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MOLECULAR DETECTION OF *INV*A VIRULENCE AND *FLO*R RESISTANCE GENES IN *SALMONELLA ENTERICA* ISOLATED FROM HAWKED RTE ROASTED/SPICED MEAT (SUYA) IN ILORIN, NIGERIA

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ABSTRACT

Salmonella remains a significant public health concern, particularly in populations where ready-to-eat products, such as roasted/spiced meat (suya), are popular. The consumption of products contaminated with bacteria strains harbouring virulence and resistance genes may result in illnesses such as gastroenteritis, typhoid fever or septicaemia that are tough to control. The *inv*A and *flor*R genes code for cell proteins needed for invasion of the host epithelial cells and antimicrobial resistance to florfenicol (FFC)/chloramphenicol (CM), an antibiotic commonly used in veterinary practice, respectively. This study aimed to detect the presence of *inv*A virulence and *flo*R antimicrobial resistance genes in the *Salmonella enterica* isolated from 102 Ready to Eat (RTE) suya samples obtained from hawkers within Ilorin, Nigeria, using PCR. The *Salmonella* strains were sub-cultured, while pure colonies were inoculated into tryptone soya broth, incubated for 24 hours at 37 °C, and transported to the Bioformatics Laboratory, Ibadan, for PCR analysis. DNA was extracted from the isolates and amplified using primers targeting specific parts of the organism's genome. The PCR products were run on agarose gel electrophoresis and visualized with the gel imagery device. All five (100%) isolates displayed the 284bp *inv*A and 926bp *flo*R genes' bands. This study confirmed the presence of invasion and antimicrobial resistance genes in all *Salmonella enterica* from RTE spiced roasted meat obtained from the study area. There is a need for continued surveillance and control measures to prevent foodborne illnesses that may be due to contamination by virulent and antimicrobial-resistant *Salmonella* in food.

Keywords: *Salmonella enterica*, suya, *inv*A, *flo*R, PCR

INTRODUCTION

Salmonellosis, caused by *Salmonella* species, is a major cause of acute bacterial gastroenteritis in humans and animals (Lamichhane *et al*., 2024). According to the World Health Organisation (WHO), *Salmonella* is one of the top four global causes of diarrhoea (Sumana *et al*., 2025). It causes typhoidal salmonellosis (TS) and non-typhoidal salmonellosis (NTS), which present as different syndromes in humans and animals. The severity of the infection varies according to the host's immune

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system and the bacterial serotype. The organism has been reported to be of serious public health importance worldwide (Akinyemi *et al.,* 2021; Nazir *et al*., 2025). *Salmonella* ranks third on a list of the 14 foodborne pathogens that cause sickness, with economic impact and an annual cost of around $3.3 billion. Every year, between 200 million and 1 billion cases of *Salmonella* infections are reported globally, resulting in 93 million gastroenteritis cases and 155,000 fatalities. Of these, about 85% are linked to eating tainted food (Lamichhane *et al.*, 2024; Keddy *et al*., 2025). In 2009, 1,722 cases of foodborne illness were attributed to *Salmonella* in European nations, while foodborne diarrhoeal infections are responsible for the deaths of approximately 1.8 million people annually in developing nations. Annually, approximately 120,000 cases of food poisoning and 1 million cases of diarrhoea occur in Thailand (Akbar and Anal, 2015), posing a significant threat to public health. Salmonellosis is second to campylo-bacteriosis in Europe as the most common and widely distributed foodborne disease. Typhoid fever usually causes mortality in 5% to 30% of typhoid-infected individuals in the developing world. It was recorded that 16 to 17 million cases occur annually, resulting in about 600,000 deaths (Gashaw and Jambo, 2022).

The mortality rates vary from region to region, but can be as high as 5 to 7% despite the use of appropriate antibiotics. On the other hand, non-typhoidal *Salmonella* cases reportedly account for 1.3 billion cases, with 3 million deaths worldwide (Gashaw and Jambo, 2022).

Salmonella spp. can harbour virulence genes (Gharaibeh *et al*., 2024), which contribute to significant traits such as establishment in the host, ensuring disease initiation, as well as cellular invasion (Ramatla *et al.,* 2020). *Salmonella* pathogenicity is mediated by numerous genes, such as *inv*A, *spiC* and *pipD,* which code for effectors that induce successful host infection. Pathogenicity of *Salmonella* is expressed in three ways: host cell invasion, intracellular survival and colonisation (Brown *et al*., 2005). Several virulence genes play a crucial role in *Salmonella* pathogenesis, and they are distributed across different segments of the genome, such as the chromosome, plasmids, integrated bacteriophage DNA, *Salmonella* pathogenicity islands (SPIs), and *Salmonella* genomic islands (SGIs) (Felix *et al*., 2025). The majority of the virulence genes are gathered within SPI-1 and SPI-2. The SPI-1 encodes factors vital for cell adhesion, while SPI-2 encodes factors essential for replication and intracellular survival. The SPIs play important roles in invasion, antibiotic resistance, adhesion, systemic infection, fimbrial expression, toxin production, intracellular survival, and magnesium/iron uptake (Ramatla *et al.*, 2020).

Salmonella enterica harbours several virulence genes that encode products that assist organisms expressing their virulence in the host (He *et al*., 2025). Among the virulence genes, *inv*, *sef* and *pef* are considered for adhesion and invasion of the pathogen in the host system, and the *spv* gene enhances the establishment of a systemic disease state in the host cells (Lan *et al*., 2018). While the *stn* virulence gene codes for enterotoxin production, *sop* and *pip* genes are associated with actual expressions of host pathogenic processes (Murugkar *et al.,* 2003).

The *inv*A stands out as one of the extensively examined virulence factors and serves as a biomarker for detecting *Salmonella*. This is attributed to its possession of sequences distinctive to the *Salmonella* genus (Teklemariam *et al*., 2023). Invasion A, found in the outer membrane of *Salmonella* spp., plays a role in entering host epithelial cells within the intestines, thereby initiating infection (Moreau *et al.,* 2025). The *inv* locus in *S. enterica* serovar Typhimurium underwent characterization, revealing that *inv*A is

crucial for exhibiting virulence in the intestine (Moreau *et al.,* 2025). It influences the host cell by delivering type III secreted effectors, resulting in a mutant phenotype, and it is crucial for the invasion of epithelial cells (El-Sharkawy *et al.,* 2017). The presence of the *inv*A gene has been validated exclusively in *Salmonella* species, making it a key component in the genetic diagnosis of *Salmonella* species (Ramatla *et al.*, 2020). The use of *inv*A gene is recognized as an international standard procedure for the detection of the genus *Salmonella* (Malorny *et al.,* 2003).

Chloramphenicol (CM) and its fluorinated derivative, florfenicol (FFC), belong to the class of antibiotics, phenicols (Michael *et al*., 2006). The broad-spectrum antibiotic, CM, has been banned in food-producing animals because of its potentially fatal side effects, which include carcinogenicity and toxicity to sensitive individuals (Alimutairi *et al*., 2025). Florfenicol was derived from CM and has been approved for use in animal production in many countries. Florfenicol is used only for the treatment of animal diseases and has played a crucial role in the control of *Salmonella* infections in poultry, cattle, fish and other animals because of its effectiveness against CM-resistant isolates (Cannon *et al*., 1990; Xiao *et al*., 2025). Florfenicol was first approved by the US Food and Drug Administration in 1996 for treating bovine respiratory infections (Cloeckaert *et al*., 2000). The misuse of FFC in recent years has led to a significant increase in resistance to *Salmonella*, substantially posing challenges to the prevention and treatment of *Salmonella* infections (Mei *et al*., 2021).

The genes reported to mediate the resistance of *Salmonella* to phenicols include *flo*, *flo*R, *fex*A, *cfr* and *pp-flo*. Most FFC-resistant strains have been reported to exhibit multidrug resistance (MDR) to ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline (Xiao *et al*., 2025). The gene *flo*R is a plasmid or chromosome-encoded CM exporter found in many bacterial species. It mediates *Salmonella* resistance to FFC and facilitates horizontal transfer among bacteria of the same and different species/genera through plasmids (Lu *et al*., 2018). Over 90% of *Salmonella* strains have been reported to exhibit FFC resistance (Lu *et al*., 2012).

Suya is a ready-to-eat lean meat that is boneless, made from beef, goat, mutton or chicken, coated with spices/oil, stacked on sticks and roasted, smoked or barbecued using fire from charcoal (Inyang *et al.*, 2005). In most parts of Africa, suya is a well-sought-after, highly cherished delicacy that cuts across race, educational level, religion, age and socio-economic status (Adesoji *et al*., 2019). There had been reports of MDR bacteria, especially *Salmonella,* in suya samples in Nigeria (Adesoji *et al*., 2019; Oludairo *et al*., 2022).

This study aimed to genetically characterize five *Salmonella enterica* isolates recovered from RTE roasted meat (Suya) in Ilorin, Nigeria, for the presence of *inv*A virulence and *flo*R resistant genes.

MATERIALS AND METHODS

Five *Salmonella enterica* isolates were obtained from suya samples from hawkers within the Ilorin metropolis (Oludairo *et al*., 2022) and subcultured on *Salmonella-Shigella* agar and incubated at 37 °C for 24 hours. Pure colonies of the growth were inoculated into nutrient broths and incubated at 37 °C for 24 hours. The growths in the broth were transported in insulated containers with ice packs to the Bioformatics laboratory, Sango, Ibadan.

The DNA extraction of the *Salmonella* spp. was done using a DNA extraction kit (ZR Fungal/Bacterial DNA Miniprep), after which the virulence and resistance genes were amplified using primers targeting specific regions of the bacteria's genome (Lee *et al*., 2012). The primers used were *inv*A Forward 5’ GTG AAA TTA TCG

CCA CGT TCG GGC AA 3' and Reverse 5' TCA TCG CAC CGT CAA AGG AAC C 3' (Rahn *et al*., 1992; Yanestria *et al*., 2019) and *flo*R Forward 5' TCCTGAACACGACGCCCGCTAT 3' and Reverse 5' TCACCGCCAATGTCCC-GACGAT 3' (Lu *et al*., 2019).

Twenty-five microliters of the master mix manufactured by Fermentas TM, Germany, composed of Taq polymerase, $MgCl_2$ and dNTPs, were mixed with 2.5 µl forward primer, 2.5 µl reverse primer, 10 µl template DNA and 10 µl nuclease-free water in a PCR microtube. Initial denaturation was carried out at 95 °C for 2 minutes and 30 cycles of amplification consisting of denaturation at 94º for 30 seconds. Annealing was done at 53 °C for 30 seconds, extension at 72 °C for 60 seconds, and final extension was at 72 °C for 7 minutes (Oludairo *et al*., 2013). The PCR was performed in a DNA thermal cycler (Applied Biosystems, Gene Amp PCR system 9700).

At the end of the run, 10 µl of the final reaction product was separated by electrophoresis on ethidium bromide-stained 2% agarose gel along with a 50bp DNA ladder at 80-150 V for about 1.5 hours. The products were visualized to determine the presence or otherwise of the *inv*A virulence and *flo*R resistance genes with expected amplicons of 284bp and 926bp (Kim and Aoki, 1996) by using a Bioimaging system, ChemiGenius (Manufactured by Syngene Europe, Beacon House, Nuffield Road, Cambridge CBITF) (Oludairo *et al*., 2013).

RESULTS

The presence of *inv*A virulence gene (284bp) was recorded in all (100%) *Salmonella enterica* isolates from hawked suya (62b, 64b, 69b, 88a, 101a) used in this study. The isolates were from samples obtained from Sabo-Oke/Maraba Junction, Sabo-Oke, Tipper Garage/Unilorin PS and Zamfara hostel, Ilorin metropolis (Table 1), (Figure 1). The resistance gene, *flo*R, with an amplicon of 926bp, was also detected in the five (100%) *Salmonella enterica* from suya, on which PCR was carried out in this study (Table 1), (Figure 2).

Table 1: A summary of *Salmonella* isolates, samples, location of samples, and number positive for *inv*A and *flo*R genes.

S/N	*Salmonella* isolates	Location of samples from which isolates were obtained	*inv*A *and flo*R gene test results	Number (%) of *Salmonella enterica* positive for *inv*A	Number (%) of *Salmonella enterica* positive for *flo*R
1	62b	Sabo-oke/ Maraba Junction	++		
2	64b	Sabo-oke/ Maraba Junction	++		
3	69b	Sabo-oke Junction	++	5 (100%)	5 (100%)
4	88a	Tipper Garage / Unilorin PS	++		
5	101a	Zamfara hostel	++		

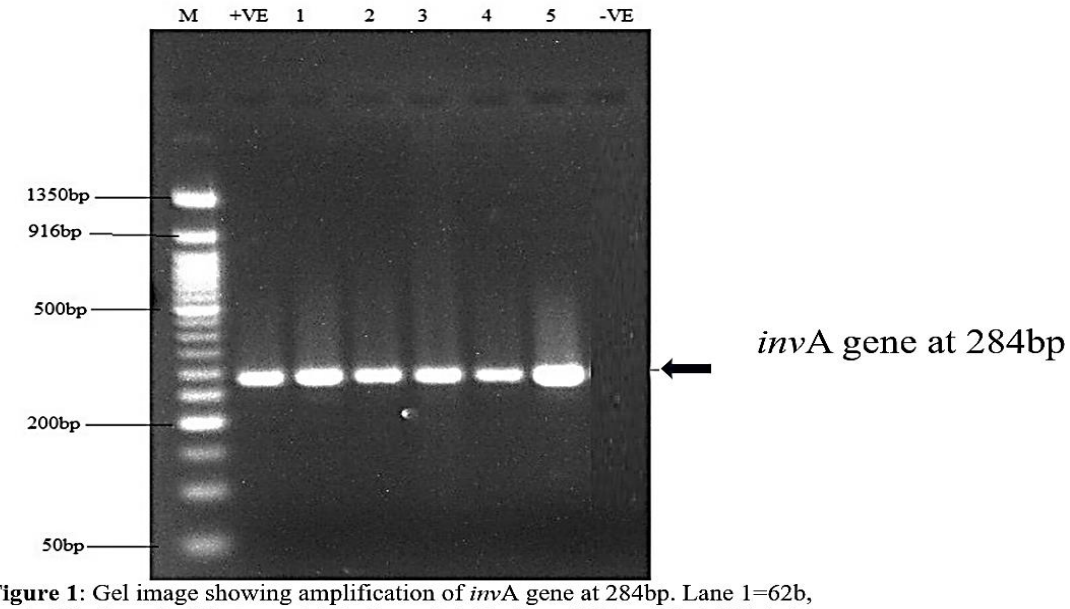

Figure 1: Gel image showing amplification of *inv*A gene at 284bp. Lane 1=62b, Lane 2=64b, Lane 3=69b, Lane 4=88a, Lane 5=101a, Lane M is a 50bp DNA ladder, Lane +ve is the positive control, and Lane -ve is the negative control.

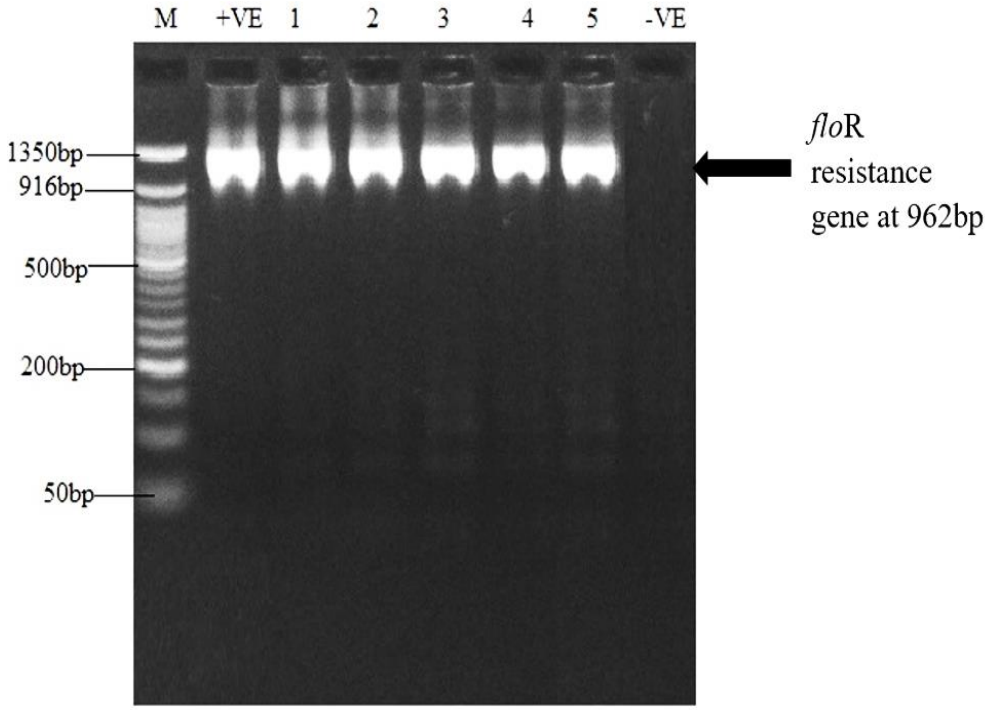

Figure 2: Gel image showing amplification of *flo*R at 962bp. Lane 1=62b; Lane 2=64b; Lane 3=69b; Lane 4=88A; Lane 5=101a; Lane M=50bp DNA ladder; Lane +VE=Positive control; Lane -VE=Negative control

DISCUSSION

The display of the 284bp *inv*A gene band in all the isolates in this study could indicate that the isolates can invade the host cell epithelial walls through the gene's coding for a protein of a type III secretion system (T3SS), which is crucial for the bacterium's ability to enter and infect host cells (Yanestria *et al*., 2019). It could also be an attestation of the sensitivity and specificity of the PCR diagnostic technique in the detection of *Salmonella,* and an assertion of the invA gene being a confirmatory test in *Salmonella* identifi-cation (Abhadionmhen *et al*., 2024).

The result of this study is in agreement with the findings of many researchers who reported the detection of the *invA* gene in all or some of the *Salmonella* spp. isolated. Abhadionmhen *et al.* (2023) detected the *invA* gene in 100% of the samples screened from food vendors. Another study in Jos, Nigeria, where *Salmonella* was isolated from vegetables, detected the *inv*A gene in 24 (96%) out of 25 pure culture isolates (Adeniyi *et al*., 2016). In a study in Lagos, Smith *et al.* (2015) detected the *invA* gene in 70 (96.1%) out of the 73 *Salmonella* isolates recorded. In a study on the molecular characterization of specific *Salmonella* species in Jos, Nigeria, Uneze *et al.* (2021) reported that seven (70%) out of 10 confirmed *Salmonella* species were positive for the *inv*A gene. Also, in another study on the isolation of *Salmonella* from animal sources in Southern Taraba, Abhadionmhen *et al.* (2023) reported that *inv*A gene was recorded in 79 (80.8%) of the 98 *Salmonella* isolates.

Salmonella isolates that did not display the *inv*A band may lack the ability to invade the host epithelial cells. If they are, however, able to express, other invasive genes like the *mis*L and *hil*A may be responsible rather than the *inv*A gene (Abhadionmhen *et al*., 2023). It has therefore been suggested that molecular detection of the *inv*A gene in *Salmonella* spp. should be a 'complementary' rather than a 'confirmatory' test. Therefore, when *invA* gene is not detected after PCR, *Salmonella* isolates that have been validated culturally and biochemically should not be regarded as non-*Salmonella* (Resendiz-Nava *et al*., 2019; Uneze *et al*., 2021).

The presence of *flo*R in all the *Salmonella enterica* screened in this study could be due to the inappropriate use of CM, FFC and other derivatives of CM in animals. The ability of the gene to be transferred to other bacteria of the same and different species/genera through plasmids could also promote its widespread presence and propagation (Kim and Aoki, 1996).

The abuse of CM, especially in animal health and production, coupled with its toxicity in sensitive individuals, led to the ban on its use in many countries (Almutairi *et al*., 2025). The prohibition on the use of CM led to the production of its broader and less toxic synthetic derivatives, FFC and thiamphenicol (Guo *et al*., 2024). The misuse of FFC and other derivatives of CM has led to an increased the resistance to phenicols. Over 90% of *Salmonella* strains have been reported to exhibit FFC resistance (Lu *et al*., 2012). These widespread reports of AMR could have been mediated by the *flo*R resistance gene.

The MDR reported in many *Salmonella* spp. may be predisposed by *flo*R, since most strains of the organism resistant to FFC have been reported to exhibit MDR to up to five commonly used antibiotics (Xiao *et al*., 2025). The weight and implication of the presence of the gene in suya in this study are further stressed by the report that AMR-resistant genes can be transmitted to humans through the consumption of meat-harbouring resistant bacteria, which also underscores the one health dimension of the situation (Conceicao *et al*., 2023).

The identification of *flo*R resistance gene in this study is in agreement with the studies of Li *et al.* (2016) and Liu et al. (2019), who reported the identification of the gene in pork meat. Xiong *et al.* (2019) also identified the resistance gene in aquatic products, while Thong and Modarressi (2011) reported its presence in retail meats and street foods.

The detection of the *inv*A virulence and *flo*R resistant genes in all *Salmonella* species isolated from suya samples in this study highlights a critical issue in ensuring the provision of wholesome food and the protection of public health in Nigeria, considering the potential multiplier effect of virulent and resistant strains of *Salmonella* and the disease they cause through the consumption of the commonly taken snack, suya.

CONCLUSION

The report of the detection of *inv*A virulence and *flo*R resistance genes in all *Salmonella* isolates from hawked suya within Ilorin, Nigeria, underscores the peculiarity of the invasion gene, which is also regarded as a reliable marker in the identification of *Salmonella,* in addition to the prevalence of CM and its derivatives' indicator resistant gene in *Salmonella* strains in the study area. This study further verified the distribution of virulence and antimicrobial resistance genes among the screened *Salmonella enterica* and the attendant risks on the consumption of food laden with *Salmonella* spp. harbouring the genes by the public, in addition to its challenge of food safety. The gene's continued presence could also have major implications on local, regional and international food trade and export.

Ongoing research into the genetic diversity and prevalence of *Salmonella* invasion and antimicrobial resistance genes should be intensified and encouraged. Government, non-governmental organizations, researchers and academic institutions should fund and support this line of research. Further research, surveillance and monitoring systems should be collaboratively conducted and implemented locally and internationally to explore the molecular characterization of *Salmonella* strains in different populations in Africa and worldwide. A centralized database of the circulating *Salmonella* virulence and antimicrobial resistance genes with accessibility to the public, health authorities and researchers should be established, maintained and updated. This will provide comprehensive data and information that can help track trends of the genes in circulation and guide policy decisions with informed knowledge.

Government agencies responsible for food safety and public health should enhance and enforce the food safety rules and regulations. Regular inspections and strict adherence to hygiene standards, like safe food handling practices can significantly reduce the occurrence of *Salmonella,* along with its virulence and anti-microbial resistance genes. Routine screening of food products and regular testing of meat and other food products for the presence of *Salmonella* should be conducted. This would help identify contaminated food items early and prevent their distribution. Public health campaigns, extension work and training among stakeholders in the food chain should focus on improved hygiene, proper cooking temperatures for food like suya, to ensure the destruction of bacteria with virulence and antimicrobial resistance genes.

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