

# Molecular characterization of virulence and resistance genes in *Salmonella* strains isolated from chickens sold at the informal chicken market in Gauteng Province, South Africa

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## Funding information

Gauteng Department of Agriculture and Rural Development

## Abstract

This cross-sectional study determined the occurrence of virulence and antimicrobial resistance genes in *Salmonella* strains recovered from chicken obtained from informal markets in Gauteng province, South Africa. The study also assessed the relationship between these characteristics, the source, the type of samples, and the serotypes of *Salmonella* isolates. A total of 151 samples (cloacal swabs, chicken carcasses, and carcass drips) were randomly collected from 47 informal market outlets in six townships in Gauteng province. *Salmonella* spp. was isolated and identified based on ISO 6579:2002 methods and confirmed by polymerase chain reaction (PCR) targeting *invA* gene fragment. Conventional PCR was used to detect 12 virulence and 18 antimicrobial resistance (AMR) genes in *Salmonella* spp. The most frequently detected virulence genes were *invA* (100%), *shdA* (91%), *mgtB* (87.7%), and *sopE* (81%), but considerably low for *spvC* (2.2%), *sefC* (1.5%), and *pefC* (0.4%). The differences in detection frequency were statistically significant ( $p < 0.05$ ). Tetracycline-resistant genes *tetA* (34.7%) and *tetB* (16%) were the most frequently detected, while Beta-lactam-resistant genes *blaTEM* (0.4%), *blaCMY-2* (0.4%) and quinolones resistant gene *qnrS* (0.4%) were detected in low frequency ( $p < 0.05$ ). The locations of the outlets and the types of samples were significantly associated with detecting some virulence and AMR genes. Significant but moderately to substantial positive correlations were observed for *qnrS*, *sul2*; *shdA*, and *mgtB* genes. The *pipA* and *spiC* were, however, substantially negatively correlated. Our findings show that detecting these virulence and AMR genes in *Salmonella* isolates serves as a potential health hazard to the public, environment, and poultry farming in Gauteng, South Africa.

## KEYWORDS

chicken, informal market, *Salmonella*, South Africa, virulence and antimicrobial resistance genes

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## 1 | INTRODUCTION

*Salmonella* spp. are important zoonotic pathogens capable of causing infections and clinical salmonellosis (Ehunwa et al., 2021). Salmonellosis in animals and humans poses food safety, public health, and economic challenges, including the cost of prevention and treatment of salmonellosis, loss of production incurred, and its potential mortality in humans and animals cannot be ignored (He et al., 2023; Sanni et al., 2023). Although *Salmonella* spp. can cause asymptomatic infections, clinical salmonellosis is due to the organism's pathogenicity through the expression of the organism's virulence genes (Casaux et al., 2023; Sedrakyan et al., 2022).

Chickens serve as the main reservoirs of *Salmonella* spp. and can enter the human food chain through the farm-to-fork continuum (Al-Hadidi et al., 2022; Wang et al., 2023), following the consumption of improperly cooked contaminated chicken and chicken products. Chicken-borne salmonellosis outbreaks and sporadic cases of gastroenteritis in humans have been documented (Wessels et al., 2021).

The pathogenicity of *Salmonella* strains has been related to numerous virulence genes present in chromosomal *Salmonella* pathogenicity islands (SPIs) (Lerminiaux et al., 2020; Rodriguez-Riviera et al., 2015; Sirken, 2013). These virulence genes may be encoded in *Salmonella* pathogenicity islands and the high pathogenicity island (HPI); mobile genetic elements like virulence plasmids and bacteriophages, as well as genes encoding fimbriae, flagella, and toxins (Jiao et al., 2020; McMillan et al., 2020; Saleh et al., 2023; Wang, Wang, et al., 2020). Virulence genes encoded in SPI-1 to SPI-5, detection frequency, and their activities in the pathogenicity of *Salmonella* spp. recovered from chickens or cases of human salmonellosis have been well documented in the literature (Kumar et al., 2021; Pavon et al., 2022; Zishiri et al., 2016). The frequency and types of virulence genes in *Salmonella* isolated from poultry are variable across countries (Kumar et al., 2021; Lozano-Villegas et al., 2023; Zishiri et al., 2016).

In most developing countries, the lack of and non-compliance with regulations on access to and prescription of antibiotics by livestock farmers drive antimicrobial-resistant pathogens, including *Salmonella*, and pose public health risks (Castrooi-Vargas et al., 2020; Emes et al., 2023). *Salmonella* genomic island 1, which contains an antibiotic-resistance gene cluster identified in several *Salmonella* enterica serovars, is known to be mobilized explicitly by *IncA* and *IccC* conjugative plasmids (Cummins et al., 2020; Douard et al., 2010). The SGI1 antibiotic resistance gene cluster, a complex class 1 integron, confers the typical multidrug resistance phenotype of epidemic *S. enterica* Typhimurium DT104. Antimicrobial agents such as tetracycline, sulfamethoxazole, ampicillin, chloramphenicol-florfenicol, streptomycin, and spectinomycin resistance genes are linked to class 1 integrons contained in SGI-1. *IncHI1* and *IncHI2* plasmids have been linked to quinolone resistance-encoding genes, extended-spectrum beta-lactamase (ESBL) genes, heavy metal resistance genes, and the carriage of typical complex class 1 integron (Cain & Hall, 2012; Garcia-Fernandez et al., 2008; McMillan et al., 2020).

In South Africa, limited information exists on the characteristics with respect to virulence and antimicrobial resistance genes in the

strains of chicken-originated *Salmonella* isolates compared to those from other parts of the world (Bahramianfard et al., 2019; Kumar et al., 2021; Siddiky et al., 2022). The South African informal chicken market is a highly patronized source of chickens nationwide, regardless of the economic status of the populace. A previous study has shown that chickens and other products from informal market outlets in South Africa have a high prevalence of pathogens, largely attributable to poor sanitary practices, poor water quality, absence or inadequate wastewater, and solid waste disposal (Adigun et al., 2021). The operations at these outlets are deemed illegal and not approved to slaughter, process or retail foods destined for human consumption and, therefore, not subjected to oversight by the health personnel. Although there are reports that confirmed the occurrence of antimicrobial (AMR) and virulence genes in *Salmonella* isolates recovered from commercial poultry farms (Ramatla et al., 2020; Zishiri et al., 2016), similar information is scanty or non-existent in the informal market in the country. The non-enforcement of existing legislation on using AMR agents exacerbates the situation. This is because there are regulations that govern the use of antimicrobials in South Africa [i.e., the Medicines and Related Substances Control Act (Act 101)], which prohibits access to antimicrobials without prescription, and juxtaposes it with the Farm Feeds, Agriculture Remedies, Fertilizers and Stock Remedies Act (Act No. 36 of 1947). This Act provides for the readily available, over-the-counter purchase of inexpensive antimicrobials by farmers for animal treatment (Theobald et al., 2019; Van et al., 2020), which drives over-use by poultry farmers.

The current study investigated the carriage of virulence and AMR genes in *Salmonella* spp. isolated from chicken carcasses slaughtered, processed, and retailed in the informal market in Gauteng province. The study also assessed the effects of the township, sample type and sources, and the serovars of the *Salmonella* isolates on the carriage of virulence and AMR genes. Finally, any correlation or association between the virulence and AMR genes detected was investigated.

## 2 | MATERIALS AND METHODS

### 2.1 | Informal chicken market

In South Africa, the outlets of the informal chicken market are located across the country but are preferably concentrated in the townships. These outlets are popularly patronized because they are conveniently located and provide a cost-effective source of protein to the population (Adigun et al., 2021). These outlets receive live chickens for slaughter through contractors from commercial poultry farms, Developing Poultry Farmers Organization (DPFO) farms, and some backyard farms. Three categories of chickens (broilers, spent hens, and culled breeders) are slaughtered, processed, and sold at these outlets. The details of the operations at the outlets of the informal chicken market in Gauteng province, South Africa, have been reported by others (Adigun et al., 2021).

The informal chicken market outlets are unauthorized and unregulated, with no oversight. Therefore, unlike commercial poultry processing plants or abattoirs where routine quality control and inspections are conducted by health personnel, such activities do not take place at the informal chicken market, thus raising health concerns among consumers. There are reports that the bacterial contamination of chickens from the informal chicken market in the country is associated with unacceptable sanitary practices (Adigun et al., 2021; Oguttu et al., 2014).

## 2.2 | Study design

The cross-sectional study was designed to be conducted in informal chicken outlets in Gauteng province, South Africa. The Veterinary Public Health section of the Gauteng Department of Agriculture and Rural Development (GDARD) provided the list of operational outlets and locations. Additionally, colleagues from the University of Pretoria community who knew the locations of the informal market outlets provided us with the information. Overall, 61 outlets were identified in the process. Pre-study assessment visits were made to these outlets to obtain general information on the size of the outlet, types of chicken slaughtered, daily throughput, operational practices, number of workers, and GPS locations as earlier described (Adigun et al., 2021), and the willingness of the outlet owners to participate in the proposed study. During these outlet visits, owners were apprised of the research project and its objectives. They were also assured that the project was being conducted by students and researchers from the University of Pretoria and that their locations, activities, and study results would be treated with the strictest confidentiality. A pretested questionnaire was then administered to each outlet owner. A total of 61 outlet owners consented to participate in the study and the outlets were initially categorized based on the following criteria based on the questionnaire-generated data, and the number of samples to be collected from each category was determined based on proportionally representative samples by size of outlet, daily throughput, operational practices, and geographical location to achieve the sample size for study. The four categories used were as follows: large operations (over five drums or buckets) for rinsing carcasses and over five persons involved in the operation and collecting five whole chicken samples per outlet; medium-sized operations with 2–5 drums or buckets of water for rinsing carcasses and 2–5 persons involved in the operation (collected three samples per outlet); Small operations with one bucket of water for rinsing carcasses and only one person running the operation (two samples collected per outlet); and home operations with mechanical device for de-feathering and 2–5 persons involved in the operation (collected three samples per outlet) (Adigun et al., 2021). Based on the aforementioned criteria, 47 outlets were selected. A minimum sample size of 150 was determined using a formula by Thrusfield (2007) with a precision value of 8% and a prevalence of 50%. A total of 151 samples were collected from the 47 outlets based on the outlet-specific information obtained during the initial visits to the outlets. Following the aforementioned number

of samples to be collected from each type of outlet, simple random sampling was used to collect the chicken carcasses into sterile bags from the outlets. The retail outlet owners who are small business entrepreneurs did not exhibit any evidence of bias or apprehension during sample collection. The locations of the informal chicken outlets from where the 151 samples originated are shown in Supplementary data, Figure S1.

## 2.3 | Origin of *Salmonella* isolates

The isolates used in this study originated from an earlier investigation where the details of the phenotypic and molecular procedures used to isolate and confirm *Salmonella* recovered from the informal chicken market outlets were described (Mokgophi, Gcebe, Fasina, Jambwa, & Adesiyun, 2021).

## 2.4 | Molecular identification and characterization of *Salmonella* isolates

### 2.4.1 | DNA extraction

DNA was extracted using the heating method (Mokgophi, Gcebe, Fasina, Jambwa, & Adesiyun, 2021), where individual colonies of *Salmonella* isolates were suspended in 2 mL microcentrifuge tubes containing 200  $\mu$ L of sterile distilled water. The cell suspensions were heated at 95°C in a heating block (Labnet International Inc., New Jersey, USA) for 10 min and allowed to cool at room temperature (22  $\pm$  2°C) for 5 min. The cell suspension was then centrifuged at 15,493 $\times$ g for 5 min. The supernatant was transferred into a clean tube and stored at –20°C for virulence gene and resistance gene screening by polymerase chain reaction (PCR). The cell precipitate was discarded.

### 2.4.2 | Confirmation of *Salmonella* isolates using PCR

The *invA* primer set was used separately as part of the confirmatory process for all biochemically identified *Salmonella* isolates (Mokgophi, Gcebe, Fasina, Jambwa, & Adesiyun, 2021). PCR conditions were as described by Mokgophi, Gcebe, Fasina, Jambwa, and Adesiyun (2021).

### 2.4.3 | Serovars of *Salmonella* isolates used

The serovars of the isolates of *Salmonella* used in the current study were determined in an earlier study (Mokgophi, Gcebe, Fasina, Jambwa, & Adesiyun, 2021). Briefly, both the traditional slide agglutination and conventional PCR assays were used to determine the serovars of the isolates. Initially, the PCR assay described by Kim et al. (2006), which consisted of two five-plex and two monoplex reactions,

was used to determine the serovars of the isolates. Overall, of the 268 isolates of *Salmonella*, 157 were typable and the frequency distribution of the serovars was as follows: Bovismorbificans, 83 (52.9%), Enteritidis, 20 (12.7%), Hadar, 18 (11.5%), Dublin, 13 (8.3%), Mbandaka, 11 (7%), Saintpaul, 6 (3.8%), Thompson, 3 (1.9%), Infantis, 2 (1.3%), and Agona, 1 (0.6%).

## 2.4.4 | Detection of virulence and antimicrobial resistance genes in *Salmonella* isolates

### Detection of virulence genes by PCR

We assayed virulence genes from SPI-1 (*invA* and *sopE*), SPI-2 (*spIC*), SPI-3 (*mgtB*), SPI-4 (*spi4D*), SPI-5 (*pipA*), SPI-6 (*pagN*), SEF14 (*sefC*), plasmid (*spvC* and *pefC*), flagellin (*fliC*), and CS54 (*shdA*) island. Commercially synthesized primer sets (Supplementary data, Table S2) were used in the following combination of three sets of multiplex PCR: set 1 (*pefC*, *mgtB*, *shdA*, *spi4D*, and *pagN*), set 2 (*spIC*, *pipA* and *sefC*), and set 3 (*sopE*, *spvC*, and *fliC*) (Inqaba Biotec Laboratory, South Africa). The virulence genes assessed in this study were obtained from information in published articles, as cited in Supplementary data, Table S1. PCR for each set was conducted in a final volume of 50  $\mu$ L reaction containing 0.75  $\mu$ L of Supertherm Taq DNA polymerase (Separations Scientific, South Africa), 4.75  $\mu$ L of 10 $\times$  reaction buffer, 3  $\mu$ L of MgCl<sub>2</sub> (25 mM), 1  $\mu$ L of dNTPs (10 mM), 0.5  $\mu$ L of each 20 mM primer and 25.5  $\mu$ L of PCR-grade water for set 1 and 27.5  $\mu$ L for set 2 and 3. A 10  $\mu$ L volume template DNA was added. *S. Typhimurium* ATCC 25923 and *S. Enteritidis* ATCC 13076 were used as the positive control, while *Escherichia coli* ATCC 25922 and PCR water were used as the negative control. PCR amplification was performed in a thermal cycler (Bio-Rad, Germany) under the following conditions: initial denaturation at 94°C for 1 min, followed by 44 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min; then final extension at 72°C for 10 min. A 15  $\mu$ L of PCR products were mixed with 5  $\mu$ L of the loading dye, loaded onto a 1.5% TAE (Bio-Rad, Germany) ethidium bromide-stained agarose gel, and electrophoresed for 1 h at 120 volts. Gels were visualized with a UV transilluminator in a gel documentation system (SynGene, Europe).

### Detection of antimicrobial resistance (AMR) genes by PCR

The antimicrobial resistance genes assayed are encoded by tetracycline (*tetA* and *tetB*), trimethoprim (*dfrrI*, *dfrrII*, and *dfrrIII*), sulfonamides (*sul1*, *sul2*, and *sul3*), phenicols (*cat1*, *flo*, and *cmlA*),  $\beta$ -lactamase (*bla-TEM*, *blacmy-2*, *blaSHV* and *blapSE*) and quinolones (*qnrA*, *qnrB*, and *qnrS*). Conventional PCR assays were performed to screen for the presence of 18 antimicrobial (AMR) genes (Supplementary data, Table S2). The mPCR to detect resistance genes was performed in six sets consisting of 3–4 genes/primer sets, as follows: set 1 (*tetA* and *tetB*), set 2 (*dfrrI*, *dfrrII*, and *dfrrIII*), set 3 (*sul1*, *sul2*, and *sul3*), set 4 (*cat1*, *flo*, and *cmlA*), set 5 (*blaTEM*, *blacmy-2*, *blaSHV* and *blapSE*), and set 6 (*qnrA*, *qnrB*, and *qnrS*). The selection of target AMR genes was based on previous studies. The primers (Inqaba Biotec Laboratory, South Africa) were optimized as shown in Supplementary data,

Table S2. Briefly, each PCR assay was done in a total volume of 25  $\mu$ L which contained 12.5  $\mu$ L 2 $\times$  Taq master mix, 4.5  $\mu$ L nuclease-free water (except  $\beta$ -lactamase: 3.5  $\mu$ L and tetracycline 5.5  $\mu$ L), 5  $\mu$ L template DNA and 0.5  $\mu$ L of each primer. *S. Typhimurium*, *S. Anatum*, and *S. Heidelberg* were used as positive controls while *E. coli* A0157 and water were used as the negative control. The PCR cycling conditions consisted of an initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 30 sec, various annealing temperatures for the respective genes, and extension at 72°C for 1 min followed by a final extension at 72°C for 10 min. The PCR products were electrophoresed on a 3% agarose gel, and results were captured using a gel documentation system (Vacutec, Johannesburg, South Africa).

## 2.5 | Correlation of the virulence and AMR genes in *Salmonella* isolates

Relevant *Salmonella* spp. data were extracted from Microsoft Excel spreadsheet v 2016 (Microsoft Corporation, Redmond, Washington, United States) and coded as follows: (Negative = 0; Positive = 1) for the AMR genes ( $n = 7$ ) or virulence genes ( $n = 12$ ). Pairwise correlation analysis was conducted to determine the correlated association of the evaluated AMR genes and virulence genes to determine whether the presence of a gene served as a predictor for the presence of another gene in the study (Supplementary data, Table S3).

## 2.6 | Data analysis

Data were analyzed using the Statistical Package for Social Sciences (version 23, IBM Corp., Somers, NY). Chi-square analysis was conducted to determine whether there were statistically significant differences in the frequency of virulence and antimicrobial resistance genes among *Salmonella* isolates recovered by townships, types of samples, and serotypes of *Salmonella*. The level of significance was determined at an alpha level of 0.05.

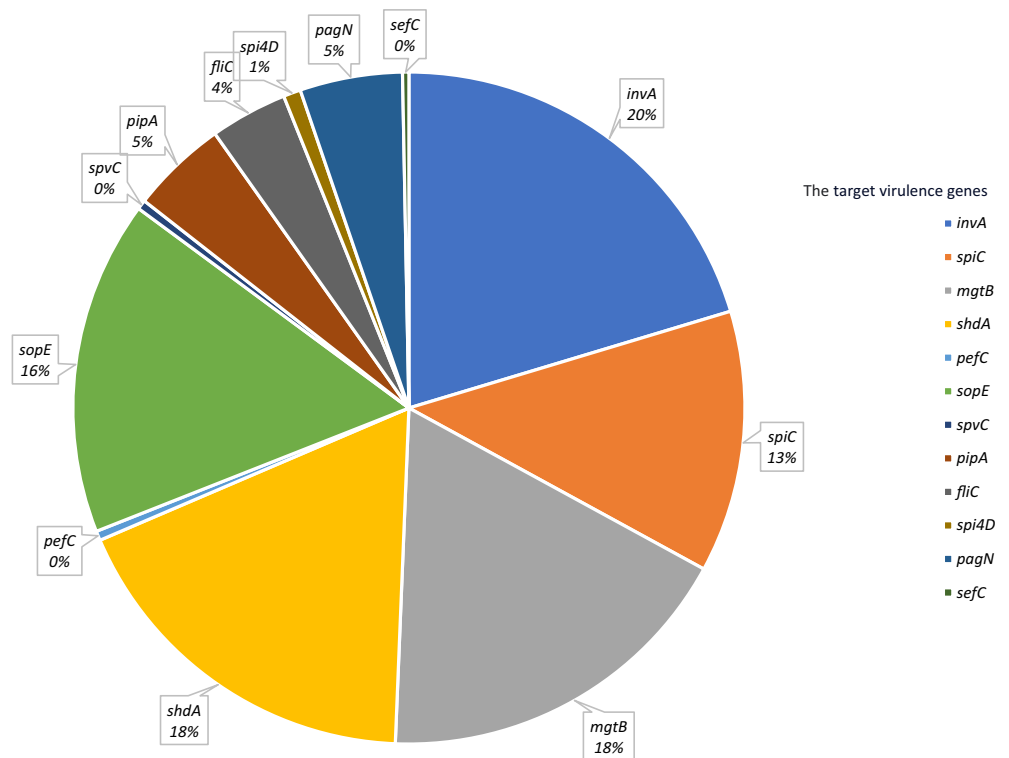
For the pairwise correlation analysis, significant  $p$  values were set at 0.05 using Bonferroni-adjusted significance level in Stata 15.0 (StataCorp LLC, College Station, Texas, United States).

## 3 | RESULTS

Overall, for the 268 isolates of *Salmonella* from chicken carcasses assayed for 12 virulence genes, the proportion of detection is shown in Figure 1. Significantly, the virulence gene *invA* was found in all the isolates of *Salmonella*, and all 12 virulence genes were found in one or more of the genes assayed.

Across the six townships, the frequencies of virulence genes were high for *invA*, 100% (268/268), *shdA*, 88.4% (237/268), *mgtB*, 87.3% (234/268), and *sopE*, 79.1% (212/268) (Table 1). However, virulence genes *pefC*, *spvC*, and *sefC* were detected at statistically significant ( $p < 0.05$ ) lower frequency of 2.2% (6/268), 1.9% (5/268), and 1.9%

**FIGURE 1** Proportion of virulence genes in *Salmonella* strains isolated from chicken carcasses sold at the informal chicken market in Gauteng Province, South Africa ( $n = 1317$ ).



(5/268). Within each of the 12 virulence genes, the frequency varied significantly ( $p < 0.05$ ) according to the townships, except for *pefC* ( $p = 0.740$ ).

For the 12 virulence genes, using the sample type origin of the *Salmonella* isolates, the frequency of only *shdA* ( $p = 0.022$ ) and *fliC* ( $p = 0.003$ ) were statistically significantly affected, an indication of the sample type had little effect on the frequency of the virulence genes detected.

All the 157 serotyped isolates of *Salmonella* distributed across nine serovars possessed one or more virulence genes (Table 2). Except for the *invA* gene, which was used for the confirmation of *Salmonella* isolates, the frequency of virulence genes was high for *spiC* (91.7%), *shdA* (87.8%), *mgtB* (83.4%) and *sopE* (77.7%) but low to *pefC* (0.6%) and *sefC* (2.5%) and the differences were statistically significant ( $p < 0.05$ ). Within the 11 serovars, the frequency of virulence gene-positive serovars was high for *S. Bovismorbificans*, 90.9% (10/11), *S. Enteritidis*, 90.9% (10/11), *S. Dublin*, 81.8% (9/11) and *S. Hadar*, 72.7% (8/11), but comparatively low for *S. Infantis*, 45.5% (5/11) and *S. Agona*, 36.4% (4/11) ( $p = 0.029$ ). The occurrence of individual virulence genes was variable, with the highest gene frequency being: *shdA* (92.8%) and *spiC* (96.4%) for serovar *Bovismorbificans*; *mgtB* (75%) and *spiC* (85%) for serovar *Enteritidis*; *sopE* (92.3%) and *spiC* (92.3%) for serovar *Dublin*; *mgtB* (88.9%) and *shdA* (100%) for serotype *Hadar*; *shdA* (100%) and *sopE* (100%) for serovar *Mbandaka*; *spiC* (100%) and *shdA* (83.3%) for serovar *Saintpaul*; *spiC* (100%) and *shdA* (100%) for serovar *Thompson*; *spiC* (100%), *mgtB* (100%), and *sopE* (100%) for serotype *Infantis*; and *spiC* (100%), *mgtB* (100%), *shdA* (100%) and *sopE* (100%) for serovar *Agona*. The differences in the distribution of the virulence genes by the serovars of *Salmonella* were statistically

significant ( $p < 0.05$ ). Across the nine serovars, the frequency of the virulence genes was high and varied considerably, but the differences were only statistically significant for *pagN* ( $p = 0.0185$ ).

The patterns of virulence genes detected in *Salmonella* isolates from carcass swabs, cloacal swabs, and carcass drips are shown in Table 3. Across the three types of samples from where the isolates originated, 22 main patterns were detected, comprising 3–7 virulence genes. For *Salmonella* isolates from carcass swabs ( $n = 78$ ), a total of 33 virulence gene patterns were detected, and the predominant patterns were *invA-spiC-mgtB-shdA-sopA* (17.9%) and *invA-mgtB-shdA-sopE-pipA* (16.3%), each pattern with 5 virulence genes. Of the 71 isolates of *Salmonella* spp. from cloacal swabs, 28 virulence gene patterns were detected, with the predominant patterns being *invA-spiC-mgtB-shdA-sopE-pagN* (15.5%; 6 genes), *invA-spiC-mgtB-shdA-sopE* (14.1%; 5 genes) and *invA-spiC-mgtB-shdA-sopE-fliC* (14.1%; 6 genes). Among the 119 isolates of *Salmonella* spp. from carcass drips, 28 virulence gene patterns were detected, of which the most frequent virulence pattern was *invA-spiC-mgtB-shdA-sopE* (31.1%; 5 genes). The frequency of the predominant virulence patterns varied significantly ( $p = 0.041$ ) by sample type, being 17.9% (14/78), 15.5% (11/71), and 31.1% (37/119) for carcass swabs, cloacal swabs, and carcass drips, respectively. Across the three sample types, among the 22 main patterns, statistically significant differences in the frequencies of virulence patterns were detected for *invA-spiC-mgtB-shdA-sopE* [5] ( $p = 0.020$ ), *invA-spiC-mgtB-shdA-sopE-pagN* [6] ( $p = 0.047$ ), *invA-spiC-mgtB-shdA-sopE-fliC* [6] ( $p = 0.027$ ), and *invA-spiC-mgtB-shdA* [4] ( $p = 0.001$ ). Finally, for the virulence patterns exhibited by one *Salmonella* isolate each, the frequency was variable, but the differences were not statistically significant ( $p = 0.059$ ) according to the sample type at 20.5%

**TABLE 1** Frequency of detection of selected virulence-associated genes in *Salmonella* isolates by township and sample type using PCR.

Township	No. of isolates		No. (%) of isolates positive for virulence gene												
	Tested	No. (%) positive <sup>a</sup>	invA	spIC	mgdB	shdA	pefC	sopE	spvC	pipA	fljC	spi4d	pagN	sefC	
Atteridgeville	8	8 (100.0)	8 (100.0)	8 (100.0)	7 (87.5)	7 (87.5)	0 (0.0)	6 (75.0)	0 (0.0)	1 (12.5)	0 (0.0)	1 (12.5)	2 (25.0)	0 (0.0)	
Ga-Rankuwa	5	5 (100.0)	5 (100.0)	3 (60.0)	3 (60.0)	4 (80.0)	0 (0.0)	2 (40.0)	0 (0.0)	2 (40.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Tembisa	1	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Alexandra	13	13 (100.0)	13 (100.0)	11 (84.6)	11 (84.6)	9 (69.2)	0 (0.0)	10 (77.8)	1 (7.7)	1 (7.7)	3 (23.1)	0 (0.0)	2 (15.4)	1 (7.7)	
Germiston	31	31 (100.0)	14 (45.2)	23 (74.2)	23 (74.2)	0 (0.0)	22 (71.8)	1 (3.2)	1 (3.2)	14 (45.2)	0 (0.0)	3 (9.7)	3 (9.7)	1 (3.2)	
Soweto	210	210 (100.0)	127 (60.5)	190 (90.5)	193 (91.9)	1 (0.5)	171 (81.4)	3 (1.4)	3 (1.4)	42 (20.0)	44 (21.0)	7 (3.3)	59 (28.1)	2 (1.8)	
p-value	1.000		1.000	0.001	0.0001	0.0001	0.740	0.0001	0.005	0.0001	0.0001	0.0005	0.0001	0.0005	
Total	268	268 (100.0)	166 (61.9)	234 (87.3)	237 (88.4)	1 (0.4)	212 (79.1)	5 (1.9)	5 (1.9)	61 (22.8)	49 (18.3)	11 (4.1)	66 (24.6)	4 (1.5)	
Type of sample	No. of isolates		No. (%) of isolates positive for virulence gene												
	Tested	No. (%) positive <sup>a</sup>	invA	spIC	mgdB	shdA	pefC	sopE	spvC	pipA	fljC	spi4d	pagN	sefC	
Carcass swabs	78	78 (100.0)	78 (100.0)	48 (61.5)	70 (89.7)	73 (93.6)	0 (0.0)	56 (71.8)	2 (2.6)	21 (26.9)	14 (17.9)	5 (3.2)	17 (21.8)	1 (1.3)	
Cloacal swabs	71	71 (100.0)	71 (100.0)	39 (54.9)	56 (78.9)	53 (74.6)	1 (1.4)	55 (46.2)	1 (1.4)	12 (16.9)	21 (29.6)	3 (4.2)	20 (28.2)	1 (1.4)	
Carcass drip	119	119 (100.0)	119 (100.0)	79 (66.4)	108 (77.3)	111 (90.8)	0 (0.0)	101 (84.9)	2 (1.7)	28 (23.5)	14 (11.8)	3 (5.9)	29 (24.4)	2 (1.7)	
p-value	1.00		1.000	0.577	0.533	0.002	0.264	0.056	0.880	0.482	0.003	0.412	0.417	0.880	
Total	268	268 (100.0)	166 (61.9)	234 (87.3)	237 (88.4)	1 (0.4)	212 (79.1)	5 (1.9)	5 (1.9)	61 (22.8)	49 (18.3)	11 (4.1)	66 (24.6)	4 (1.5)	

<sup>a</sup>Positive for one or more virulence genes.

TABLE 2 Frequency of virulence genes in *Salmonella* isolates by serotype.

Serotype	No. (%) of isolates positive for		No. (%) of isolates positive for												
	Tested	Isolates positive <sup>a</sup>	InvA	ShdA	SopE	MgtB	SpIC	pagN	PipA	FliC	spi4d	SpvC	SefC	PefC	
Bovismorbificans	83	83 (100.0)	83 (100.0)	77 (92.8)	70 (84.3)	71 (85.5)	80 (96.4)	22 (26.5)	30 (36.1)	24 (28.9)	4 (4.8)	2 (2.4)	1 (1.2)	0 (0.0)	
Enteritidis	20	20 (100.0)	20 (100.0)	13 (65.0)	10 (50.0)	15 (75.0)	17 (85.0)	9 (45.0)	8 (40.0)	12 (60.0)	4 (20.0)	2 (10.0)	1 (5.0)	0 (0.0)	
Dublin	13	13 (100.0)	13 (100.0)	9 (69.2)	12 (92.3)	10 (76.9)	12 (92.3)	6 (46.2)	11 (84.6)	2 (15.4)	2 (15.4)	0 (0.0)	1 (7.7)	0 (0.0)	
Hadar	18	18 (100.0)	18 (100.0)	18 (100.0)	10 (55.6)	16 (88.9)	14 (77.8)	3 (16.7)	5 (27.8)	6 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)	
Mbandaka	11	11 (100.0)	11 (100.0)	11 (100.0)	11 (100.0)	10 (90.9)	9 (81.8)	8 (72.7)	0 (0.0)	4 (36.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Saintpaul	6	6 (100.0)	6 (100.0)	5 (83.3)	4 (66.7)	4 (66.7)	6 (100.0)	0 (0.0)	4 (66.7)	0 (0.0)	0 (0.0)	2 (33.3)	0 (0.0)	0 (0.0)	
Thompson	3	3 (100.0)	3 (100.0)	3 (100.0)	2 (66.7)	2 (66.7)	3 (100.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Infantis	2	2 (100.0)	2 (100.0)	1 (50.0)	2 (100.0)	2 (100.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	
Agona	1	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

<sup>a</sup>Positive for one or more virulence genes.

**TABLE 3** Virulence gene patterns exhibited by *Salmonella* isolates recovered from chickens.

Virulence gene pattern [No. of genes]	No. (%) of <i>Salmonella</i> isolates from			p-value
	Carcass swab (n = 78)	Cloacal swab (n = 71)	Carcass drip (n = 119)	
<i>invA-spiC-mgtB-shdA-sopE</i> [5]	14 (17.9)	10 (14.1)	37 (31.1)	<0.005
<i>invA-spiC-mgtB-shdA-sopE-pagN</i> [6]	5 (6.4)	11 (15.5)	8 (6.7)	<0.005
<i>invA-mgtB-shdA-sopE-pipA</i> [5]	13 (16.7)	7 (9.9)	12 (10.1)	<0.005
<i>invA-spiC-mgtB-shdA-sopE-fliC</i> [6]	4 (5.1)	10 (14.1)	6 (5.0)	<0.005
<i>invA-spiC-mgtB-shdA</i> [4]	4 (5.1)	3 (4.2)	0 (0.0)	0.80
<i>invA-mgtB-shdA-pagN</i> [4]	3 (3.8)	1 (1.4)	1 (0.8)	<0.005
<i>invA-mgtB-shdA-sopE</i> [4]	2 (2.6)	5 (7.0)	9 (7.6)	<0.005
<i>invA-mgtB-shdA-sopE-pagN</i> [5]	2 (2.6)	3 (4.2)	6 (5.0)	<0.005
<i>invA-spiC-mgtB-shdA-spi4D</i> [5]	2 (2.6)	0 (0.0)	0 (0.0)	0.17
<i>invA-spiC-mgtB-fliC</i> [4]	2 (2.6)	0 (0.0)	0 (0.0)	0.17
<i>invA-spiC-mgtB-shdA-sopE-spi4D</i> [6]	2 (2.6)	0 (0.0)	0 (0.0)	0.17
<i>invA-mgtB-shdA-pipA</i> [4]	2 (2.6)	0 (0.0)	5 (4.2)	0.59
<i>invA-mgtB-shdA</i> [3]	2 (2.6)	0 (0.0)	1 (0.8)	0.4
<i>invA-spiC-mgtB-shdA-sopE-pipA</i> [6]	0 (0.0)	0 (0.0)	2 (1.7)	0.25
<i>invA-spiC-mgtB-shdA-pagN</i> [5]	2 (2.6)	0 (0.0)	0 (0.0)	0.17
<i>invA-spiC-sopE</i> [3]	0 (0.0)	1 (1.4)	2 (1.7)	0.76
<i>invA-sopE-pipA</i> [3]	1 (1.3)	4 (5.6)	2 (1.7)	<0.005
<i>invA-spiC-mgtB-shdA-sopE-sefC</i> [6]	1 (1.3)	0 (0.0)	2 (1.7)	0.82
<i>invA-spiC-mgtB-sopE-fliC</i> [5]	0 (0.0)	2 (2.8)	0 (0.0)	0.07
<i>invA-spiC-mgtB-shdA-sopE-fliC-pagN</i> [7]	1 (1.3)	2 (2.8)	4 (3.4)	<0.005
<i>invA-mgtB-shdA-sopE-pipA-pagN</i> [6]	0 (0.0)	0 (0.0)	4 (3.4)	0.12
<i>invA-spiC-mgtB-sopE</i> [4]	0 (0.0)	0 (0.0)	2 (1.7)	0.27
Other	16 (20.8) <sup>a</sup>	16 (22.5) <sup>b</sup>	12 (10.1) <sup>c</sup>	0.041

<sup>a</sup>Other patterns (one isolate each) include the following: *invA-spiC-pipA*, *invA-spiC-shdA-fliC*, *invA-spiC*, *invA-spiC-mgtB-shdA-sopE-spvC-fliC*, *invA-spiC-mgtB-shdA-pefC-spi4D*, *invA-spiC-mgtB-shdA-fliC*, *invA-mgtB-shdA-pipA-fliC*, *invA-shdA-sopE*, *invA-spiC-mgtB-shdA-sopE-spvC*, *invA-mgtB-shdA-sopE-fliC-pagN*, *invA-spiC-shdA-sopE-spi4D*, *invA-mgtB-sopE-pipA*, *invA-mgtB-shdA-spvC*, *invA-spiC-mgtB-shdA-pefC-fliC-pagN*, *invA-mgtB-shdA-pipA*, and *invA-spiC-shdA-sopE*.

<sup>b</sup>Other pattern (one isolate each) include: *invA-spiC-fliC*, *invA-spiC-sopE-fliC*, *invA-spiC-sopE-spi4D-pagN*, *invA-mgtB-shdA-spvC-spi4D-pagN*, *invA-sopE-pipA-sefC*, *invA-spiC-mgtB-shdA-fliC*, *invA-mgtB-shdA-pipA-fliC*, *invA-sopE-fliC*, *mgtB-shdA-sopE-fliC-pagN*, *invA-mgtB-sopE*, *invA-spiC-mgtB-shdA-pefC-sopE-fliC*, *invA-spiC-sopE-fliC-spi4D-pagN*, *invA-mgtB-shdA-sopE-pipA-fliC*, *invA-spiC-mgtB-shdA-pagN*, *invA-mgtB-shdA-sopE-pipA-fliC*, and *invA-spiC-mgtB-shdA-pefC-sopE-fliC*.

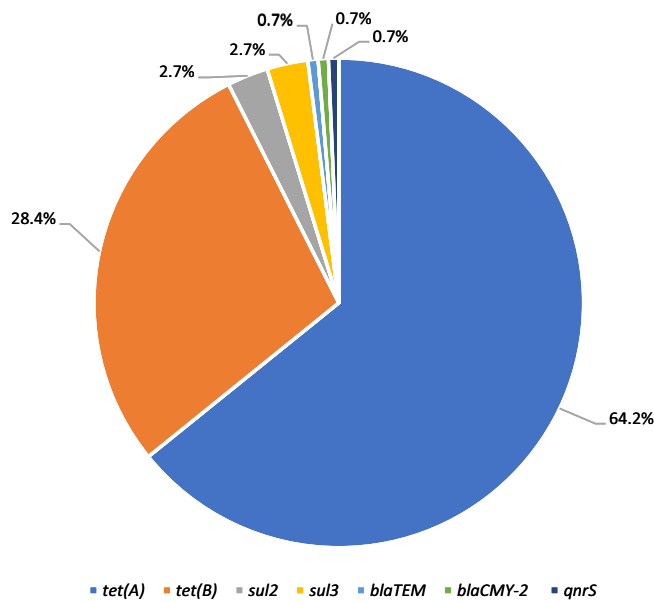
<sup>c</sup>Other pattern (one isolate each) include: *invA-spiC-shdA-sopE-pipA-fliC*, *invA-spiC*, *invA-spiC-mgtB-shdA-pipA-fliC*, *invA-sopE-fliC*, *invA-spvC-mgtB-shdA-sopE-spvC*, *invA-mgtB-shdA-sopE-fliC-pagN*, *invA-spiC-spi4D-pagN*, *invA-spiC-shdA*, *invA-spiC-spvC-pipA*, *invA-mgtB-shdA-sopE*, *invA-sopE-fliC*, and *invA-mgtB-shdA-sopE-pagN-sefC*.

(16/78), 21.1% (15/71), and 10.1% (12/119) for carcass swabs, cloacal swabs, and carcass drips, respectively.

Of the 18 AMR genes assayed, seven (38.9%) were detected in the *Salmonella* isolates recovered from the informal chicken market. Of the 268 *Salmonella* isolates evaluated for 18AMR genes, the proportion of the seven genes detected is shown in Figure 2. The proportion was high for *tet(A)*, 63% and *tet(B)*, 29% (Tetracycline), but low for *blaTEM* (<1%), *blaCMY-2* (<1%) (beta-lactam), and *qnrS* (<1%) (Quinolone). Overall, of the 268 isolates of *Salmonella* tested, 114 (42.5%) possessed one or more of the seven AMR genes detected. Among the *Salmonella* isolates tested, the frequency of detection of AMR genes was predominant for *tet(A)*, 34.7%, and *tet(B)*, 16%, which encode tetracycline resistance but low to *sul2*, 1.5%, and *sul3*, 1.5% (Sulphonamides), *blaTEM*, 0.4% and *blacmy-2*, 0.4%

(Beta-lactam), and *qnrS*, 0.4% (quinolone). The differences were statistically significantly different ( $p < 0.001$ ). For the six classes of AMR (tetracycline, trimethoprim, sulphonamides, phenols, beta-lactam, and quinolones) assayed in the current study, AMR genes were detected in four (tetracycline, sulphonamides, beta-lactam, and quinolones). AMR genes encoding tetracycline resistance, *tet(A)* and *tet(B)*, were predominantly detected as evident by their detection, either singly or together in the 114 positive for AMR genes, in 81.6% (93/114) and 37.7% (43/114) of the isolates, respectively. All the *Salmonella* isolates were negative for the following AMR genes: *Dfr1*, *Dfrx11*, *Dfrx111* (trimethoprim), *sul1* (sulphonamides), *cat1*, *flo*, *cm1A* (phenicol), *blaSHV*, *blaPSE* (beta-lactam), *qnrA* and *qnrB* (quinolone).

The frequency distribution of AMR genes according to the townships of the outlets and the type of samples is shown in Table 4.



**FIGURE 2** Proportion of AMR genes in *Salmonella* strains isolated from chicken carcasses sold at the informal chicken market in Gauteng Province, South Africa ( $n = 148$ ).

Across the six townships from where the isolates of *Salmonella* originated, the frequency of isolates of *Salmonella* positive for one or more of the AMR genes detected ranged from 0% (Tembisa) to 46.2% (Soweto) ( $p = 0.0001$ ). Overall, the detection frequency of one or more of the seven AMR genes by sample type was 42.3%, 31.8%, and 49.6% for carcass swabs, cloacal swabs, and carcass drips, respectively ( $p = 0.043$ ). Similarly, none of the three sample types had a statistically significant ( $p > 0.05$ ) effect on the occurrence of AMR genes.

Table 5 shows the frequency of AMR genes among *Salmonella* serovars. For the 157 isolates that were serotypable, of which nine serotypes were identified, 40.1% (63/157) were carriers of one or more AMR genes and a range from 0% (*S. Enteritidis* and *S. Dublin*) to 61.1% (*S. Hadar*). The differences were statistically significant ( $p < 0.05$ ). Overall, the frequency of *tet(A)* and *tet(B)* genes was 26.8% (42/157) and 9.6% (15/157), respectively, but comparatively low for *blaTEM*, 0.6% (1/157), *blacmy-2*, 0.6% (1/157) and *qnrS*, 0.6% (1/157). The differences were statistically significant ( $p < 0.001$ ). The differences in the frequency of the seven AMR genes varied significantly ( $p < 0.05$ ) across the serotypes, except for *sul3* ( $p = 0.23$ ) and *qnrS* ( $p = 0.62$ ). Among the nine serotypes of *Salmonella* tested, 6 (66.7%) had *Salmonella* isolates that possessed AMR genes, while all the

**TABLE 4** Frequency of resistance genes in *Salmonella* isolates recovered from the informal market by township and type of samples.

Variable	No. of isolates tested	No. (%) of isolates positive <sup>b</sup>	No. (%) of isolates positive <sup>a</sup> for resistance genes						
			tetA <sup>a</sup>	tetB	sul2	sul3	blaTEM	blacmy-2	qnrS
Township									
Atteridgeville	8	2 (25.0)	1 (12.5)	0 (0.0)	2 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ga-Rankuwa	5	2 (40.0)	2 (40.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tembisa	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Alexandra	13	2 (15.4)	2 (15.4)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)
Germiston	31	11 (35.5)	10 (32.3)	2 (6.5)	1 (3.3)	0 (0.0)	1 (3.3)	1 (3.3)	0 (0.0)
Soweto	210	97 (46.2)	78 (37.1)	40 (19.0)	0 (0.0)	4 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)
<i>p</i> -value		0.0001	0.0001	<0.005	<0.05	0.60	<0.01	<0.01	0.0001
Total	268	114 (42.5)	93 (34.7)	43 (16.0)	4 (1.5)	4 (1.5)	1 (0.4)	1 (0.4)	1 (0.4)
Variable	No. of isolates tested	No. (%) of isolates positive <sup>b</sup>	No. (%) of isolates positive <sup>a</sup> for resistance genes						
			tetA	tetB	sul2	sul3	blaTEM	blacmy-2	qnrS
Type of sample									
Carcass swab	78	33 (42.3)	28 (35.9)	18 (23.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cloacal swab	71	22 (31.8)	16 (22.5)	6 (8.5)	2 (2.8)	3 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)
Carcass drip	119	59 (49.6)	49 (41.2)	19 (16.0)	2 (1.7)	1 (0.8)	1 (0.8)	1 (0.8)	1 (0.8)
<i>p</i> -value		0.043	0.072	0.083	0.518	0.092	1.000	1.000	1.000
Total	268	114 (42.5)	93 (34.7)	43 (16.0)	4 (1.5)	4 (1.5)	1 (0.4)	1 (0.4)	1 (0.4)

<sup>a</sup>All the isolates were negative for *Dfr1*, *Dfrx11*, *Dfrx111*, *sul1*, *cat1*, *flo*, *cm1A*, *blaSHV*, *blaPSE*, *qnrA*, and *qnrB*.

<sup>b</sup>Positive for one or more of the 18 resistance genes assayed.

**TABLE 5** Frequency of AMR genes in *Salmonella* isolates by serotype.

Serotype <sup>a</sup>	No. of isolates	No. (%)	No. (%) of isolates positive <sup>b</sup> for						
	Tested	Positive for AMR genes <sup>c</sup>	<i>tetA</i>	<i>tetB</i>	<i>sul2</i>	<i>sul3</i>	<i>blaTEM</i>	<i>blacmy-2</i>	<i>qnrS</i>
Bovismorbificans	83	43 (51.8)	29 (34.9)	11 (13.3)	1 (1.2)	1 (1.2)	0 (0.0)	0 (0.0)	1 (1.2)
Enteritidis	20	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dublin	13	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hadar	18	11 (61.1)	6 (33.3)	3 (16.7)	1 (5.6)	1 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)
Mbandaka	11	6 (56.5)	4 (36.4)	1 (9.1)	1 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Saintpaul	6	1 (16.7)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Thompson	3	1 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Infantis	2	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Agona	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>p</i> -value		0.404	0.0001	<0.005	<0.005	0.23	0.002	0.002	0.62
Total	157	63 (40.1)	42 (26.8)	15 (9.6)	3 (1.9)	2 (1.3)	1 (0.6)	1 (0.6)	1 (0.6)

<sup>a</sup>Fifty-one *tetA* genes were detected in unknown serotype isolates; 28 *tetB* were detected in unknown serotype isolates; *Sul2* gene was detected in one unknown serotype isolate while *sul3* gene in unknown serotype isolates; was detected in two unknown serotype isolates; Each *blaTEM* and *blacmy-2* gene was detected in only one unknown serotype isolate.

<sup>b</sup>Out of the 18 resistance genes tested for in all the isolates, 11 were negative (*Dfr1*, *Dfrx11*, *Dfrx111*, *sul1*, *cat1*, *flo*, *cm1A*, *blaSHV*, *blaPSE*, *qnrA*, and *qnrB*).

<sup>c</sup>Isolates positive for one or more antimicrobial resistance genes.

Pattern [No. of AMR genes]	Sources of <i>Salmonella</i> isolates			<i>p</i> -value
	Carcass swab ( <i>n</i> = 78)	Cloacal swab ( <i>n</i> = 71)	Drip ( <i>n</i> = 119)	
<i>TetA</i> [1]	19 (24.4)	14 (19.7)	37 (31.1)	0.206
<i>TetB</i> [1]	9 (11.5)	5 (7.0)	9 (7.6)	0.538
<i>TetA-TetB</i> [2]	9 (11.5)	0 (0.0)	10 (8.4)	0.003
<i>TetA-Sul2</i> [2]	1 (1.3)	0 (0.0)	1 (0.8)	1.000
<i>TetA-Sul3</i> [2]	0 (0.0)	1 (1.4)	0 (0.0)	0.477
<i>TetB-Sul3</i> [2]	0 (0.0)	1 (1.4)	0 (0.0)	0.477
<i>TetA-Sul2-QnrS</i> [3]	0 (0.0)	0 (0.0)	1 (0.8)	1.000
<i>sul3</i> [1]	0 (0.0)	1 (1.4)	1 (0.8)	0.711
<i>blaTEM</i> [1]	0 (0.0)	0 (0.0)	1 (0.8)	1.000
<i>blacmy-2</i> [1]	0 (0.0)	0 (0.0)	1 (0.8)	1.000
Total	38 (48.7)	22 (31.0)	61 (51.3)	0.019

**TABLE 6** Patterns of AMR genes in *Salmonella* isolates from chickens in the informal market, Gauteng province, South Africa.

isolates of *S. Enteritidis* (*n* = 20), *S. Dublin* (*n* = 13), and *S. Agona* (*n* = 1) were negative. For the two predominant AMR genes, the frequency of the *tet(A)* gene was comparatively high among the isolates of *S. Mbandaka* (36.4%) and *S. Bovismorbificans* (34.9%); for *tet(B)*, 16.7% and 13.3% of *S. Hadar* and *S. Bovismorbificans*, respectively.

The frequency distribution of AMR gene patterns in *Salmonella* isolates recovered from carcass swabs, cloacal swabs, and carcass drips is shown in Table 6. Overall, 10 patterns were exhibited by the isolates, with patterns consisting of 3-AMR genes, 2-AMR genes, and 1-AMR gene detected in 1, 4, and 5 patterns, respectively. For the 78 isolates of *Salmonella* from carcass swabs, 40% (4/10) of the patterns were detected, with the predominant pattern being *tetA*, 24.4%

(19/78). Five (50%) of the 10 AMR patterns were detected among isolates from the 71 cloacal swabs, and the most frequently detected pattern was *tet(A)*, 19.7% (14/71). For the 119 isolates from carcass drips, 80% (8/10) of the patterns were found, and the most frequently found were *tet(A)*, 31.1% (37/119). A comparison of the frequency of AMR patterns according to the sample type was statistically significantly (*p* = 0.004) different for pattern *tetA-tetB*, 11.5% (9/78), 0% (0/71), and 8.4% (10/119) for carcass swabs, cloacal swabs, and carcass drips, respectively. Overall, for the 10 patterns, the frequency of isolates exhibited in the patterns was 48.7% (38/78), 31% (22/71), and 51.3% (61/119) for carcass swabs, cloacal swabs, and carcass drips, respectively. The differences were not statistically significant (*p* = 0.019). For the 268 isolates of *Salmonella* from the three sample

**TABLE 7** Pairwise correlation analysis of antimicrobial resistance genes and virulence genes from *Salmonella* spp., Gauteng Province.

	tet(A)	tet(B)	Sul2	Sul3	blaTEM 2	blaCMY-2	qnrS	invA	spIC	mgfB	shdA	pefC	sopE	spvC	pipA	fliC	spi4D	pagN	sefC	
tet(A)	1.000																			
tet(B)	0.110	1.000																		
Sul2	0.102	-0.053	1.000																	
Sul3	0.037	-0.053	-0.015	1.000																
blaTEM	-0.045	-0.026	-0.008	-0.008	1.000															
blaCMY-2	-0.045	-0.026	-0.008	-0.008	-0.004	1.000														
qnrS	0.083	-0.026	<b>0.497*</b>	-0.008	-0.004	-0.004	1.000													
invA	-	-	-	-	-	-	-	1.000												
spIC	0.031	-0.087	0.096	0.010	-0.079	0.048	0.048	-	1.000											
mgfB	-0.092	-0.051	0.047	0.041	0.023	0.023	0.023	-	0.003	1.000										
shdA	0.050	0.060	0.045	0.039	0.022	0.022	0.022	-	-0.018	<b>0.633*</b>	1.000									
pefC	0.046	0.004	-0.019	<b>0.224*</b>	-0.009	-0.009	-0.009	-	0.118	0.058	-0.261*	1.000								
sopE	-0.028	0.042	-0.090	-0.034	-0.120*	0.031	-0.120*	-	-0.015	0.056	<b>0.162*</b>	-0.235*	1.000							
spvC	-0.007	-0.066	-0.019	-0.016	-0.009	-0.009	-0.009	-	0.041	-0.018	-0.024	-0.023	-0.110	1.000						
pipA	0.061	0.010	-0.006	0.027	-0.033	-0.033	0.113	-	<b>-0.569*</b>	-0.113	-0.081	-0.083	0.013	-0.022	1.000					
fliC	-0.049	-0.099	-0.059	<b>0.133*</b>	-0.029	-0.029	-0.029	-	<b>0.230*</b>	-0.051	-0.130*	<b>0.124*</b>	0.002	0.059	-0.189*	1.000				
spi4D	0.013	0.118	-0.026	-0.022	-0.013	-0.013	-0.013	-	<b>0.123*</b>	-0.204*	-0.101	0.096	-0.127*	0.096	-0.113	-0.050	1.000			
pagN	-0.039	0.019	-0.070	-0.061	-0.035	-0.035	-0.035	-	-0.025	0.086	0.097	-0.027	-0.013	0.032	<b>-0.226*</b>	-0.044	0.058	1.000		
sefC	-0.092	-0.053	-0.015	-0.013	-0.008	-0.008	-0.008	-	-0.031	-0.045	-0.052	-0.019	0.063	-0.019	0.006	-0.059	-0.026	0.002	1.000	

Note: All bold values were significant; AMR genes (n = 7) were indicated in gray color and virulence genes (n = 12) were indicated in blue. \*Significance level of p < 0.05.

types, AMR gene pattern *tet(A)* was most frequently detected, 70 (26.1%) while *tet(A)-sul2-qnrS* was least found, 1 (0.4%) ( $p < 0.001$ ).

In the correlation analysis (Table 7), the AMR genes, the *qnrS*, and *sul2* were moderately positively correlated ( $r = 0.497$ ;  $p < 0.05$ ). Similarly, while the virulence genes *shdA* and *mgtB* were substantially positively correlated ( $r = 0.633$ ;  $p < 0.05$ ), the *pipA* and *spiC* were substantially negatively correlated ( $r = -0.569$ ;  $p < 0.05$ ). There were weak positive correlations between the following pairs of genes *pefC* and *sul3* ( $r = 0.224$ ;  $p < 0.5$ ), *fliC* and *sul3* ( $r = 0.133$ ;  $p < 0.05$ ), *fliC* and *spiC* ( $r = 0.230$ ;  $p < 0.05$ ), *spi4D* and *spiC* ( $r = 0.123$ ;  $p < 0.05$ ), *sopE* and *shdA* ( $r = 0.162$ ;  $p < 0.05$ ), and *fliC* and *pefC* ( $r = 0.124$ ;  $p < 0.05$ ). However, weak negative correlations existed between the following pairs of genes *sopE* and *blaTEM* ( $r = -0.120$ ;  $p < 0.05$ ), *sopE* and *qnrS* ( $r = -0.120$ ;  $p < 0.05$ ), *spi4D* and *mgtB* ( $r = -0.204$ ;  $p < 0.05$ ), *pefC* and *shdA* ( $r = -0.261$ ;  $p < 0.05$ ), *sopE* and *pefC* ( $r = -0.235$ ;  $p < 0.05$ ), and *fliC* and *shdA* ( $r = -0.130$ ;  $p < 0.05$ ). The same weak negative correlations were observed between the *fliC* and *pipA* ( $r = -0.189$ ;  $p < 0.05$ ); *spi4D* and *mgtB* ( $r = -0.204$ ;  $p < 0.05$ ); *spi4D* and *sopE* ( $r = -0.127$ ;  $p < 0.05$ ) and *pagN* and *pipA* ( $r = -0.226$ ;  $p < 0.05$ ). The frequency relationships of virulence genes and AMR genes are shown in Supplementary data, Table S3.

## 4 | DISCUSSION

Our study on the 268 *Salmonella* isolates recovered from chicken carcasses from informal market outlets in Gauteng province, South Africa, has explored virulence and resistance genes, including their potential to cause clinical salmonellosis and antimicrobial resistance in humans. All the isolates were carriers of at least one virulence gene using assays (SPI, plasmids, flagellin, CS54 island, and fimbriae), while 114 (42.5%) possessed one or more of the seven (38.9%) of the 18 AMR genes detected, Kumar et al. (2021), in a similar study conducted on 'pluck shops'-processed poultry in Trinidad and Tobago, reported that of the 46 *Salmonella* isolates recovered, all were positive for one or more of the detected 12 (92.3%) of the 13 virulence genes tested while only 4 (33.3%) of the 12 AMR genes tested were detected. Variable frequencies of virulence and AMR genes have been reported for *Salmonella* isolates recovered from samples from the 'wet markets' elsewhere by others (Long et al., 2022; Pavon et al., 2022; Siddiky et al., 2022). The differences in the frequencies of virulence and resistance genes across the 'wet market' outlets in several countries may reflect the sample size, prevalence of *Salmonella*, types of genes tested for, and the types and use of antimicrobial agents in the poultry industry.

It is noteworthy that all the 12 virulence genes tested for were detected in one or more isolates of the *Salmonella* recovered from chicken carcasses, albeit at different frequencies but with the predominance of *invA*, *shdA*, *mgtB*, *sopE*, and *spiC* at frequencies ranging from 61% to 100%. Three previously published comparable reports from South Africa on isolates of *Salmonella* recovered from different sources and assayed are available for analysis. Zishiri et al. (2016) investigated *Salmonella* isolates recovered from the caeca of broilers

from commercial farms where the four virulence genes (*spiC*, *pipA*, *misL*, and *orfL*) were detected. Mthembu et al. (2019) similarly tested *Salmonella* isolates from fecal, oral, environmental, water, and feed samples from livestock, including chickens, and detected all five (*invA*, *iroB*, *spiC*, *pipD*, and *int1*) of the virulence genes assayed. Finally, Ramatla et al. (2020) have detected all the seven virulence genes (*invA*, *spy*, *hilA*, *misL*, *sdfI*, *orfL*, and *spiC*) tested for in *Salmonella* isolates recovered from chicken feces from poultry farms. Our findings agree with the reports above and those from Egypt (Awad et al., 2020), China (Ren et al., 2016), and Brazil (Dantas et al., 2020). However, Kumar et al. (2021) detected 12 (92.3%) of 13 virulence genes assayed in *Salmonella* isolates recovered from 'pluck shop' outlets in Trinidad and Tobago, and Elkenany et al. (2019) found 8 (80%) of 10 virulence genes assayed in Egypt. Furthermore, the frequency of the individual virulence genes assayed was in agreement with our findings, where the frequency of the virulence genes detected varied significantly by the sources and types of samples (0.4%: *sefC*- 100%: *invA*) (Awad et al., 2020; Kumar et al., 2021; Wang, Wang, et al., 2020). It has been suggested that these variations may influence the type of *Salmonella* infection manifested, whether systemic or enteric (Ulaja, 2013).

In addition to the high frequency of virulence genes detected in our study, the high frequency of multi-virulence gene patterns (10.1%–22.5%; 3–7 genes per pattern) across sample types is equally important. It is known that each virulence gene has a specific role acting alone or in combination with other genes in the pathogenesis of salmonellosis, thus increasing the pathogenic potential of the isolates of *Salmonella* spp. (Foley et al., 2013; Imre et al., 2013).

The virulence gene *shdA*, a restricted gene to serotypes of *Salmonella enterica* subspecies I, was detected at an overall frequency of 88.4% and in 77.2% of the multi-virulence gene patterns, has been reported to possibly assist with the binding of fibronectin for intestinal persistence, or the gene may encode a thin aggregative fimbria required for prolonged fecal shedding of *Salmonella* (Kingsley et al., 2000). Kumar et al. (2021) documented the detection of *shdA* in 70.9% of *Salmonella* from chickens in Trinidad.

The *mgtB* gene, found in 87.3% of our isolates and 90.9% of the patterns, has been reported at higher frequencies by others elsewhere such as 100% in Trinidad and China (Kumar et al., 2021; Zhang et al., 2018). However, lower frequencies of the *mgtB* gene have been reported by others (Liu et al., 2012). The gene is encoded in SPI-3, and the gene product is required for magnesium transport and macrophage survival (Smith et al., 1998). It has been suggested that the *mgtB* might have contributed to the pathogenicity observed in the farms where the sampled poultry originated (Choi et al., 2017).

Both *invA* and *sopE* are encoded by SPI-1, which is involved in host invasion, and the frequency of detection of *sopE* (79.1%) in the current study is lower than the 99% found in chickens in Brazil (Borges et al., 2013) but higher than the 67.7% detected in Trinidad (Kumar et al., 2021). *SopE*, an effector for translocation, contributes to the expression of *Salmonella* invasion by stimulating membrane ruffling (Bakshi et al., 2000).

The frequency of *spiC* (61.9%) in the current study was high. A previous study from South Africa confirmed *spiC* as the most prevalent virulent gene (47%) among *Salmonella* isolates in commercial broiler chicken farms (Zishiri et al., 2016). The gene is encoded by SPI-2, which interferes with intercellular membrane trafficking to alter it, hindering the correct cellular function (Uchiya & Nikai, 2008), thus playing a role in the pathogenesis of *Salmonella* spp.

A significant association between virulence genes *shdA* and *mgtB* was observed, highlighting *Salmonella* isolates' pathogenic potentials from Gauteng chicken carcasses. The fact that the low prevalence of *spvC* (De Oliveira et al., 2003), *sefC*, and *pefC* (Shome et al., 2006) detected from *Salmonella* spp. in the current study indicates the genes could have implications for virulence and pathogenicity. Currently, the foremost challenge concerning virulence genes is determining how *Salmonella* spp. acquire virulence factors, and what are the most important genetic traits that confer virulence to *Salmonella* spp.

The frequency of virulence genes varied significantly across the townships or locations of the outlets studied except for gene *pefC*. This finding reflects the types and serovars of the infecting *Salmonella* and the practices that could have been affected by the types of farms, types of chickens, and their distribution across the townships, similar to the findings of others (Mthembu et al., 2019; Pavon et al., 2022).

In our study, the sample type was significantly associated with detecting virulence genes, except *pefC*, *spi4D*, and *sefC*, a finding contrary to the report of Mthembu et al. (2019) who demonstrated that the sample type and animal source did not significantly predict the prevalence of *Salmonella* virulence genes. The differences between both studies may reflect the type of samples being compared.

The distribution of the virulence genes among the nine serovars assessed demonstrated a distinction in the frequency of specific virulence genes in some serovars of *Salmonella* in the current study. For example, the predominance of *shdA* (92.8%) and *spiC* (96.4%) for serovar Bovismorbificans, and *mgtB* (75%) and *spiC* (85%) for serovar Enteritidis. The high frequency of detection of specific virulence genes in serovars of *Salmonella* found in the current study is well documented in the literature (Foley et al., 2013; Kumar et al., 2021). It has been reported that there was a correlation or association between serotypes and virulence genotypes in most *Salmonella* isolates, except *S. Pullorum*, which showed complicated genetic diversity (Liu et al., 2002; Wang, Wang, et al., 2020). The differences in the frequencies of virulence genes among serovars of *Salmonella* may account for their degree of involvement in clinical salmonellosis in humans and animals, as earlier reported (Lan et al., 2018).

In the current study, the predominant AMR genes detected were *tet(A)* and *tet(B)*, which encode resistance to tetracycline and accounted for 81.6% and 37.7%, respectively, of the AMR gene-positive isolates of *Salmonella* detected. This is important because, in South Africa, although there are legislations on the types and use of antimicrobial agents on livestock, there are challenges in enforcing the policy by appropriate agencies. Therefore, the livestock industry does not enforce veterinary drug use (Eagar et al., 2012; Theobald et al., 2019; Van et al., 2020). Furthermore, the existing Fertilizers, Farm, Feed and Agricultural and Stock Remedies Act (Act 36, 1947),

which makes it legal for specific antimicrobial agents (such as tetracyclines, sulfonamides, and trimethoprim) to be purchased without a prescription has further compounded the uncontrolled use and abuse of these antimicrobial agents as growth promoters and in therapy. This practice is known to lead to developing resistant pathogens and increasing antimicrobial residues in livestock tissues (Eagar et al., 2012; Van et al., 2020). It can, therefore, not be over-emphasized that to successfully control or reduce the prevalence of AMR in the country, there is a need to proactively enforce existing legislation and institute measures to educate livestock farmers on the prudent use of non-prescription AMR drugs.

Our detection of overwhelming frequencies of the two AMR genes, *tet(A)* and *tet(B)* are therefore not surprising since genetic resistance may result from chromosomal mutations or acquired genes harbored on transposons and plasmids (Deekshit et al., 2012). Therefore, the carriage of these genes may reflect the pattern of antimicrobial use in the poultry industry in the country. Interestingly, an earlier study on the same 268 *Salmonella* isolates determined phenotypically, the prevalence of resistance to tetracycline and doxycycline is 65.3% and 59.2%, respectively using the disc diffusion method (Mokgophi, Gcebe, Fasina, & Adesiyun, 2021), thus providing evidence of concordance between phenotypic and genotypic AMR profiles in the *Salmonella* isolates, as similarly documented by others (Bharat et al., 2022; Schwan et al., 2021). The high frequencies of *tet(A)* and *tet(B)* genes in our study is of public health significance since tetracycline is a commonly used antimicrobial agent in human and veterinary medicine in the country. Compared to the only earlier published report on the AMR genes in the country conducted on *Salmonella* isolates recovered from broiler chicken cecal samples from commercial farms, considerably higher frequencies were detected for *sul1* (43%), *sul2* (42%), *sul3* (17.6%), *tet(A)* (44%), and *tet(B)* (28%) (Zishiri et al., 2016). The differences in the findings between both studies may be partly due to the sources, types, and number of samples tested, as well as a reflection of the patterns of antimicrobial use on the farms from where the chickens originated.

Other AMR genes were detected at low frequencies from our study, and similar detections have been confirmed by other researchers (Table 5; Langata et al., 2019; Maka et al., 2015; Pravelquesi et al., 2021; Sabry et al., 2020).

In our study, except for AMR gene *sul3* ( $p = 0.60$ ), the township or geographical location of the informal market outlets had a statistically significant effect on the occurrence of AMR genes in our *Salmonella* isolates. These findings could be explained, in part, by the operations of the informal poultry outlets in the country. The practice by the operators of the informal market outlets receiving different types of chickens (broilers, spent hens, and culled breeders) from commercial poultry farms (large, medium, and small), small-medium-sized DPFO farms, and some backyard poultry farms. It cannot be over-emphasized that chickens reared in these variable poultry farm management systems are subjected to different antimicrobial use, with a potential to increase resistance to antimicrobial agents in pathogens such as *Salmonella* spp. and *E. coli*, among others.

In our study, for the two predominant AMR genes, the frequency of the *tet(A)* gene was comparatively high among the isolates of *S. Mbandaka* (36.4%) and *S. Bovismorbificans* (34.9%); for *tet(B)*, 16.7% and 13.3% of *S. Hadar* and *S. Bovismorbificans*, respectively. These findings may reflect the differences in the AMR genes of *Salmonella* serovars and the frequency of use or abuse of antimicrobial agents across the farms from where the chickens originated. Findings similar to ours were reported for studies conducted in Trinidad and Tobago (Kumar et al., 2021), Iran (Fardsanei et al., 2018), and the United States (Santos et al., 2007).

Our correlation analysis of the relationship among the seven AMR genes and 12 virulence genes revealed a largely non-significant correlation, thus concluding that many of them were non-predictors for other genes. However, significantly, we observed some pair associations between *qnrS* and *sul2* ( $r = 0.497$ ;  $p < 0.05$ ), *shdA* and *mgtB* ( $r = 0.633$ ;  $p < 0.05$ ), and *pipA* and *spiC* ( $r = -0.569$ ;  $p < 0.05$ ). Although *pipA* and *spiC* are responsible for *Salmonella* virulence, they were significantly poor predictors of each other in this study, having displayed significant negative correlations as similarly documented by others (Gao & Tikekar, 2023; Uchiya & Nikai, 2008). Partly like our findings is the report of a previous empirical evaluation of environmental samples of aquaculture farms where antimicrobials are used, and positive correlations were established between *sul1*, *sul2*, *sul3*, *qnrS*, and other genes (Cheng et al., 2021). Furthermore, Pavon et al. (2022) reported five virulence gene pair associations in *Salmonella* isolates from the chicken wet market and abattoirs in the Philippines, namely, *avrA* and *hilA*, *avrA* and *spi4R*, *hilA* and *spi4R*, *sseC* and *spi4R*, and *mgtC* and *pipB*. In addition, the authors reported that the animal sources predicted the presence of virulence genes, *sseC*, and *pipB*. In contrast, location type for *hilA* and *spi4R* suggested that these factors may contribute to the type and pathogenicity of *Salmonella* present.

Some limitations of the study include the fact that the strains of *Salmonella* recovered chickens sold at the informal market in Gauteng province may not fully represent *Salmonella* strains in other settings or sources in South Africa and in other countries; hence, the data generalizability should be viewed with caution. A larger sample size could provide a more comprehensive understanding of strain variability. Although 18 AMR genes were tested in our study, a broader analysis of AMR genes using whole genome sequencing (WGS) could provide a complete picture of antimicrobial resistance patterns. Furthermore, the cross-sectional design used in this study provides a snapshot of *Salmonella* strains at a specific time point; perhaps, longitudinal studies may offer insights into broader spatio-temporal trends and changes in genetic profiles over time.

## 5 | CONCLUSIONS AND RECOMMENDATIONS

In conclusion, among the isolates of *Salmonella* recovered from chicken carcasses sampled from the outlets of the informal market in Gauteng province, South Africa, the carriage of virulence genes was high for *invA*, *shdA*, *mgtB*, and *sopE*. In contrast, two AMR genes (*tetA*

and *tetB*) encoding tetracycline resistance were predominantly detected. These findings have implications for food safety, pathogenicity, virulence of *Salmonella* isolates, clinical salmonellosis in humans, and antimicrobial therapy in consumers of *Salmonella*-contaminated chicken and chicken products from informal market outlets. Finally, assessing the comprehensive data generated from this study to reflect practices on poultry farms, particularly on the AMR genes, is imperative for potential intervention, particularly regarding tetracycline use.

It is recommended that future studies consider the limitations mentioned above and be extended to cover all nine provinces in South Africa, possibly using WGS to determine the whole spectrum of the virulence and AMR genes in the *Salmonella* strains and a larger sample size. From the viewpoint of resource limitation, country-wide cross-sectional study findings may be followed with a longitudinal study in selected provinces of South Africa.

## ACKNOWLEDGMENTS

We want to thank Olivia Lentsoane and Kudakwashe Jabwa for helping with molecular characterization and the ARC-OVR Bacteriology Section for providing the facilities used for our study. We also thank the Gauteng Department of Agriculture and Rural Development (GDARD), Gauteng Province, for funding this study.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in University of Pretoria, Research Data Repository at <http://repository.up.ac.za>.

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**How to cite this article:** Mokgophi, T. M., Gcebe, N., Fasina, F., & Adesiyun, A. A. (2024). Molecular characterization of virulence and resistance genes in *Salmonella* strains isolated from chickens sold at the informal chicken market in Gauteng Province, South Africa. *Journal of Food Safety*, 44(2), e13110. <https://doi.org/10.1111/jfs.13110>