Pathogens and for the Enumeration of Colon Bacilli in Milk and Water. Pathology and Bacteriology. 1935;40:581-99.

Leininger HV. Equipment, media, reagents, routine tests and stains. Compendium of methods for the microbiological examination of foods. 1976:79-80.

Lenchenko E, Khai V, Vatnikov Y. The effect of Antibacterial Drugs on the Formation *Salmonella* Biofilms. International Journal of Pharmaceutical Research (09752366). 2021 Jan 1;13(1).

Lenders MH, Weidtkamp-Peters S, Kleinschrodt D, Jaeger KE, Smits SH, Schmitt L. Directionality of substrate translocation of the hemolysin A Type I secretion system. Scientific reports. 2015 Jul 27;5(1):12470.

Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, Van Den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, Van de Sande-Bruinsma N, Scharinga J, Bonten MJ. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clinical Microbiology and Infection. 2011 Jun;17(6):873-80.

Li H, Wang H, D'Aoust JY, Maurer J. *Salmonella* species. Food microbiology: fundamentals and frontiers. 2012 Dec 28:223-61.

Li L, Cheng CK, Cheung MK, Law PT, Ling JM, Kam KM, Cheung WM, Kwan HS. Draft Genome Sequence of *Salmonella* enterica Serovar Typhimurium ST 1660/06, a Multidrug-Resistant Clinical Strain Isolated from a Diarrheic Patient. Journal of bacteriology. 2012;194(22):6319-20.

Li S, He Y, Mann DA, Deng X. Global spread of *Salmonella* Enteritidis via centralized sourcing and international trade of poultry breeding stocks. Nature communications. 2021 Aug 25;12(1):5109.

Li Y, Xie X, Xu X, Wang X, Chang H, Wang C, Wang A, He Y, Yu H, Wang X, Zeng M. Nontyphoidal *Salmonella* infection in children with acute gastroenteritis: prevalence, serotypes, and antimicrobial resistance in Shanghai, China. Foodborne pathogens and disease. 2014 Mar 1;11(3):200-6.

Liang B, Xie Y, He S, Mai J, Huang Y, Yang L, Zhong H, Deng Q, Yao S, Long Y, Yang Y. Prevalence, serotypes, and drug resistance of nontyphoidal *Salmonella* among paediatric patients in a tertiary hospital in Guangzhou, China, 2014–2016. Journal of Infection and Public Health. 2019 Mar 1;12(2):252-7.

Linares JF, Gustafsson I, Baquero F, Martinez JL. Antibiotics as intermicrobial signaling agents instead of weapons. Proceedings of the National Academy of Sciences. 2006 Dec 19;103(51):19484-9.

Liu H, Whitehouse CA, Li B. Presence and persistence of *Salmonella* in water: the impact on microbial quality of water and food safety. Frontiers in Public Health. 2018 May 30;6:159.

Liu J, Guo JT, Li YG, Johnston RN, Liu GR, Liu SL. The type VI secretion system gene cluster of *Salmonella* typhimurium: required for full virulence in mice. Journal of basic microbiology. 2013 Jul;53(7):600-7.

Liu X, Yao H, Zhao X, Ge C. Biofilm Formation and Control of Foodborne Pathogenic Bacteria. Molecules. 2023 Mar 7;28(6):2432.

Llanes C, Kirchgesner V, Plesiat P. Propagation of TEM- and PSE-type β-lactamases among amoxicillin-resistant *Salmonella* spp. isolated in France, Antimicrob Agents Chemother, 1999, vol. 43 (pg. 2430-6)

Lodowska J, Wolny D, Węglarz L, Dzierżewicz Z. The structural diversity of lipid A from gram-negative bacteria. Advances in Hygiene and Experimental Medicine. 2007 Feb 22;61.

Low D, Braaten B, Van der Woude M. Fimbriae. Escherichia coli and *Salmonella* : cellular and molecular biology, 2nd ed. ASM Press, Washington, DC. 1996:146-57.

Luu RA, Gurnani K, Dudani R, Kammara R, van Faassen H, Sirard JC, Krishnan L, Sad S. Delayed expansion and contraction of CD8+ T cell response during infection with virulent *Salmonella* typhimurium. The Journal of Immunology. 2006 Aug 1;177(3):1516-25.

Lynch M, Painter J, Woodruff R, Braden C, Centers for Disease Control and Prevention. Surveillance for foodborne-disease outbreaks--United States, 1998-2002. Morbidity and mortality weekly report. Surveillance summaries (Washington, DC: 2002). 2006 Nov 10;55(10):1-42.

MacKenzie KD, Palmer MB, Köster WL, White AP. Examining the link between biofilm formation and the ability of pathogenic *Salmonella* strains to colonize multiple host species. Frontiers in veterinary science. 2017 Aug 25;4:138.

MacKenzie KD, Wang Y, Musicha P, Hansen EG, Palmer MB, Herman DJ, Feasey NA, White AP. Parallel evolution leading to impaired biofilm formation in invasive *Salmonella* strains. PLoS genetics. 2019 Jun 24;15(6):e1008233.

MacWilliams MP. Indole test protocol. American Society for Microbiology, Washington, DC. 2012. Cited from website:

https://asm.org/getattachment/200d3f34-c75e-4072-a7e6-df912c792f62/indole-test-protocol-3202.pdf

Maguire HC, Codd AA, Mackay VE, Rowe B, Mitchell E. A large outbreak of human salmonellosis traced to a local pig farm. Epidemiology & Infection. 1993 Apr;110(2):239-46.

Magwedere K, Rauff D, De Klerk G, Keddy KH, Dziva F. Incidence of nontyphoidal *Salmonella* in food-producing animals, animal feed, and the associated environment in South Africa, 2012–2014. Clinical Infectious Diseases. 2015 Nov 1;61(suppl_4):S283-9.

Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends in microbiology. 2001 Jan 1;9(1):34-9.

Mah TF. Biofilm-specific antibiotic resistance. Future microbiology. 2012 Sep;7(9):1061-72.

Mahon BE, Pönkä A, Hall WN, Komatsu K, Dietrich SE, Siitonen A, Cage G, Hayes PS, Lambert-Fair MA, Bean NH, Griffin PM. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. Journal of Infectious Diseases. 1997 Apr 1;175(4):876-82.

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM, International Collaboration on Enteric Disease "Burden of Illness" Studies. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clinical infectious diseases. 2010 Mar 15;50(6):882-9.

Marković KG, Grujović MŽ, Koraćević MG, Nikodijević DD, Milutinović MG, Semedo-Lemsaddek T, Djilas MD. Colicins and microcins produced by Enterobacteriaceae: Characterization, mode of action, and putative applications. International Journal of Environmental Research and Public Health. 2022 Sep 19;19(18):11825.

Martins M, McCusker M, Amaral L, Fanning S. Mechanisms of antibiotic resistance in *Salmonella* : efflux pumps, genetics, quorum sensing and biofilm formation. Letters in Drug Design & Discovery. 2011 Feb 1;8(2):114-23.

Maruzani R, Sutton G, Nocerino P, Marvasi M. Exopolymeric substances (EPS) from *Salmonella* enterica: polymers, proteins and their interactions with plants and abiotic surfaces. Journal of Microbiology. 2019 Jan;57:1-8.

Marzel A, Desai PT, Goren A, Schorr YI, Nissan I, Porwollik S, Valinsky L, McClelland M, Rahav G, Gal-Mor O. Persistent infections by nontyphoidal *Salmonella* in humans: epidemiology and genetics. Clinical Infectious Diseases. 2016 Apr 1;62(7):879-86.

Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. Indian journal of medical microbiology. 2006 Jan 1;24(1):25-9.

McDermott MF, Kaper JB, Olsen SJ, et al. Emergence of Extended Spectrum Beta-Lactamase–Producing Escherichia coli and Klebsiella pneumoniae Carrying IncX3 Plasmids Among Human Isolates of Nontyphoidal *Salmonella* in the United States, 2009–2014. Clinical Infectious Diseases. 2018;67(5):668-676.

McLure A, Shadbolt C, Desmarchelier PM, Kirk MD, Glass K. Source attribution of salmonellosis by time and geography in New South Wales, Australia. BMC Infectious Diseases. 2022 Dec;22(1):1-3.

Mei X, Zhai X, Lei C, Ye X, Kang Z, Wu X, Xiang R, Wang Y, Wang H. Development and application of a visual loop-mediated isothermal amplification combined with lateral flow dipstick (LAMP-LFD) method for rapid detection of Salmonella strains in food samples. Food Control. 2019 Oct 1;104:9-19.

Meletis G. Carbapenem resistance: overview of the problem and future perspectives. Therapeutic advances in infectious disease. 2016 Feb;3(1):15-21.

Menezes GA, Khan MA, Harish BN, Parija SC, Goessens W, Vidyalakshmi K, Baliga S, Hays JP. Molecular characterization of antimicrobial resistance in non-typhoidal *salmonellae* associated with systemic manifestations from India. Journal of medical microbiology. 2010 Dec;59(12):1477-83.

Meng CY, Smith BL, Bodhidatta L, Richard SA, Vansith K, Thy B, Srijan A, Serichantalergs O, Mason CJ. Etiology of diarrhea in young children and patterns of antibiotic resistance in Cambodia. The Pediatric infectious disease journal. 2011 Apr 1;30(4):331-5.

Mireles JR, Toguchi A, Harshey RM. *Salmonella* enterica serovar Typhimurium swarming mutants with altered biofilm-forming abilities: surfactin inhibits biofilm formation. Journal of bacteriology. 2001 Oct 15;183(20):5848-54.

Miriagou V, Tassios PT, Legakis NJ, Tzouvelekis LS. Expanded-spectrum cephalosporin resistance in non-typhoid *Salmonella* . International journal of antimicrobial agents. 2004 Jun 1;23(6):547-55.

Mohakud NK, Panda RK, Patra SD, Sahu BR, Ghosh M, Kushwaha GS, Misra N, Suar M. Genome analysis and virulence gene expression profile of a multi drug resistant *Salmonella* enterica serovar Typhimurium ms202. Gut Pathogens. 2022 Jun 28;14(1):28.

Mohanty S, Renuka K, Sood S, Das BK, Kapil A. Antibiogram pattern and seasonality of *Salmonella* serotypes in a North Indian tertiary care hospital. Epidemiology & Infection. 2006 Oct;134(5):961-6.

Monack DM, Mueller A, Falkow S. Persistent bacterial infections: the interface of the pathogen and the host immune system. Nature Reviews Microbiology. 2004 Sep 1;2(9):747-65. Antony

Morgan E, Campbell JD, Rowe SC, Bispham J, Stevens MP, Bowen AJ, Barrow PA, Maskell DJ, Wallis TS. Identification of host-specific colonization factors of *Salmonella* enterica serovar Typhimurium. Molecular microbiology. 2004 Nov;54(4):994-1010.

Mottet A, Tempio G. Global poultry production: current state and future outlook and challenges. World's Poultry Science Journal. 2017 Jun;73(2):245-56.

Nadimpalli M, Fabre L, Yith V, Sem N, Gouali M, Delarocque-Astagneau E, Sreng N, Le Hello S. CTX-M-55-type ESBL-producing *Salmonella* enterica are emerging among retail meats in Phnom Penh, Cambodia. Journal of Antimicrobial Chemotherapy. 2019 Feb 1;74(2):342-8.

Nair DV, Venkitanarayanan K, Kollanoor Johny A. Antibiotic-resistant *Salmonella* in the food supply and the potential role of antibiotic alternatives for control. Foods 2018, 7: 167.

Nazari Moghadam M, Rahimi E, Shakerian A, Momtaz H. Prevalence of Salmonella Typhimurium and Salmonella Enteritidis isolated from poultry meat: virulence and antimicrobial-resistant genes. BMC microbiology. 2023 Jun 15;23(1):168.

Nedialkova LP, Denzler R, Koeppel MB, Diehl M, Ring D, Wille T, Gerlach RG, Stecher B. Inflammation fuels colicin Ib-dependent competition of Salmonella serovar Typhimurium and E. coli in enterobacterial blooms. PLoS pathogens. 2014 Jan 2;10(1):e1003844.

Nga TV, Parry CM, Le T, Lan NP, Diep TS, Campbell JI, Hoang NV, Dung LT, Wain J, Dolecek C, Farrar JJ. The decline of typhoid and the rise of non-typhoid *Salmonellae* and fungal infections in a changing HIV landscape: bloodstream infection trends over 15 years in southern Vietnam. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2012 Jan 1;106(1):26-34.

Nguyen TV, Le PV, Le CH, Weintraub A. Antibiotic resistance in diarrheagenic Escherichia coli and Shigella strains isolated from children in Hanoi, Vietnam. Antimicrobial Agents and Chemotherapy. 2005 Feb;49(2):816-9.

Nhu NT, Vinh H, Nga TV, Stabler R, Duy PT, Thi Minh Vien L, van Doorn HR, Cerdeno-Tarraga A, Tho Harb mson N, Campbell J, Van Minh Hoang N. The sudden dominance of bla CTX–M harbouring plasmids in Shigella spp. circulating in Southern Vietnam. PLoS neglected tropical diseases. 2010 Jun 8;4(6):e702.

Nielsen HL, Thomsen PK, Litrup E, Torpdahl M, Overballe-Petersen S, Hansen F, Christensen AK, Hasman H. A case of blaNDM-1-positive *Salmonella* Kottbus, Denmark, November 2020. Eurosurveillance. 2021 Jul 1;26(26):2100569.

Notti RQ, Stebbins CE. The structure and function of type III secretion systems. Virulence Mechanisms of Bacterial Pathogens. 2016 Jun 22:241-64.

Nuccio SP, Bäumler AJ. Evolution of the chaperone/usher assembly pathway: fimbrial classification goes Greek. Microbiology and Molecular Biology Reviews. 2007 Dec;71(4):551-75.

Obe T, Richards AK, Shariat NW. Differences in biofilm formation of *Salmonella* serovars on two surfaces under two temperature conditions. Journal of Applied Microbiology. 2022 Mar 1;132(3):2410-20.

Ochiai RL, Acosta CJ, Danovaro-Holliday MC, Baiqing D, Bhattacharya SK, Agtini MD, Bhutta ZA, Canh DG, Ali M, Shin S, Wain J. A study of typhoid fever in five Asian countries: disease burden and implications for controls. Bulletin of the world health organization. 2008 Apr;86(4):260-8.

Ogasawara N, Tran TP, Ly TL, Nguyen TT, Iwata T, Okatani AT, Watanabe M, Taniguchi T, Hirota Y, Hayashidani H. Antimicrobial susceptibilities of *Salmonella* from domestic animals, food and human in the Mekong Delta, Vietnam. Journal of Veterinary Medical Science. 2008;70(11):1159-64.

Ogunremi D, Kelly H, Dupras AA, Belanger S, Devenish J. Development of a new molecular subtyping tool for *Salmonella* enterica serovar Enteritidis based on single nucleotide polymorphism genotyping using PCR. Journal of Clinical Microbiology. 2014 Dec;52(12):4275-85.

Okpa BO, Gberikon GM, Aguoru CU, Ogbonna IO. ESBL production and multidrug resistance of *Salmonella* serovars isolates in Benue State. American Journal of Molecular Biology. 2020 Jul 10;10(03):200.

Oladeinde BH, Omoregie R, Olley M, Anunibe JA. Urinary tract infection in a rural community of Nigeria. North American journal of medical sciences. 2011 Feb 1;3(2):75.

Olson ME, Ceri H, Morck DW, Buret AG, Read RR. Biofilm bacteria: formation and comparative susceptibility to antibiotics. Canadian journal of veterinary research. 2002 Apr;66(2):86.

O'mahony M, Cowden J, Smyth B, Lynch D, Hall M, Rowe B, Teare EL, Tettmar RE, Rampling AM, Coles M, Gilbert RJ. An outbreak of *Salmonella* saint-paul infection associated with beansprouts. Epidemiology & Infection. 1990 Apr;104(2):229-35.

Onwuezobe IA, Oshun PO, Odigwe CC. 2012. Antimicrobials for treating symptomatic non-typhoidal *Salmonella* infection. Cochrane Database Syst Rev 11:CD001167.

Ormsby MJ, White HL, Metcalf R, Oliver DM, Feasey NA, Quilliam RS. Enduring pathogenicity of African strains of *Salmonella* on plastics and glass in simulated peri-urban environmental waste piles. Journal of Hazardous Materials. 2024 Jan 5;461:132439.

Oxman JM, Boadla LR, Sells N. An isolated case of nosocomial acquisition of invasive non-typhoidal *Salmonella* . IDCases. 2023 Jun 9:e01816.

Pandey N, Cascella M. Beta-Lactam Antibiotics. [Updated 2023 Jun 4]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545311/

Papavasileiou K, Papavasileiou E, Tseleni-Kotsovili A, Bersimis S, Nicolaou C, Ioannidis A, Chatzipanagiotou S. Comparative antimicrobial susceptibility of biofilm versus planktonic forms of *Salmonella* enterica strains isolated from

children with gastroenteritis. European journal of clinical microbiology & infectious diseases. 2010 Nov;29:1401-5.

Paredes J, Alonso-Arce M, Schmidt C, Valderas D, Sedano B, Legarda J, Arizti F, Gómez E, Aguinaga A, Del Pozo JL, Arana S. Smart central venous port for early detection of bacterial biofilm related infections. Biomedical microdevices. 2014 Jun;16:365-74.

Parreira VR, Arns CW, Yano T. Virulence factors of avian Escherichia coli associated with swollen head syndrome. Avian Pathology. 1998 Apr 1;27(2):148-54.

Parry CM, Threlfall EJ. Antimicrobial resistance in typhoidal and nontyphoidal *Salmonellae*. Current opinion in infectious diseases. 2008 Oct 1;21(5):531-8.

Patel TA, Armstrong M, Morris-Jones SD, Wright SG, Doherty T. Imported enteric fever: case series from the hospital for tropical diseases, London, United Kingdom. The American journal of tropical medicine and hygiene. 2010 Jun;82(6):1121.

Pavelquesi SL, de Oliveira Ferreira AC, Rodrigues LF, de Souza Silva CM, da Silva IC, Orsi DC. Prevalence and antimicrobial resistance of Salmonella spp. isolated from chilled chicken meat commercialized at retail in Federal District, Brazil. Journal of Food Protection. 2023 Sep 1;86(9):100130.

Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. Journal of medical microbiology. 2015 Apr;64(4):323-34.

Pezoa D, Blondel CJ, Silva CA, Yang HJ, Andrews-Polymenis H, Santiviago CA, Contreras I. Only one of the two type VI secretion systems encoded in the *Salmonella* enterica serotype Dublin genome is involved in colonization of the avian and murine hosts. Veterinary research. 2014 Dec;45:1-9.

Pfaller MA, Segreti J. Overview of the epidemiological profileand laboratory detection of extended-spectrum β-Lactamases. Clinical Infectious Diseases. 2006 Apr 15;42(Supplement_4):S153-63.

Philippon A, Labia RO, Jacoby GE. Extended-spectrum beta-lactamases. Antimicrobial agents and chemotherapy. 1989 Aug;33(8):1131-6.

Phu Huong Lan N, Le Thi Phuong T, Nguyen Huu H, Thuy L, Mather AE, Park SE, Marks F, Thwaites GE, Van Vinh Chau N, Thompson CN, Baker S. Invasive non-typhoidal *Salmonella* infections in Asia: clinical observations, disease outcome and dominant serovars from an infectious disease hospital in Vietnam. PLoS neglected tropical diseases. 2016 Aug 11;10(8):e0004857.

Pollock HM, Dahlgren BJ. Clinical evaluation of enteric media in the primary isolation of *Salmonella* and Shigella. Applied Microbiology. 1974 Jan;27(1):197-201.

Popoff MY, Bockemühl J, Gheesling LL. Supplement 2002 (no. 46) to the Kauffmann–White scheme. Research in microbiology. 2004 Sep 1;155(7):568-70.

Pratt LA, Kolter R. Genetic analysis of Escherichia coli biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Molecular microbiology. 1998 Oct;30(2):285-93.

Pugsley AP, Schwartz M, Lavina M, Moreno F. On the effect of ompR on colicin E2 production. FEMS Microbiology Letters. 1983 Jun 1;19(1):87-92.

Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC, Heidelberg JF, Mekalanos JJ. Identification of a conserved bacterial protein secretion system in Vibrio cholerae using the Dictyostelium host model system. Proceedings of the National Academy of Sciences. 2006 Jan 31;103(5):1528-33.

Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. Annual review of biochemistry. 2002 Jul;71(1):635-700.

Ramatla T, Tawana M, Onyiche TE, Lekota KE, Thekisoe O. One health perspective of *Salmonella* serovars in South Africa using pooled prevalence: Systematic review and meta-analysis. International Journal of Microbiology. 2022 Apr 20;2022.

Reeves MW, Evins GM, Heiba AA, Plikaytis BD, Farmer 3rd JJ. Clonal nature of *Salmonella* typhi and its genetic relatedness to other *Salmonellae* as shown by multilocus enzyme electrophoresis, and proposal of *Salmonella* bongori comb. nov. Journal of clinical microbiology. 1989 Feb;27(2):313-20.

Rehman T, Yin L, Latif MB, Chen J, Wang K, Geng Y, Huang X, Abaidullah M, Guo H, Ouyang P. Adhesive mechanism of different *Salmonella* fimbrial adhesins. Microbial pathogenesis. 2019 Dec 1;137:103748.

Reiner K. Catalase test protocol. American society for microbiology. 2010 Nov 11:1-6.

Romling U, Bokranz W, Rabsch W, Zogaj X, Nimtz M, Tschäpe H. Occurrence and regulation of the multicellular morphotype in *Salmonella* serovars important in human disease. International journal of medical microbiology. 2003 Jan 1;293(4):273-85.

Russell AB, Wexler AG, Harding BN, Whitney JC, Bohn AJ, Goo YA, Tran BQ, Barry NA, Zheng H, Peterson SB, Chou S. A type VI secretion-related pathway in Bacteroidetes mediates interbacterial antagonism. Cell host & microbe. 2014 Aug 13;16(2):227-36.

S. McDevitt. Methyl Red and Voges-Proskauer Test Protocols (2009) Cited from website: http://www.asmscience.org/content/education/protocol/protocol.3204

Sabbagh SC, Forest CG, Lepage C, Leclerc JM, Daigle F. So similar, yet so different: uncovering distinctive features in the genomes of *Salmonella* enterica serovars Typhimurium and Typhi. FEMS microbiology letters. 2010 Apr 1;305(1):1-13.

Sabbagh SC, Lepage C, McClelland M, Daigle F. Selection of *Salmonella* enterica serovar Typhi genes involved during interaction with human macrophages by screening of a transposon mutant library. PloS one. 2012 May 4;7(5):e36643.

Sahuquillo-Arce JM, Hernández-Cabezas A, Yarad-Auad F, et al. Carbapenemases: a worldwide threat to antimicrobial therapy. World J Pharmacol. 2015;4(1):75–95.

Sallam KI, Kasem NG, Abdelkhalek A, Elshebrawy HA. Extensively drug-, ciprofloxacin-, cefotaxime-, and azithromycin-resistant *Salmonella* enterica serovars isolated from camel meat in Egypt. International Journal of Food Microbiology. 2023 Dec 20:110538.

Salmon DE, Smith T. Report on swine plague. USDA Bureau of Animal Ind. 2nd Annual Report. USDA, Washington, DC. 1885:237-9.

Sarkar A, Pazhani GP, Chowdhury G, et al. Attributes of carbapenemase encoding conjugative plasmid pNDM-SAL from an extensively drug-resistant *Salmonella* enterica Serovar Senftenberg. Front Microbiol. 2015;6:969.

Sasaki Y, Ikeda T, Yonemitsu K, Kuroda M, Ogawa M, Sakata R, Uema M, Momose Y, Ohya K, Watanabe M, Hara-Kudo Y. Antimicrobial resistance profiles of Campylobacter jejuni and *Salmonella* spp. isolated from enteritis patients in Japan. Journal of Veterinary Medical Science. 2023;85(4):463-70.

Sauer K, Stoodley P, Goeres DM, Hall-Stoodley L, Burmølle M, Stewart PS, Bjarnsholt T. The biofilm life cycle: Expanding the conceptual model of biofilm formation. Nature Reviews Microbiology. 2022 Oct;20(10):608-20.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. Foodborne illness acquired in the United States—major pathogens. Emerging infectious diseases. 2011 Jan;17(1):7-15

Scallan E, Mahon BE, Hoekstra RM, Griffin PM. Estimates of illnesses, hospitalizations and deaths caused by major bacterial enteric pathogens in young children in the United States. The Pediatric infectious disease journal. 2013 Mar 1;32(3):217-21.

Schneider T, Hahn-Löbmann S, Stephan A, Schulz S, Giritch A, Naumann M, Kleinschmidt M, Tusé D, Gleba Y. Plant-made Salmonella bacteriocins salmocins for control of Salmonella pathovars. Scientific Reports. 2018 Mar 6;8(1):4078.

Sears KT, Nasrin S, Baliban SM, Council DN, Pasetti MF, Tennant SM. Evaluation of Three Candidate Live-Attenuated *Salmonella* enterica Serovar Typhimurium Vaccines to Prevent Non-Typhoidal *Salmonella* Infection in an Infant Mouse Model. Vaccines. 2023 Oct 4;11(10):1562.

Sedrakyan AM, Ktsoyan ZA, Arakelova KA, Zakharyan MK, Hovhannisyan AI, Gevorgyan ZU, Mnatsakanyan AA, Kakabadze EG, Makalatia KB, Chanishvili NA, Pirnay JP. Extended-spectrum β-lactamases in human isolates of multidrug-resistant non-typhoidal *Salmonella* enterica. Frontiers in Microbiology. 2020 Dec 22;11:592223.

Seiffert SN, Hilty M, Perreten V, Endimiani A. Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: an emerging problem for human health?. Drug Resistance Updates. 2013 Feb 1;16(1-2):22-45.

Sengkhui S, Klubthawee N, Aunpad R. A novel designed membrane-active peptide for the control of foodborne *Salmonella* enterica serovar Typhimurium. Scientific Reports. 2023 Mar 2;13(1):3507.

Seral C, Weill FX. Prevalence and characterization of extended-spectrum beta-lactamases-producing *Salmonella* enterica isolates in Saragossa, Spain (2001-2008). Microbial Drug Resistance (Larchmont, NY). 2011 Jan 16;17(2):207-13.

Sethuvel DP, Anandan S, Ragupathi NK, Veeraraghavan B, Vinod O, Walia K. Association of blaCTX-M15 and qnr genes in multidrug-resistant *Salmonella* Typhimurium and Shigella spp from India. J Infect Dev Countr. 2015;9:1294–7.

Shahane V, Muley V, Kagal A, Bharadwaj R. Non-typhoidal Salmonellosis: emerging infection in Pune? Indian J Med Microbiol. 2007;25:173–174.

Shaheen A, Tariq A, Iqbal M, Mirza O, Haque A, Walz T, Rahman M. Mutational diversity in the quinolone resistance-determining regions of type-II topoisomerases of *Salmonella* serovars. Antibiotics. 2021 Nov 26;10(12):1455.

Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, Langley JM, Wanke C, Warren CA, Cheng AC, Cantey J. 2017 Infectious Diseases Society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. Clinical Infectious Diseases. 2017 Dec 15;65(12):e45-80.

Shields P, Cathcart L. Oxidase test protocol. American Society for Microbiology. 2010 Nov 11:1-9.

Shimoni Z, Pitlik S, Leibovici L, Samra Z, Konigsberger H, Drucker M, Agmon V, Ashkenazi S, Weinberger M. Nontyphoid *Salmonella* bacteremia: age-related differences in clinical presentation, bacteriology, and outcome. Clinical infectious diseases. 1999 Apr 1;28(4):822-7. Arii

Shuai GU, Chaomin WA. 13 cases of non-typhoid *Salmonella* septicemia in children: A case series report. Chinese Journal of Evidence-Based Pediatrics. 2023 Apr 25;18(2):129.

Siala, M., Barbana, A., Smaoui, S., Hachicha, S., Marouane, C., Kammoun, S., Gdoura, R. and Messadi-Akrout, F., 2017. Screening and detecting Salmonella in different food matrices in Southern Tunisia using a combined enrichment/real-time PCR method: Correlation with conventional culture method. *Frontiers in microbiology*, *8*, p.2416.

Siddique A, Azim S, Ali A, Andleeb S, Ahsan A, Imran M, Rahman A. Antimicrobial resistance profiling of biofilm forming non typhoidal *Salmonella* enterica isolates from poultry and its associated food products from Pakistan. Antibiotics. 2021 Jun 28;10(7):785.

Simons A, Alhanout K, Duval RE. Bacteriocins, antimicrobial peptides from bacterial origin: Overview of their biology and their impact against multidrug-resistant bacteria. Microorganisms. 2020 Apr 27;8(5):639.

Sirichote P, Bangtrakulnonth A, Tianmanee K, Unahalekhaka A, Oulai A, Chittaphithakchai P, Kheowrod W, Hendriksen RS. Serotypes and antimicrobial resistance of *Salmonella* enterica ssp in central Thailand, 2001-2006. Southeast Asian Journal of Tropical Medicine and Public Health. 2010 Nov 1;41(6):1405.

Sirichote P, Hasman H, Pulsrikarn C, Schønheyder HC, Samulionienė J, Pornruangmong S, Bangtrakulnonth A, Aarestrup FM, Hendriksen RS. Molecular characterization of extended-spectrum cephalosporinase-producing *Salmonella* enterica serovar Choleraesuis isolates from patients in Thailand and Denmark. Journal of clinical microbiology. 2010 Mar;48(3):883-8.

Soe GB, Overturf GD. Treatment of typhoid fever and other systemic salmonelloses with cefotaxime, ceftriaxone, cefoperazone, and other newer cephalosporins. Reviews of infectious diseases. 1987 Jul 1;9(4):719-36.

Soliani L, Rugna G, Prosperi A, Chiapponi C, Luppi A. *Salmonella* Infection in Pigs: Disease, Prevalence, and a Link between Swine and Human Health. Pathogens. 2023 Oct 21;12(10):1267.

Sood S, Kapil A, Das B, Jain Y, Kabra SK. Re-emergence of chloramphenicol sensitive *Salmonella* typhi. The Lancet. 1999 Apr 10;353(9160):1241-2.

Sriyapai T, Pulsrikarn C, Chansiri K, Sriyapai P. Molecular characterization of extended-spectrum cephalosporin and fluoroquinolone resistance genes in *Salmonella* and Shigella isolated from clinical specimens in Thailand. Heliyon. 2022 Dec 1;8(12).

Stanaway JD, Parisi A, Sarkar K, Blacker BF, Reiner RC, Hay SI, Nixon MR, Dolecek C, James SL, Mokdad AH, Abebe G. The global burden of non-typhoidal *Salmonella* invasive disease: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet Infectious Diseases. 2019 Dec 1;19(12):1312-24.

Steenackers H, Hermans K, Vanderleyden J, De Keersmaecker SC. *Salmonella* biofilms: an overview on occurrence, structure, regulation and eradication. Food Research International. 2012 Mar 1;45(2):502-31.

Stepanović S, Ćirković I, Ranin L, Svabić-Vlahović M. Biofilm formation by *Salmonella* spp. and Listeria monocytogenes on plastic surface. Letters in applied microbiology. 2004 May 1;38(5):428-32.

Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. Journal of microbiological methods. 2000 Apr 1;40(2):175-9.

Stepanović S, Vuković D, Hola V, Bonaventura GD, Djukić S, Ćirković I, Ruzicka F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Apmis. 2007 Aug;115(8):891-9.

Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. The Lancet. 2001 Jul 14;358(9276):135-8.

Su L, Chiu CH. *Salmonella* : clinical importance and evolution of nomenclature. Chang Gung medical journal. 2007 May 1;30(3):210-19.

Su LH, Chiu CH, Chu C, Ou JT. Antimicrobial resistance in nontyphoid *Salmonella* serotypes: a global challenge. Clinical infectious diseases. 2004 Aug 15;39(4):546-51.

Sudhanthirakodi S. Non-typhoidal *Salmonella* isolates from livestock and food samples, Kolkata, India. Journal of Microbiology and Infectious Diseases. 2016 Jan 9;6(3):113-20.

Swanson SJ, Snider C, Braden CR, Boxrud D, Wünschmann A, Rudroff JA, Lockett J, Smith KE. Multidrug-resistant *Salmonella* enterica serotype Typhimurium associated with pet rodents. New England Journal of Medicine. 2007 Jan 4;356(1):21-8.

Tack B, Phoba MF, Barbe B, Kalonji LM, Hardy L, Van Puyvelde S, Ingelbeen B, Falay D, Ngonda D, van der Sande MA, Deborggraeve S. Non-typhoidal *Salmonella* bloodstream infections in Kisantu, DR Congo: emergence of O5-negative *Salmonella* Typhimurium and extensive drug resistance. PLoS neglected tropical diseases. 2020 Apr 2;14(4):e0008121.

Tack B, Vanaenrode J, Verbakel JY, Toelen J, Jacobs J. Invasive non-typhoidal *Salmonella* infections in sub-Saharan Africa: a systematic review on antimicrobial resistance and treatment. BMC medicine. 2020 Dec;18:1-22.

Tahoun A, Mahajan S, Paxton E, Malterer G, Donaldson DS, Wang D, Tan A, Gillespie TL, O'Shea M, Roe AJ, Shaw DJ. *Salmonella* transforms follicle-associated epithelial cells into M cells to promote intestinal invasion. Cell host & microbe. 2012 Nov 15;12(5):645-56.

Takaya A, Suzuki M, Matsui H, Tomoyasu T, Sashinami H, Nakane A, Yamamoto T. Lon, a stress-induced ATP-dependent protease, is critically important for systemic *Salmonella* enterica serovar typhimurium infection of mice. Infection and Immunity. 2003 Feb;71(2):690-6.

Taneja N, Appannanavar SB, Kumar A, Varma G, Kumar Y, Mohan B, Sharma M. Serotype profile and molecular characterization of antimicrobial resistance in non-typhoidal *Salmonella* isolated from gastroenteritis cases over nine years. Journal of medical microbiology. 2014 Jan 1;63(1):66-73.

Taneja N, Mohan B, Khurana S, Sharma M. Antimicrobial resistance in selected bacterial enteropathogens in North India. Indian J Med Res. 2004;120:39–43.

Tankeshwar, A (2022). Triple Sugar Iron (TSI) Agar: Principle, Results, and Interpretation. Cited from web: https://microbeonline.com/triple-sugar-iron-agar-tsi-principle-procedure-and-interpretation/

Tauxe RV, Pavia AT. Salmonellosis: nontyphoidal. In Evans, A.S., Brachman, P.S. Eds. Bacterial infections of humans: epidemiology and control. 1998:613-30.

Taylor WI. Isolation of shigellae. I. Xylose lysine agars; new media for isolation of enteric pathogens. American Journal of Clinical Pathology. 1965;44(4):471-5.

Thames HT, Pokhrel D, Willis E, Rivers O, Dinh TT, Zhang L, Schilling MW, Ramachandran R, White S, Sukumaran AT. *Salmonella* Biofilm Formation under Fluidic Shear Stress on Different Surface Materials. Foods. 2023 May 8;12(9):1918.

Thielman NM, Guerrant RL. Acute infectious diarrhea. New England Journal of Medicine. 2004 Jan 1;350(1):38-47.

Thomsen LE, Chadfield MS, Bispham J, Wallis TS, Olsen JE, Ingmer H. Reduced amounts of LPS affect both stress tolerance and virulence of *Salmonella* enterica serovar Dublin. FEMS microbiology letters. 2003 Nov 1;228(2):225-31.

Tiew WT, Janapatla RP, Chang YJ, Chen YC, Yang HP, Su LH, Chen CL, Chiu CH. Emergence and spread in Taiwan of multidrug-resistant serotypes of nontyphoidal *Salmonella* . Infection. 2022 Apr;50(2):475-82.

Townsend SM, Kramer NE, Edwards R, Baker S, Hamlin N, Simmonds M, Stevens K, Maloy S, Parkhill J, Dougan G, Bäumler AJ. *Salmonella* enterica serovar Typhi possesses a unique repertoire of fimbrial gene sequences. Infection and immunity. 2001 May 1;69(5):2894-901.

Tsai MH, Huang YC, Lin TY, Huang YL, Kuo CC, Chiu CH. Reappraisal of parenteral antimicrobial therapy for nontyphoidal *Salmonella* enteric infection in children. Clin Microbiol Infect. 2011;17:300–5.

Turki Y, Ouzari H, Mehri I, Aissa RB, Hassen A. Biofilm formation, virulence gene and multi-drug resistance in *Salmonella* Kentucky isolated in Tunisia. Food Research International. 2012 Mar 1;45(2):940-6.

Van Schothorst M, Renaud A, Van Beek C. *Salmonella* isolation using RVS broth and MLCB agar. Food microbiology. 1987 Jan 1;4(1):11-18.

van Sorge NM, Zialcita PA, Browne SH, Quach D, Guiney DG, Doran KS. Penetration and activation of brain endothelium by *Salmonella* enterica serovar Typhimurium. Journal of Infectious Diseases. 2011 Feb 1;203(3):401-5.

Van TT, Chin J, Chapman T, Tran LT, Coloe PJ. Safety of raw meat and shellfish in Vietnam: an analysis of Escherichia coli isolations for antibiotic resistance and virulence genes. International journal of food microbiology. 2008 Jun 10;124(3):217-23.

Van TT, Moutafis G, Istivan T, Tran LT, Coloe PJ. Detection of *Salmonella* spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. Applied and environmental microbiology. 2007 Nov 1;73(21):6885-90.

Van TT, Nguyen HN, Smooker PM, Coloe PJ. The antibiotic resistance characteristics of non-typhoidal *Salmonella* enterica isolated from food-producing animals, retail meat and humans in South East Asia. International Journal of Food Microbiology. 2012 Mar 15;154(3):98-106.

Vasicek EM, Gunn JS. Invasive Non-Typhoidal *Salmonella* Lineage Biofilm

Formation and Gallbladder Colonization Vary But Do Not Correlate Directly with Known Biofilm-Related Mutations. Infection and Immunity. 2023 May 16;91(5):e00135-23.

Verma S, Kalyan RK, Gupta P, Khan MD, Venkatesh V. Molecular Characterization of Extended Spectrum β-Lactamase Producing Escherichia coli and Klebsiella pneumoniae Isolates and Their Antibiotic Resistance Profile in Health Care-Associated Urinary Tract Infections in North India. Journal of Laboratory Physicians. 2022 Oct 20;15(02):194-201.

Vestby LK, Møretrø T, Langsrud S, Heir E, Nesse LL. Biofilm forming abilities of *Salmonella* are correlated with persistence in fish meal-and feed factories. BMC veterinary research. 2009 Dec;5:1-6.

Vinayaka AC, Golabi M, Than TL, Wolff A, Bang DD. Point-of-care diagnosis of invasive non-typhoidal Salmonella enterica in bloodstream infections using immunomagnetic capture and loop-mediated isothermal amplification. New Biotechnology. 2022 Jan 25;66:1-7.

Vlieghe ER, Phe T, De Smet B, Veng CH, Kham C, Bertrand S, Vanhoof R, Lynen L, Peetermans WE, Jacobs JA. Azithromycin and ciprofloxacin resistance in *Salmonella* bloodstream infections in Cambodian adults. PLoS neglected tropical diseases. 2012 Dec 13;6(12):e1933.

Vo AT, Van Duijkeren E, Fluit AC, Heck ME, Verbruggen A, Maas HM, Gaastra W. Distribution of *Salmonella* enterica serovars from humans, livestock and meat in Vietnam and the dominance of *Salmonella* Typhimurium phage type 90. Veterinary microbiology. 2006 Mar 10;113(1-2):153-8.

Vo AT, Van Duijkeren E, Gaastra W, Fluit AC. Antimicrobial resistance, class 1 integrons, and genomic island 1 in *Salmonella* isolates from Vietnam. PloS one. 2010 Feb 26;5(2):e9440.

Wadhwa N, Berg HC. Bacterial motility: machinery and mechanisms. Nature reviews microbiology. 2022 Mar;20(3):161-73. Jahan

Wagner C, Polke M, Gerlach RG, Linke D, Stierhof YD, Schwarz H, Hensel M. Functional dissection of SiiE, a giant non-fimbrial adhesin of *Salmonella* enterica. Cellular microbiology. 2011 Aug;13(8):1286-301.

Wang CH, Huang ML, Zhuo ZQ, Wang ZX, Chen L, Song YQ, Yu H. Clinical features and antimicrobial resistance of invasive non-typhoid *Salmonella* infection in children at Xiamen. Zhonghua er ke za zhi= Chinese Journal of Pediatrics. 2023 Aug 1;61(8):685-9.

Wang J, Zhu X, Zhao Y, Xue Y, Zhang Z, Yan L, Chen Y, Robertson ID, Guo A, Aleri JW. Risk factors associated with *Salmonella* in dairy cattle farms in Henan and Hubei provinces, China. Animal diseases. 2023 Jul 11;3(1):20.

Wang M, Luo Z, Du H, Xu S, Ni B, Zhang H, Sheng X, Xu H, Huang X. Molecular characterization of a functional type VI secretion system in *Salmonella* enterica serovar Typhi. Current microbiology. 2011 Jul;63:22-31.

Wang S, Yang D, Wu X, Wang Y, Wang D, Tian M, Li T, Qi J, Wang X, Ding C, Yu S. Autotransporter MisL of *Salmonella* enterica serotype Typhimurium facilitates bacterial aggregation and biofilm formation. FEMS microbiology letters. 2018 Sep;365(17):fny142.

Wang S, Yang D, Wu X, Yi Z, Wang Y, Xin S, Wang D, Tian M, Li T, Qi J, Ding C. The ferric uptake regulator represses type VI secretion system function by binding directly to the clpV promoter in *Salmonella* enterica serovar Typhimurium. Infection and Immunity. 2019 Oct;87(10):10-128.

Wang X, Biswas S, Paudyal N, Pan H, Li X, Fang W, Yue M. Antibiotic resistance in *Salmonella* Typhimurium isolates recovered from the food chain through national antimicrobial resistance monitoring system between 1996 and 2016. Frontiers in microbiology. 2019 May 7;10:985.

Wannaprasat W, Padungtod P, Chuanchuen R. Class 1 integrons and virulence genes in *Salmonella* enterica isolates from pork and humans. International journal of antimicrobial agents. 2011 May 1;37(5):457-61.

Weinberger M, Keller N. Recent trends in the epidemiology of non-typhoid *Salmonella* and antimicrobial resistance: the Israeli experience and worldwide review. Current Opinion in Infectious Diseases. 2005 Dec 1;18(6):513-21.

Wen SC, Best E, Nourse C. Non-typhoidal *Salmonella* infections in children: Review of literature and recommendations for management. Journal of paediatrics and child health. 2017 Oct;53(10):936-41.

Wickham ME, Brown NF, Provias J, Finlay BB, Coombes BK. Oral infection of mice with *Salmonella* enterica serovar Typhimurium causes meningitis and infection of the brain. BMC infectious diseases. 2007 Dec;7:1-6.

Willems E, Verhaegen J, Magerman K, Nys S, Cartuyvels R. Towards a phenotypic screening strategy for emerging β-lactamases in Gram-negative bacilli. International journal of antimicrobial agents. 2013 Feb 1;41(2):99-109.

Williams V, Fletcher M. Pseudomonas fluorescens adhesion and transport through porous media are affected by lipopolysaccharide composition. Applied and Environmental Microbiology. 1996 Jan;62(1):100-4.

Wingender J, Flemming HC. Biofilms in drinking water and their role as reservoir for pathogens. International journal of hygiene and environmental health. 2011 Nov 1;214(6):417-23.

Woerther PL, Burdet C, Chachaty E, Andremont A. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M. Clinical microbiology reviews. 2013 Oct;26(4):744-58.

Woods DF, Reen FJ, Gilroy D, Buckley J, Frye JG, Boyd EF. Rapid multiplex PCR and real-time TaqMan PCR assays for detection of *Salmonella* enterica and the highly virulent serovars Choleraesuis and Paratyphi C. Journal of clinical microbiology. 2008 Dec;46(12):4018-22.

World Health Organization. 2019. *Critically important antimicrobials for human medicine*, 6th revision. World Health Organization, Geneva, Switzerland.

World Health Organization. Guidance on regulations for the transport of infectious substances 2021-2022: applicable as from 1 January 2021. World Health Organization; 2021.

Wu LJ, Luo Y, Shi GL, Li ZY. Prevalence, clinical characteristics and changes of antibiotic resistance in children with nontyphoidal *Salmonella* infections from 2009–2018 in Chongqing, China. Infection and Drug Resistance. 2021 Apr 13:1403-13.

Yang B, Qu D, Zhang X, Shen J, Cui S, Shi Y, Xi M, Sheng M, Zhi S, Meng J. Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. International journal of food microbiology. 2010 Jun 30;141(1-2):63-72.

Yang C, Chen K, Ye L, Heng H, Yang X, Wai-chi Chan E, Chen S. Prevalence and molecular characterization of cefotaxime-resistant *Salmonella* strains recovered from retail meat samples in Shenzhen, China, during 2014–2017. Microbiology Spectrum. 2023 Oct 17;11(5):e04886-22.

Yin X, Dudley EG, Pinto CN, M'ikanatha NM. Fluoroquinolone sales in food animals and quinolone resistance in non-typhoidal *Salmonella* from retail meats: United States, 2009–2018. Journal of Global Antimicrobial Resistance. 2022 Jun 1;29:163-7.

Yousefi-Mashouf R, Moshtaghi AA. Frequency of typhoidal and non-typhoidal *Salmonella* species and detection of their drugs resistance patterns. Journal of Research in Health Sciences. 2023 Aug 30;7(1):49-56.

Zeng X, Lin J. Beta-lactamase induction and cell wall metabolism in Gram-negative bacteria. Frontiers in microbiology. 2013 May 22;4:128.

Zhang J, Jin H, Hu J, Yuan Z, Shi W, Ran L, et al. Serovars and antimicrobial resistance of non-typhoidal *Salmonella* from human patients in Shanghai, China, 2006–2010. Epidemiol Infect. 2014; 142(4): 826–832. Available: https://pubmed.ncbi.nlm.nih.gov/23842508/

Zhang X, Liu B, Ding X, Bin P, Yang Y, Zhu G. Regulatory Mechanisms between Quorum Sensing and Virulence in *Salmonella* . Microorganisms. 2022 Nov 9;10(11):2211.

Ziech RE, Lampugnani C, Perin AP, Sereno MJ, Sfaciotte RA, Viana C, Soares VM, Pinto JP, Bersot LD. Multidrug resistance and ESBL-producing *Salmonella* spp. isolated from broiler processing plants. brazilian journal of microbiology. 2016 Jan;47:191-5.

APPENDIX -1

LIST OF IMPORTANT MEDIA AND REAGENTS USED

S. No.	Material	Manufacturer
1.	Antibiotic discs	Tulip diagnostic (P) Ltd., Goa, India
2.	Antisera polyvalent and monovalent against 'O' & 'H' antigens of *Salmonella*	National *Salmonella* and *Escherichia* Centre, CRI Kasauli, HP, India. And Denka Seiken Co. Ltd., Japan
3.	Catalase reagent	SD Fine Chem Ltd., Mumbai, India
4.	Sodium citrate	
5.	Sodium chloride	
6.	Agar	
7.	Extended Spectrum Beta Lactamase (ESBL) detection Kit	Hi Media Labs. Pvt. Ltd., Mumbai, India
8.	Glucose	
9.	Glucose phosphate medium	
10.	Agarose	
11.	Ethidium bromide	
12.	α-Naphthol	
13.	MR reagent	
	Nitrate broth	
14.	Urea medium base	
15.	Urea solution	
16.	Nutrient broth	
17.	Oxidase reagent	
18.	Peptone Water	
19.	Muller Hinton agar (MHA)	
20.	Simmon Citrate Medium	
21.	Sucrose	
22.	Lactose	Loba Chemie Pvt. Ltd., Mumbai, India
23.	Luria Bertani (LB) Broth	SRL Pvt. Ltd., SRL Pvt. Ltd., Mumbai, India
24.	MacConkey agar medium	Microxpres, Tulip diagnostic (P) Ltd., Goa, India
25.	Nutrient agar	
26.	Trypticase soy broth	
27.	Formalin	Rankem, APM India Pvt. Ltd.
28.	Ammonium dihydrogen phosphate	

S. No.	Material	Manufacturer
29.	Ethidium bromide (0.625mg/ml)	Gbiosciences, USA
30.	Agarose l (Low EEO)	
31.	Magnesium sulphate	Qualigens Fine Chemicals, Thermo Fisher Scientific India Pvt. Ltd., MH, India
32.	Dipotassium phosphate	
33.	Acetic acid	
34.	Disodium hydrogen phosphate	
35.	Ethyl alcohol	Merck Life Sciences Pvt. Ltd., India
36.	Potassium dihydrogen phosphate	
37.	Triple Sugar Iron Medium	
38.	Plasmid DNA extraction kit (QIAprep Spin miniprep kit)	QIAGEN India Pvt. Ltd., GmBH, Germany.
39.	Primers for $bla_{\text{CTX-M15}}$, $bla_{\text{CMY-2,}}$ bla_{TEM} & bla_{SHV} genes	Eurofins Genomics India Pvt. Ltd., India
40.	PCR Ready to use master mix (FIREPol®)	Solis BioDyne, Tartu, Estonia

Appendix 2

A. Composition of Media

1. Nutrient Broth:

Ingredients	Quantity
Peptone	5 g
Yeast Extract	1.5 g
Beef Extract	1.5 g
Sodium Chloride	5 g
Distilled water	1000 ml
pH	7.4 ± 0.2

2. Nutrient Agar:

Ingredients	Quantity
Peptone	5 g
Yeast Extract	1.5 g
Beef Extract	1.5 g
Sodium Chloride	5 g
Agar	15 g
Distilled water	1000 ml
pH	7.4 ± 0.2

3. Peptone water:

Ingredients	Quantity
Sodium Chloride	5 g
Peptic digest of animal tissue	10 g
Distilled water	1000 ml
pH	7.2 ± 0.2

4. Glucose Phosphate Medium:

Ingredients	Quantity
Glucose	5 g
Dipotassium Hydrogen Phosphate	5 g
Peptone	7 g
Distilled water	1000 ml
pH	6.9 ± 0.2

5. Luria Bertani Broth, Miller:

Ingredients	Quantity
Tryptone	10 g
Yeast extract	5 g
Sodium chloride	10 g
Distilled water	1000 ml
pH	7.5 ± 0.2

6. Trypticase Soya Broth with glucose:

Ingredients	Quantity
Dipotassium hydrogen Phosphate	2.5 g
Pancreatic Digest of Casein	17g
Peptic Digest of Soybean	3 g
Glucose	2.5 g
Sodium Chloride	5 g
pH	7.3 ± 0.2

7. MacConkey Agar:

Ingredients	Quantity
Peptone	20 g
Sodium taurocholate	5 g
Agar	20 g
Neutral red	0.4 g
Lactose	15 g
Distilled water	1000 ml
pH	7.1 ± 0.2

8. 1% Sugar media (Glucose/Sucrose/Lactose/Mannitol):

Ingredients	Quantity
Sugar	1 g
Distilled water	100 ml

9. Simmon's Citrate Media:

Ingredients	Quantity
Magnesium sulphate	0.2 g
Ammonium dihydrogen phosphate	1 g
Dipotassium phosphate	1 g
Sodium citrate	2 g
Sodium chloride	5 g
Agar	15 g
Bromothymol blue	0.08 g
Final pH	6.8 ± 0.2

10. Triple Sugar Iron (TSI) Medium:

Ingredients	Quantity
Peptone	20 g
Yeast Extract	3 g
Beef extract	3 g
Glucose	1 g
Lactose	10 g
Sucrose	10 g
Ferrous ammonium sulphate	0.2 g
Sodium chloride	5 g
Sodium thiosulphate	0.3 g

Phenol red	0.025 g
Agar	15 g
Distilled water	1000 ml
pH	7.4 ± 0.2

11. Christensen's Urease Medium:

A. Urease Base:

Ingredients	**Quantity**
Peptone	1 g
Agar	15 g
Distilled water	500 ml

Sterilize by autoclaving at 121°C for 15 minutes.

B. Urea Solution:

Sodium chloride	5 g
Potassium dihydrogen phosphate	2 g
Phenol red	1.2 g
Urea	20 g
Glucose	1 g
Distilled water	500ml

Mix to prepare a homogeneous solution and sterilize by filtration through 0.22µ filter. Finally add urea solution aseptically to urease base cooled to 55°C, mix to obtain a homogeneous solution and dispense in sterile glass tubes (10ml/tube).

12. Nitrate Reduction medium:

Ingredients	**Quantity**
Peptic digest of animal tissue	5 g
Meat extract	3 g
Potassium nitrate	1 g
Sodium chloride	30 g
Distilled water	1000 ml
pH	7.0 ± 0.2

13. Hugh & Leifson's(O/F) medium:

Ingredients	**Quantity**
Peptone	2 g
Sodium chloride	5 g
Dipotassium dihydrogen phosphate	0.3 g
Agar	3 g
Bromothymol blue (1% solution)	3 ml
Distilled water	1000ml
pH	7.1 ± 0.1

Sterilized at 121°C for 15 minutes. Separately prepared and filter sterilized 1%w/v Glucose solution, mixed with above solution aseptically. Dispensed in sterile glass tubes (~2ml/tube). To half of the tubes filled, added sterile liquid paraffin to form an overlay over the medium. The other half of the tubes were used as such without paraffin.

14. Mueller Hinton (MH) Agar:

Ingredients	**Quantity**
Meat infusion solids	2 g
Casein acid hydrolysate	17.5 g
Starch	1.5 g
Agar	17 g
Distilled water	1000ml
pH	7.3 ± 0.1

15. Mueller Hinton (MH) Broth:

Ingredients	**Quantity**
Meat infusion solids	2 g
Casein acid hydrolysate	17.5 g
Starch	1.5 g
Distilled water	1000 ml
pH	7.3 ± 0.1

B. Solution and Reagents:

1. Nitrate reduction reagent:

a) Solution -A

Ingredients	**Quantity**
Sulfanillic acid	8 g
5N Acetic acid	1000 ml

b) Solution -B

N,N-dimethyl-1-naphthylamine	6 ml
5N Acetic acid	1000 ml

2. Voges Proskauer reagent:

a) Solution -A

Ingredients	**Quantity**
Potassium hydroxide	40 g
Distilled water	100 ml

b) Solution-B

α-Naphthol	5 ml
Absolute alcohol	95 ml

3. Kovac's Reagent for indole:

Ingredients	**Quantity**
p-Dimethylaminobenzaldehyde	10 g
Isoamyl alcohol	150 ml
Conc. HCl	50 ml

4. Catalase reagent:

Hydrogen peroxide	3 ml
Distilled water	97 ml

5. Oxidase reagent:

Ingredients	**Quantity**
Tetramethyl-p-phenylene-diamine dihydrochloride	1 g
Distilled water	100 ml

6. Methyl Red reagent:

Ingredients	**Quantity**
Methyl red dye	0.1 g
Ethanol	300 ml
Distilled water	200 ml

7. Andrade's Indicator:

Ingredients	Quantity
Sodium hydroxide	1 N
Acid fuchsin	9.5 %

Prepared by adding 1N NaOH to 9.5% solution of acid fuchsin until the colour became yellow.

8. 1% w/v Safranin (stock solution):

Ingredients	Quantity
Safranin dye	1 g
Ethanol	100 ml

9. 0. 1% v/v Safranin (working solution):

Ingredients	Quantity
1% Stock solution of safranin	10 ml
Distilled water	90 ml

10. 30%v/v Acetic acid solution:

Ingredients	Quantity
Acetic acid (Purity ≥99.8%)	30 ml
Distilled water	70 ml

11. 1% Crystal violet:

Ingredients	Quantity
Methyl violet	10 g
Ethanol	100 ml
Distilled water	900 ml

12. Gram's Iodine:

Ingredients	Quantity
Iodine	10 g
Potassium iodide	20 g
Distilled water	1000 ml

13. 70% v/v Decolorizer:

Ingredients	Quantity
Ethanol	70 ml
Distilled water	30 ml

14. Carbol fuschin:

Ingredients	Quantity
Carbol fuschin (conc.)	1 ml
Distilled water	9 ml

15. Phosphate Buffered saline (pH 7.2)

Sodium chloride	8.5 g
Disodium hydrogen phosphate	1.91 g
Potassium dihydrogen phosphate	0.38 g
Distilled water	1000 ml
Final pH	7.2 ± 0.1

16. 0.5M EDTA solution:

Disodium EDTA	186.1 g
NaOH	50.0 g
Distilled water	To make 1L
Final pH	8.0

17. 10X TAE Buffer (Stock solution):

Tris base	48.41 g
Glacial acetic acid	11.42ml
0.5M EDTA	20ml
Triple Distilled water	To make 1L

18. 1X TAE Buffer (working solution):

10X TAE Buffer	100ml
Triple Distilled water	900ml

APPENDIX 3

LIST OF EQUIPMENT USED

S. No.	**Equipment**	**Model** No.	**Make**
1	Autoclave	GTA-001	Genist
2	Analytical balance	-	Eagle
3	Biosafety Cabinet	OL-114-A	Ocean Life Sciences
4	Electrophoresis unit	GNA-100	Genei
5	ELISA microplate reader	Sunrise	Tecan
6	Incubator	DL 2267	Scientific electronics
7	Orbital shaking Incubator	OLSC-109	Ocean Life Sciences
8	Hot air oven	-	New India Ltd.
9	Microscope	PZB-22	Quasmo
10	Laminar air flow	1554-ST	Klenzaid
11	PCR Cycler	5333 45234	Eppendorf
12	Vortex mixer	-	Arihant International
13	UV Trans-illuminator	BioVis	Expert Vision
14	pH meter	CP-901	Century
15	Micro-pipetters (0.5 – 10ul; 20-20ul and 100-1000ul)	-	Eppendorf

APPENDIX-4

ABBREVIATIONS

AMR	Antimicrobial resistance
AST	Antibiotic Susceptibility Testing
bp	Base pair
CDC	Centre for disease control
CLSI	Clinical laboratory standard institute
CNS	Central nervous system
CSF	Cerebrospinal fluid
DNA	Deoxyribose nucleic acid
ELISA	enzyme-linked immunosorbent assay
ESBL	Extended spectrum B-lactamase
HIV	human immunodeficiency virus
IMViC	Indole, Methyl red, Voges Proskauer, Citrate
LB	Luria Bertani medium
LPS	Lipopolysaccharides
MDR	Multiple drug resistance
MR	Methyl red
NR	Nitrate reduction
OD	Optical density
PCR	Polymerase chain reaction
RNA	Ribose nucleic acid
Spp	Species
TCP	Tissue culture plate method
TSB	Trypticase soy broth
TSI	Triple sugar iron
WHO	World Health Organization

APPENDIX-5
List of Publications from Thesis Work

S. No	Name of the Journal	Journal indexing (Scopus/UGC/ Web of Science)	Title of the Paper	Published Date (Date/Month /Year)	Volume & Issue Number	ISSN/ISBN Number	Impact Factor/SJR
1	Journal of Taibah University Medical Sciences	Scopus	Non-typhoidal *Salmonella* infections across India: emergence of a neglected group of enteric pathogens.	Oct, 2022	Volume -17, Issue-5	1658-3612	2.2/0.418
2	Indian Journal of Microbiology	Scopus	A Highly Drug-Resistant Salmonella Enterica Serovar Weltevreden of Human Origin from India and Detection of its Virulence Factors.	Oct, 2024	Volume -64, Issue -4	0973-7715	2.1/ 0.594
3	Frontiers in Microbiology	Scopus	Kumar G, Kumar S, Jangid H, Dutta J, Shidiki A. The Rise of Non-Typhoidal Salmonella: An Emerging Global Public Health Concern..;16:1524287.	Feb, 2025	Volume -16	1664-302X (Online)	4.0/1.69

APPENDIX-6

List of Conferences Attended

S. No.	Date of Conference	Details of the conference	Held at	Title of the research work presented
1	17-11-2017 To 18-11-2017	Innovative Strategies for Sustainable Water Management	LPU, Phagwara (Pb)	Antimicrobial resistance among *Salmonellae*
2	24-11-2023 To 25-11-2023	Microbial Bioprospecting Towards Sustainable Development Goals	LPU, Phagwara (Pb)	A highly drug resistant *Salmonella enterica* serovar Weltevreden of human origin.