

## Preliminary study of multidrug-resistant *Salmonella* Typhimurium and its monophasic variant *Salmonella* I 4,[5],12:i:- ST 36 isolated from some selected animals in Malaysia

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### ABSTRACT

*Salmonella* Typhimurium and its monophasic variant, *Salmonella* I 4,[5],12:i:- are significant zoonotic pathogens commonly associated with multidrug resistance. While *Salmonella* Typhimurium has been widely studied, the epidemiology and resistance profiles of the monophasic variant remains underexplored in Malaysia. This study analysed an archived collection of 18 strains, comprising ten *Salmonella* Typhimurium and eight *Salmonella* I 4,[5],12:i:- isolates from chickens, pigs, and ducks at the Veterinary Research Institute, Ipoh, Malaysia, between 2020 and 2023. Antimicrobial susceptibility testing using the disk diffusion method and multi-locus sequence typing were performed to characterise these isolates. A high prevalence of multidrug resistance was observed, with 90% of *Salmonella* Typhimurium and 87.5% of *Salmonella* I 4,[5],12:i:- isolates resistant to more than three antimicrobial classes, including 100% resistance to macrolides (erythromycin, tilmicosin). Notably, resistance to third-generation cephalosporins (ceftiofur and cefotaxime) was identified in one *Salmonella* I 4,[5],12:i:- strain isolated from chickens. MLST analysis revealed that all three selected isolates belonged to sequence type ST36. The findings highlight the zoonotic potential and multidrug resistance challenges posed by *Salmonella* I 4,[5],12:i:-. The detection of resistance to critical antibiotics, including third-generation cephalosporins, raises public health concerns. This study underscores the urgent need for enhanced surveillance, antimicrobial stewardship, and preventive measures to mitigate the public health risks associated with multidrug-resistant *Salmonella* strains, particularly the monophasic variant in Malaysia. Further research into resistance mechanisms and local epidemiology is essential to inform effective control strategies.

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## 1. INTRODUCTION

*Salmonella* Typhimurium is a significant animal-associated serovar known for its zoonotic potential, causing infections in humans and animals (Bawn et al., 2020). It is a group in non-typhoidal *Salmonella* (NTS), which normally causes self-limiting gastroenteritis with common disease manifestations of diarrhoea and fever (Jain et al., 2018). Livestock animals are known as a common reservoir for NTS (Bawn et al., 2020). The bacteria have been isolated from various sources, including humans, farm animals, food products, as well as domestic and wild animals (Khoo et al., 2015; Abatcha et al., 2018). Normally, these animals shed the bacteria in their faeces and contaminate the environment, particularly in poultry and pig farming systems (Chung The et al., 2023).

Since the 1990s, the monophasic variant of *Salmonella* Typhimurium, known as *Salmonella* I 4,[5],12:i:-, has emerged as a significant public health concern, with infections reported globally in humans, livestock, and poultry (Plumb et al., 2023). It lacks the expression of the second-phase flagellar 1,2 antigen,

a feature that distinguishes it from the biphasic serovar Typhimurium (Mandilara et al., 2021). The monophasic phenotype is due to a deletion in the *fljAB* region, which prevents the expression of the second flagellar antigen *FliC* (Ingle et al., 2021). This deletion is thought to be a stepwise evolutionary event that has occurred through the accumulation of mobile resistance elements. Despite the absence of the second-phase antigen, this variant exhibits a comparable capacity to cause disease in both humans and animals (Arnott et al., 2018). This monophasic variant *Salmonella* I 4,[5],12:i:- was first identified in Spain in 1997 (Echeita et al., 1999; Arrieta-Gisasola et al., 2020). Since then, it has become a predominant serovar in humans and food across several countries, including Europe, China, and Australia (Yang et al., 2015; Arnott et al., 2018; Arrieta-Gisasola et al., 2020). In 2018, a significant outbreak occurred in Danish pigs and pork, highlighting its impact on livestock and food safety (Helmuth et al., 2019). In the United States, infections caused by this variant have increased since 2010, ranking among the top ten serovars identified in broiler and ground chicken (Plumb et al., 2023). Furthermore, a multi-

country outbreak linked to chocolate products in 2022 underscored the variant's widespread public health implications (Plumb et al., 2022; Larkin et al., 2022). For several decades, the monophasic *Salmonella* I 4,[5],12:i:- has consistently been one of the most common serovars detected in poultry and a frequent cause of human salmonellosis (Lim et al., 2024).

Antimicrobial resistance (AMR) is one of the important public health threats often discussed over the years, according to the World Health Organization (WHO) and World Organisation for Animal Health (WOAH). The multidrug-resistant (MDR) of *Salmonella* Typhimurium have been covered extensively in the literature in many countries, particularly in chicken and pig farming (Plumb et al., 2023). Similarly, the multi-resistant *Salmonella* I 4,[5],12:i:- has become much more common among animals (Bawn et al., 2020). Mandilara et al. (2021) reported that *Salmonella* I 4,[5],12:i:- was mostly associated with pig (49.7%) and broiler (35.3%) based on findings from the European Food Safety Authority. However, the resistance patterns of *Salmonella* I 4,[5],12:i:- isolated from animals in Malaysia are limited and these resistance data are important for research especially in antibiotic usage, as well as in treatment or prevention of any disease outbreak. Antimicrobial therapy is usually recommended for patients with serious complications like bacteraemia or meningitis. The increasing incidence of MDR *Salmonella* poses a risk to public health, with the worst-case scenario being that antibiotic prescriptions for successful treatment in humans may have been limited due to diminished susceptibility to the drugs of choice (Lim et al., 2024).

In Malaysia, *Salmonella* Typhimurium isolates have been widely reported from different sources, including farm animals, animal products, wild animals, and humans (Khoo et al., 2015). Unlike the well-studied zoonotic importance serovar Typhimurium and Enteritidis, the isolation and identification of the *Salmonella* I 4,[5],12:i:- have not been highlighted in Malaysia. Despite global reports of monophasic *Salmonella* I 4,[5],12:i:-, there is limited data on its presence, resistance profile, or sequence type profile in animal sources in Malaysia. According to the unpublished *Salmonella* surveillance data recorded by the Department of Veterinary Services (DVS), the first identification of the monophasic variant in animal samples was in 2020 in the Veterinary Research Institute of Ipoh, Malaysia. Previously, the presence of the monophasic variant from human clinical sources in Malaysia was reported by Ngoi et al. (2018). The increasing prevalence and impact of *Salmonella* I 4,[5],12:i:- underscore the importance of continued monitoring and research into its epidemiology and pathogenic potential. This study aims to fill a gap in local surveillance by characterising MDR *Salmonella* Typhimurium and its monophasic variant *Salmonella* I 4,[5],12:i:-, from livestock in Malaysia. Specifically, it focuses on assessing their AMR profiles and sequence type (ST) lineages. This preliminary

investigation supports One Health initiatives by providing data that inform molecular epidemiology and antimicrobial stewardship at the animal-human-environment interface.

## 2. MATERIALS AND METHODS

### 2.1. Ethical and biosafety statement

This study did not involve the use of human participants or live animals and therefore did not require ethical approval. All procedures involving the handling and analysis of *Salmonella* spp. were conducted in accordance with the Biosafety Manual of Veterinary Research Institute, Ipoh, Malaysia and approved by the Institutional Research and Development Committee.

### 2.2. Bacterial isolates

The study utilised an archived collection of 18 strains, comprising ten *Salmonella* Typhimurium and eight monophasic *Salmonella* I 4,[5],12:i:- strains, isolated from farm animal species, including chickens, pigs, and ducks. These isolates were obtained between 2020 and 2023 as part of the DVS annual surveillance program and were identified at the Veterinary Research Institute (VRI), Ipoh, Malaysia (Tables 1 and 4). All available isolates identified as *Salmonella* Typhimurium or its monophasic variant via serotyping during this period were included, with efforts made to ensure representation across different species and geographic regions in Malaysia. Samples were obtained from environmental sources, cloacal and rectal swabs, and tissues collected from animal farms.

**Table 1:** Bacteria isolates in this study.

| Serovar                                     | Antigenic formulae | No. of isolate |
|---|--------------------|----------------|
| <i>Salmonella</i> Typhimurium               | 4,[5],12:i:1,2     | 10             |
| Monophasic <i>Salmonella</i> I 4,[5],12:i:- | 4,[5],12:i:-       | 8              |

*Salmonella* spp. was isolated through pre-enrichment, selective enrichment, isolation, and confirmation stages on the basis of cultural and biochemical characteristics referred from the Manual of Veterinary Laboratory Testing for Veterinary Public Health and Bacteriology (DVSM, 2016). The isolates were serotyped at the VRI using the slide agglutination method, based on the White-Kauffmann-Le Minor scheme (Grimont & Weill, 2007). The serovar identification was based on the White-Kauffmann-Le Minor scheme. Whereas, the absence of H-phase 2 flagellar in the monophasic variant was determined according to the Sven Gard method using Schwörm agar, and the phase inversion antisera, according to the standard method (Gard, 1938; SSI Diagnostica, 2023).

### 2.3. Antimicrobial susceptibility test

Disk diffusion method using Mueller-Hinton agar was used to determine the *Salmonella* Typhimurium and *Salmonella* I 4,[5],12:i:- antimicrobial susceptibility towards 11

antimicrobials belonging to eight antimicrobial classes: ampicillin (10 µg), chloramphenicol (30 µg), streptomycin (10 µg), gentamicin (10 µg), sulfamethoxazole/trimethoprim (25 µg), tetracycline (30 µg), erythromycin (15 µg), tilmicosin (15 µg), cefotaxime (30 µg), ceftiofur (30 µg), and ciprofloxacin (5 µg; Oxoid, UK). Results were obtained after incubating the samples for 16-18 hours at 37°C. The inhibition zones were measured and categorised as susceptible, intermediate, or resistant according to the breakpoints established by the Clinical and Laboratory Standards Institute (CLSI, 2020) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) using a microbiology laboratory database software (WHONET, 2024). *Escherichia coli* ATCC 25922 was used as the quality control reference strain.

**2.4. Multi-Locus Sequence Typing (MLST) analysis**

The ST of the selected two *Salmonella* Typhimurium and one *Salmonella* I 4,[5],12:i:- were assessed using the MLST, which is conducted based on the seven housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*) according to the primers listed in Table 2. The bacterial DNA was extracted using the boiling method from an overnight culture grown on blood agar, as described by Abatcha et al. (2018). A single colony of the bacteria was transferred into 100 µL nuclease-free water and incubated at 95°C in a thermal block for 10 mins. After being cooled to room temperature and centrifuged at 13,000 rpm for 5 mins, the supernatant was used as the DNA template. PCR amplifications were carried out in a 50 µL reaction mixture containing 25 µL of 2× MyTaq™ Mix (Meridian Bioscience, UK), 1 µL of each 10 µM forward and reverse primers (1st BASE, Malaysia), 5 µL of DNA template, and topped up with an appropriate amount of nuclease-free water. PCR conditions used are as follows; initial denaturation at 98°C for 30 s, 35 cycles of denaturation at 98°C for 10 s, primer annealing at 55°C for 30 s, and extension at 72°C for 30 s, and a final extension at 72°C for 10 min; as described by Khoo et al. (2023). DNA quality and PCR product yield were assessed using 1% agarose gel electrophoresis.

The PCR gel products were sent to a commercial sequencing laboratory (Apical Scientific Sdn. Bhd., Malaysia) for DNA purification and sequencing by applying the Sanger method using the list of sequencing primers mentioned in Table 2. Nucleotide sequences of the PCR products were assembled using a sequence assembly software (Seqman Pro, DNASTAR Lasergene; DNASTAR, Inc., USA). The obtained sequences of each housekeeping gene were submitted to the PubMLST public database (University of Oxford, UK) to obtain the matching locus allele number. The ST for each test isolate was determined by entering the allelic profiles combination of the seven loci into the PubMLST database for *Salmonella* as previously described by Jolley et al. (2018).

**Table 2:** The list of MLST amplification and sequencing primers used in this study.

| Genes                     | Forward and reverse primer sequence (5' – 3')     | Amplicon size (bp) | Reference          |                    |
|---------------------------|---|--------------------|--------------------|--------------------|
| MLST amplification primer |   |                    |                    |                    |
| <i>aroC</i>               | CCTGGCACCTCGCGTATAC<br>CCACACACGGATCGTGGCG        | 826                | Khoo et al. (2023) |                    |
| <i>dnaN</i>               | ATGAAATTTACCGTTGAACGTGA<br>AATTTCTCATTGAGAGGATTGC | 833                |                    |                    |
| <i>hemD</i>               | ATGAGTATTCTGATCACCCG<br>ATCAGCGACCTTAATATCTTGCCA  | 666                |                    |                    |
| <i>hisD</i>               | GAAACGTTCCATTCCGCGCAGAC<br>CTGAACGGTCATCCGTTTCTG  | 894                |                    |                    |
| <i>purE</i>               | GACACCTCAAAGCAGCGT<br>AGACGGCGATACCCAGCGG         | 510                |                    |                    |
| <i>sucA</i>               | AGCACCGAAGAGAACCGTG<br>GGTTGTTGATAACGATACGTAC     | 643                |                    |                    |
| <i>thrA</i>               | GTCACGGTGATCGATCCGGT<br>CACGATATTGATATTAGCCCG     | 852                |                    |                    |
| MLST sequencing primer    |   |                    |                    |                    |
| <i>aroC</i>               | GGCGTGACGACCGGCAC<br>AGCGCCATATGCGCCAC            |                    |                    | Khoo et al. (2023) |
| <i>dnaN</i>               | CCGATTCTCGGTAACCTGCT<br>ACGCGACGGTAATCCGGG        |                    |                    |                    |
| <i>hemD</i>               | GCCTGGAGTTTTCCACTG<br>GACCAATAGCCGACAGCGTAG       |                    |                    |                    |
| <i>hisD</i>               | GTCGGTCTGTATATCCCGG<br>GGTAATCGCATCCACCAAATC      |                    |                    |                    |
| <i>purE</i>               | ACAGGAGTTTTAAGACGCATG<br>GCAAACCTGCTCATAGCG       |                    |                    |                    |
| <i>sucA</i>               | CCGAAGAGAAACGCTGGATC<br>GGTTGTTGATAACGATACGTAC    |                    |                    |                    |
| <i>thrA</i>               | ATCCCGCGCGATCACATGAT<br>ACCGCCAGCGGCTCCAGCA       |                    |                    |                    |

*aroC*, chorismate synthase gene; *dnaN*, DNA polymerase III, β-subunit gene; *hemD*, uroporphyrinogen III synthase gene; *hisD*, histidinal dehydrogenase gene; *purE*, phosphoribosylaminoimidazole carboxylase gene; *sucA*, 2-oxoglutarate dehydrogenase decarboxylase gene; *thrA*, aspartokinase I gene.

**3. RESULT AND DISCUSSION**

The analysis revealed high levels of MDR in both *Salmonella* Typhimurium and *Salmonella* I 4,[5],12:i:-. Overall, 90.0% (9/10) of *Salmonella* Typhimurium and 87.5% (7/8) of *Salmonella* I 4,[5],12:i:- strains were MDR, which were resistant to three or more groups of antimicrobials (Table 4). *Salmonella* Typhimurium and *Salmonella* I 4,[5],12:i:- showed 100% resistance to macrolides: erythromycin and tilmicosin. Additional resistance was observed to antibiotics such as ampicillin, chloramphenicol, streptomycin, sulfamethoxazole/trimethoprim, tetracycline, and gentamicin, although at lower frequencies. Notably, one monophasic strain *Salmonella* I 4,[5],12:i:- which was STM1, isolated from chickens, showed resistance to the third-generation cephalosporin group of antibiotics: ceftiofur and cefotaxime, while all ten *Salmonella* Typhimurium isolates remained susceptible to these antibiotics (Table 3). MLST analysis revealed that all selected *Salmonella* Typhimurium and *Salmonella* I 4,[5],12:i:-isolates in this study belonged to ST36,

with exact matches at all seven housekeeping loci (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*), as described in detail in Table 5. These findings confirm the presence of ST36 in all monophasic isolates, addressing the study's objective of molecular typing and indicate the possible dominance of this sequence type in local animal reservoirs.

**Table 3:** Antimicrobial resistance of the *Salmonella* isolates in different types of antimicrobials.

| Group of antimicrobials | Antimicrobial disc            | Isolate with resistance (n)          |                           |
|-------------------------|-------------------------------|--------------------------------------|---------------------------|
|                         |                               | <i>Salmonella</i> Typhimurium n = 10 | <i>Salmonella</i> I n = 8 |
| β-lactams               | Ampicillin                    | 8 (80%)                              | 5 (62.5%)                 |
| Phenicol                | Chloramphenicol               | 6 (60%)                              | 4 (50%)                   |
| Aminoglycosides         | Streptomycin                  | 6 (60%)                              | 5 (62.5%)                 |
|                         | Gentamicin                    | 3 (30%)                              | 3 (37.5%)                 |
| Sulfonamides            | Sulfamethoxazole/trimethoprim | 7 (70%)                              | 4 (50%)                   |
| Tetracycline            | Tetracycline                  | 7 (70%)                              | 7 (87.5%)                 |
| Macrolides              | Erythromycin                  | 10 (100%)                            | 8 (100%)                  |
|                         | Tilmicosin                    | 10 (100%)                            | 8 (100%)                  |
| Cephalosporin           | Cefotaxime                    | 0 (0%)                               | 1 (12.5%)                 |
|                         | Ceftiofur                     | 0 (0%)                               | 1 (12.5%)                 |
| Fluoroquinolones        | Ciprofloxacin                 | 1 (10%)                              | 0 (0%)                    |

The emergence of MDR in *Salmonella* Typhimurium and its monophasic variant, *Salmonella* I 4,[5],12:i:-, poses significant challenges to public and animal health. MDR is defined as resistance to at least three or more antimicrobial classes, often driven by extensive antibiotic use in animal production for growth promotion, prophylaxis, and disease management (Nair et al., 2018). *Salmonella* I 4,[5],12:i:- is characterised by its MDR and has been associated with resistance to a wide range of antimicrobials. Its resistance to key antibiotics, including erythromycin, tetracycline, and macrolides, has been widely documented (Ingle et al., 2021; Chung The et al., 2023). The resistance patterns observed in this study highlight the challenge of managing infections caused by these resistant strains. The initial epidemiological marker for this lineage matched the ASSuT profile: resistance to ampicillin, streptomycin, sulfonamides, and tetracycline. In this study, both *Salmonella* Typhimurium and *Salmonella* I 4,[5],12:i:- demonstrated shared resistance patterns, including resistance to ampicillin, chloramphenicol, streptomycin, gentamicin, sulfonamides, tetracycline, and sulfamethoxazole/trimethoprim (ACSSuTTm), which is similar to the findings by Mandilara et al. (2021). Additionally, the isolates demonstrated 100% resistance to macrolides' antibiotic classes, including erythromycin and tilmicosin. Zhao et al. (2022) reported a significant proportion of *Salmonella* Typhimurium isolates from swine slaughterhouses exhibited 100% resistance to macrolides, specifically erythromycin and tilmicosin. This study found that

fluoroquinolones (ciprofloxacin), the antibiotic of choice for treating invasive salmonellosis in adults, are generally susceptible against both *Salmonella* Typhimurium and *Salmonella* I 4,[5],12:i:- isolates, making it a viable option for treating invasive salmonellosis. Despite this, proactive measures to curb MDR gene dissemination in food animals are imperative to mitigate the public health risks associated with resistant *Salmonella* strains. According to Shaikh et al. (2015), certain Enterobacterales can produce extended-spectrum beta-lactamases (ESBLs), which can break down and destroy several frequently used antibiotics, such as penicillins and cephalosporins, rendering them ineffective for treating infections. Preventive measures to inhibit the transmission of MDR genes in food and animals, such as chickens and pigs, are important as *Salmonella* spreads rapidly due to its MDR features.

The presence of all antibiotic resistance genes in commensal or free-living environmental organisms, collectively termed the resistome, may contribute to the widespread resistance to erythromycin and tetracycline observed among *Salmonella* Typhimurium and its monophasic variant (Gaissmaier et al., 2022). Few studies have highlighted the role of environmental microbes and commensal bacteria resistomes, such as lactic acid bacteria, in harbouring and disseminating resistance genes to pathogenic bacteria via the food chain (Nair et al., 2018).

The increasing resistance to the clinically important antimicrobials, such as third-generation cephalosporins, colistin, and fluoroquinolones, is concerning. Colistin, as the last-resort antibiotic for treating MDR Gram-negative bacterial infections, has been compromised by the presence of *mcr* genes reported in *Salmonella* I 4,[5],12:i:- isolates from various sources (Lima et al., 2019). Lim et al. (2024) reported six *Salmonella* Typhimurium isolates, including three ST36 chicken isolates, were phenotypically resistant to colistin and harboured the *mcr1* gene. The presence of resistance phenotypes like ACSSuTTm and ESBL enzymes in *Salmonella* Typhimurium and its monophasic variant underscores the critical need for robust antimicrobial stewardship (Shaikh et al., 2015).

The increasing global incidence of infections caused by *Salmonella* I 4,[5],12:i:-, particularly the strain ST34, has been reported in Europe, Asia, and Australia (Arnott et al., 2018; Arrieta-Gisasola et al., 2020; Chung The et al., 2023). This study did not observe ST34 but identified ST36 among the selected *Salmonella* Typhimurium and *Salmonella* I 4,[5],12:i:- isolates. This finding is an interesting observation that warrants further discussion in the context of the global epidemiology of this serovar. While ST19 remains the predominant ST worldwide,

**Table 4:** Phenotypic antimicrobial resistance profiles of the *Salmonella* isolates in poultry and pigs at different years and locations during this study.

| Isolate ID                       | Year | Species | Location        | Phenotypic antimicrobial resistance profiles | MDR |
|----------------------------------|------|---------|-----------------|--|-----|
| <i>Salmonella</i> Typhimurium    |      |         |                 |  |     |
| ST 1                             | 2020 | Pig     | Sabah           | AMP-S-TE-E-TIL                               | Yes |
| ST 2                             | 2020 | Chicken | Perak           | S-TE-E-TIL                                   | Yes |
| ST 3                             | 2021 | Chicken | Selangor        | AMP-C-S-CN-SXT-TE-E-TIL-CIP                  | Yes |
| ST 4                             | 2021 | Chicken | Selangor        | AMP-C-S-CN-SXT-TE-E-TIL                      | Yes |
| ST 5                             | 2022 | Chicken | Sabah           | AMP-SXT-TE-E-TIL                             | Yes |
| ST 6                             | 2022 | Chicken | Selangor        | AMP-C-S-SXT-E-TIL                            | Yes |
| ST 7                             | 2022 | Chicken | Sarawak         | AMP-C-SXT-TE-E-TIL                           | Yes |
| ST 8                             | 2023 | Pig     | Johor           | AMP-C-SXT-E-TIL                              | Yes |
| ST 9                             | 2021 | Duck    | Pahang          | E-TIL  | No  |
| ST 10                            | 2023 | Chicken | Sarawak         | AMP-C-S-CN-SXT-TE-E-TIL                      | Yes |
| <i>Salmonella</i> I 4,[5],12:i:- |      |         |                 |  |     |
| STM1                             | 2021 | Chicken | Negeri Sembilan | AMP-C-CN-SXT-TE-E-TIL-CTX-EFT                | Yes |
| STM2                             | 2023 | Chicken | Melaka          | AMP-C-S-CN-SXT-TE-E-TIL                      | Yes |
| STM3                             | 2022 | Pig     | Melaka          | C-S-TE-E-TIL                                 | Yes |
| STM4                             | 2022 | Duck    | Perak           | SXT-E-TIL                                    | No  |
| STM5                             | 2023 | Chicken | Perak           | AMP-C-S-CN-SXT-TE-E-TIL                      | Yes |
| STM6                             | 2021 | Chicken | Kedah           | AMP-S-TE-E-TIL                               | Yes |
| STM7                             | 2021 | Chicken | Perak           | AMP-TE-E-TIL                                 | Yes |
| STM8                             | 2022 | Duck    | Perak           | S-TE-E-TIL                                   | Yes |

AMP, ampicillin; C, chloramphenicol; S, streptomycin; CN, gentamicin; SXT, sulfamethoxazole/trimethoprim; TE, tetracycline; E, erythromycin; TIL, tilmicosin; CTX, cefotaxime; EFT, ceftiofur; CIP, ciprofloxacin.

**Table 5:** The allelic profiles and sequence type of *Salmonella* Typhimurium and *Salmonella* I 4,[5],12:i:- by MLST.

| Isolate ID                       | Year | Species | Source             | Location | <i>aroC</i> | <i>dnaN</i> | <i>hemD</i> | <i>hisD</i> | <i>purE</i> | <i>sucA</i> | <i>thrA</i> | ST |
|----------------------------------|------|---------|--------------------|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|----|
| <i>Salmonella</i> Typhimurium    |      |         |                    |          |             |             |             |             |             |             |             |    |
| ST5                              | 2022 | Chicken | Cloacal swab       | Sabah    | 18          | 14          | 12          | 9           | 5           | 18          | 21          | 36 |
| ST10                             | 2023 | Chicken | Environmental swab | Sarawak  | 18          | 14          | 12          | 9           | 5           | 18          | 21          | 36 |
| <i>Salmonella</i> I 4,[5],12:i:- |      |         |                    |          |             |             |             |             |             |             |             |    |
| STM2                             | 2023 | Chicken | Cloacal swab       | Melaka   | 18          | 14          | 12          | 9           | 5           | 18          | 21          | 36 |

regional variations in ST prevalence have emerged, with ST36 becoming dominant in specific regions or sources (Bawn et al., 2020). Lim et al. (2024) reported two predominant *Salmonella* Typhimurium STs, ST19 and ST36, in approximately equal proportions in Singapore. According to the study, ST36 was primarily associated with human intestinal and chicken isolates, exhibiting higher resistance to multiple antibiotic classes. In contrast, ST19 was linked to human extra-intestinal and non-chicken food isolates. The distribution of *Salmonella* Typhimurium STs can vary significantly across different regions due to factors such as host reservoirs, antimicrobial usage, and trade patterns. Its presence in some animals, especially poultry, is particularly concerning due to the potential for transmission through the food chain (Plumb et al., 2023).

The monophasic variant *Salmonella* I 4,[5],12:i:- is closely related to the biphasic strain *Salmonella* Typhimurium, sharing a common lineage but differing in phenotypic

characteristics due to genetic variations (Ingle et al., 2021). Biphasic strains like ST19 express both flagellar antigens *FliC* and *FliB*, whereas monophasic strains like *Salmonella* I 4,[5],12:i:- only express *FliC* (Ingle et al., 2021). The emergence of the monophasic variant as a potential superbug suggests a shift in *Salmonella* epidemiology, raising concerns about its spread and impact on public and animal health (Lund et al., 2022). Besides AMR, genetic adaptations promoting intracellular survival may contribute to the pathogenicity of *Salmonella* I 4,[5],12:i:-. The loss of flagellin expression is a key activator of host innate immunity, further exemplifying evolutionary adaptations in this serovar (Ingle et al., 2021). Isolates of this variant may have developed mechanisms to evade the host immune system and maintain viability in host cells. They demonstrate enhanced replication in human cells, potentially allowing them to evade host immune responses (Bawn et al., 2020; Ingle et al., 2021). ST36 isolates showed higher resistance to multiple antibiotic classes, including penicillins, phenicols, folate pathway inhibitors,

aminoglycosides,  $\beta$ -lactam, tetracyclines, and fluoroquinolones, compared to ST19 (Lim et al., 2024). The presence of distinct plasmid profiles among ST36 isolates, such as the Incompatibility Group X4 Plasmid (IncX4) and Colicin 156 Plasmid (Col156) plasmids, indicates significant horizontal AMR gene transfer potential. Resistance genes to third-generation cephalosporins (*bla*<sub>CTX-M-55</sub>) and colistin (*mcr-1*) have also been identified in these ST36. The higher resistance of ST36 isolates is a concerning finding, which highlights the potential challenges in treating infections caused by this type. ST36 has been associated with disease outbreaks, increased invasiveness and environmental persistence in several countries, including Denmark, Singapore, and South Korea (Gymoese et al., 2017; Lim et al., 2024; Kang et al., 2025). The detection of ST36 in Malaysian livestock raises important public health concerns due to its documented association with outbreaks and persistence in the food chain. Its presence in livestock suggests a potential zoonotic transmission route, highlighting the risk of human exposure through the consumption of contaminated meat or contact with animal waste.

The zoonotic potential of *Salmonella* Typhimurium and its monophasic variant cannot be overstated. Local circulation of *Salmonella* I 4,[5],12:i:- may play a larger role in infections than importation from overseas. This fact indicates that the strain ST36 is becoming established in local environments, which could lead to more challenges in controlling its spread. *Salmonella* I 4,[5],12:i:- is a significant public health threat due to its MDR, association with livestock, and potential for causing invasive disease. The high prevalence of MDR observed in this study aligns with global trends and highlights the importance of integrated efforts to monitor and control antimicrobial use in agriculture. The emergence of MDR in *Salmonella* strains, particularly in those isolated from animals, poses a critical public health challenge. The recent outbreak linked to contaminated chocolate products highlights the importance of implementing control measures at all stages of the food chain (Larkin et al., 2022). The improper use of antibiotics in animal husbandry are a key factor contributing to the development of resistant strains (Manyi-Loh et al., 2018). Resistance to critical antibiotics, such as third-generation cephalosporins and colistin, necessitates urgent action to prevent the spread of resistant strains. Antimicrobial stewardship programs promoting judicious antibiotic use in veterinary and agricultural practices, coupled with public awareness campaigns, are critical. Supporting research on alternative treatments, such as vaccines, can help reduce antibiotic dependence (Plumb et al., 2023). Enhanced surveillance systems to monitor resistance patterns in animal-associated isolates and genomic studies to

elucidate resistance mechanisms are essential steps in combating the growing threat of antimicrobial-resistant *Salmonella* strains (Ingle et al., 2021). These resistant pathogens can compromise the effectiveness of treatment options, leading to prolonged illness, increased healthcare costs, and higher mortality rates (Lim et al., 2024). This group of NTS with non-specific hosts adaptive characteristics, making the control effort much more challenging, especially when AMR arises and leads to public health problems (Wang et al., 2006). Its presence in food-producing animals highlights the critical need for strengthened farm-to-fork surveillance and risk mitigation strategies. This finding reinforces the importance of cross-sector collaboration under the One Health framework, integrating veterinary, public health, and food safety sectors, as outlined in the Malaysian Action Plan on Antimicrobial Resistance (MyAP-AMR) 2022-2026.

Ongoing genomic surveillance is essential for monitoring the prevalence and characteristics of *Salmonella* Typhimurium lineages, including the monophasic variant *Salmonella* I 4,[5],12:i:-. This variant requires further investigation, particularly using PCR-based testing to confirm the absence of phase 1 (*fliC*) and phase 2 (*fliB*) antigens. Additionally, the functional significance of the loss of phase 2 flagellin in *Salmonella* I 4,[5],12:i:-, including the role of *fliJAB* deletions and insertion sequence elements in transcriptional regulation and virulence, could be explored. Understanding these factors is critical for developing targeted control strategies to mitigate the risks posed by resistant strains, such as the high AMR and virulence potential of ST36 of *Salmonella* Typhimurium. The emergence of colistin resistance further highlights the urgency of antimicrobial stewardship to preserve last-resort antibiotics. Adopting a One Health approach that integrates human, animal, and environmental health perspectives is crucial to controlling the spread of this serovar and safeguarding both animal and public health (Lim et al., 2024). These findings support the call for cross-sectoral AMR stewardship policies that promote prudent antimicrobial use in animal production and molecular monitoring of high-risk lineages such as ST36. One Health-based surveillance and control measures are essential to monitor and mitigate its spread along the food production continuum. Furthermore, the persistence of ST36 in the environment and food chain may be compounded by antimicrobial resistance, underscoring the importance of implementing robust antimicrobial stewardship programs. Coordinated surveillance and policy-driven actions, as indicated in the MyAP-AMR 2022-2026 document, are essential for preventing its dissemination and protecting public health.

#### 4. CONCLUSION

This preliminary study identified monophasic *Salmonella* I 4,[5],12:i:- ST36 in livestock in Malaysia, with all isolates exhibiting multidrug resistance, including complete resistance to macrolides and concerning levels of resistance to cephalosporins. As the first documentation of ST36 in animal-origin *Salmonella* Typhimurium monophasic variant in the region, these findings provide essential data for risk assessment to support AMR surveillance. Given the zoonotic potential of these strains, there is an urgent need for integrated One Health monitoring and the implementation of responsible antimicrobial use in animal production. Future studies should include genomic analysis of resistance determinants and expanded sampling to assess the broader epidemiological dynamics of this emerging variant.

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#### Data disclaimer

The data presented in this paper is the ownership of the Veterinary Research Institute (VRI) and is shared as research finding, not as evidence of an outbreak. The findings are not correlate with reports from the World Organisation for Animal Health (WOAH) and do not meet the case definition criteria established by Protokol Veterinar Malaysia (PVM). All references and resources used in this work have been properly cited, with a comprehensive reference list provided, including URLs and access dates for online sources. This report is for informational purposes only and should not be used as the basis for claims, demands, or legal actions, and the authors shall bear no responsibility for any losses arising from its use.

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