



Molecular Characterization of *Salmonella* Species Isolated from Animal Sources in Southern-Taraba State, North-East Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author EPKI designed and supervised the research. Author AOA carried out the laboratory experiments, statistical analysis, and wrote the first draft of the manuscript while authors VIA and STCB assisted with the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The purpose of this research was to detect the *Salmonella invA* gene from chicken's droppings in Southern Taraba, North East Nigeria. The invasive "*invA*" gene facilitates the invasion of epithelial cells by *Salmonella* isolates.

Study Design: This study is experimental research involving ninety-eight (98) *Salmonella* isolates from chicken droppings in poultry farms and domestic chicken coops.

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Place and Duration of Study: This research was carried out in Wukari, Donga, Ibi and Takum towns in Southern Taraba, North East Nigeria between November 2021 and May 2023. A total of 400 (Donga,100; Wukari, 100; Ibi, 100 and Takum, 100) Chicken droppings were collected from 40 (Donga, 10; Wukari, 10; Ibi, 10 and Takum, 10) small-scale poultry farms, and 100 (Donga, 25; Wukari, 25; Ibi, 25 and Takum, 25) Chicken droppings were sampled from 20 (Donga, 5; Wukari, 5; Ibi, 5 and Takum 5) domestic chicken coops and analyzed.

Methodology: Using standard microbiological techniques, *Salmonella Shigella* Agar (SSA) was used to confirm and validate 98 (Wukari; 36, Donga; 19, Ibi; 27, and Takum; 16) *Salmonella* isolates from stock culture. Isolates were further subjected to biochemical analysis. Boiling technique was used to extract the DNA of the 98 isolates. *Salmonella invA* gene amplification process was done using Polymerase Chain Reaction (PCR). Electrophoresis was done using agarose gel, and DNA band patterns were visualized with the aid of ultraviolet light (UV) showing different patterns and interpreted with the 100 bp DNA ladder.

Results: The results of this study indicate that the *invA* gene was detected in 80.6% (Wukari; 86.1%, Donga; 68.5%, Ibi;85.2%, and Takum; 75%) of the 98 *Salmonella* isolates with an amplicon size of 284bp. This finding implies that 19 isolates which did not show the targeted band are not invasive, and even if they are, other invasive genes may be liable.

Conclusion: The detection of the invasive *invA* invasive gene from *Salmonella* isolates from the fecal droppings of apparently healthy chickens from poultry farms and domestic chicken coops is really of great concern to public health. Unless a proper hygienic protocol and biosecurity measures are strictly adhered to where applicable in food processing chain, *Salmonella* isolates harboring the *invA* traits could be opportunistically invasive among immunocompromised individuals and children.

Keywords: Chicken; *invA* gene; polymerase chain reaction PCR; poultry; *Salmonella*.

1. INTRODUCTION

The nature of *Salmonella* infection depends on the strain, serovar, infectious dose, the nature of contaminated food and most importantly the host status because, some serovar are highly pathogenic in humans than other animals; and even strains of the same serovar exhibit disparity in their pathogenicity [1]. *Salmonella* infections are more severe among children and immunocompromised individuals [2].

Salmonella pathogen may gain entrance to the digestive tract when contaminated food and water is ingested, or through direct contact with infected animals in the case of the Non Typhoidal Serovar (NTS) [3]. Irrespective of the serovars, *Salmonella* has a similar ability to invade the host cell by inducing self-entrance into cells of the intestinal epithelium using sophisticated virulent mechanism [4]. Once ingested, *Salmonella* activates the Acid Tolerance Response (ATR) in order to endure the acidic nature of the intestinal tract when it enters the small bowel [2]. Thereafter, it reaches and transverse the intestinal mucus layer and attach to the small intestinal epithelial cells, and subsequently taken up by phagocytic cells at the submucosa [3]. Engulfed *Salmonella* is internalized into large vesicles called *Salmonella*-containing vacuoles (SCV) where it can only survive and replicate [5]. The NTS travels and stops at the mesenteric

lymph nodes, while the Typhoidal Serovar (TS) which are usually invasive could defeat the host's defensive mechanism, multiply in mononuclear phagocyte system and spread to systemic sites of infections such as the spleen, blood, bone marrow and liver and causes systemic infection [1]. Unlike the TS, most NTS cannot overcome the host's defensive mechanism [6]. Hence, they remain localized to the intestine and mesenteric lymph nodes inducing inflammation which results in gastrointestinal disorder [7]. However, the NTS could be invasive among in immunocompetent patients, children and the elderly, by defeating their weak defensive system and become non-localized in the intestine, thereby spreading to other systemic sites of the body to effect infection [7,8].

Salmonella possesses several physiological abilities that enhance its virulence, thereby enabling them to inflict acute infection. One of the more important virulent factors required by *Salmonella* to accomplish pathogenesis is the type three secretion system (T3SS) encoded in the *Salmonella* pathogenicity islands (SPIs) which are bulky gene cassettes inside the bacterial chromosome that determines the specific interactions within the host [2]. So far, over 20 SPIs have been identified to enhance pathogenesis but only two (T3SS-1 and T3SS-2) have been widely studied for their respective

involvement in cell invasion of the intestinal epithelium and survive within the macrophages [9]. Other features enhancing *Salmonella* pathogenesis includes adhesions, flagella, protein adhesions and virulence plasmid (pSLT) [2,3].

Salmonella pathogenicity is intermediated by several genes and are not limited to the invasive gene (*invA*) [10]. The *invA* gene is localised in the pathogenicity island 1 (SPI1) and has been extensively investigated owing to its virulent potential. Also, many researchers have opined the *invA* gene as a biomarker for the molecular confirmation of *Salmonella* from clinical samples [11]. The invasive "*invA*" gene facilitates the coding for the protein content in the *Salmonella* internal membrane leading to the invasion of epithelial cells [12]. NTS serovars that are responsible for systemic *Salmonella* infections are known as invasive NTS (iNTS) [6,13]. This occurrence is predominantly due to the emergence of invasive clones in some NTS serotypes via conjugative plasmid transfer [6]. *Salmonella* does not significantly multiply in natural habitats, except in the digestive tract of humans and animals where they predominantly live as their natural habitats [14]. Hence, the intracellular conjugation of bacteria plays important role in the evolution of virulence and transmission of antimicrobial resistant genes in pathogens including *Salmonella* [6]. Virulence plasmids assist the invasive NTS in surviving phagocytes, thus defeating the host's defensive mechanism of immunocompetent host and spreads from the small intestine to the spleen and liver [15]. Hence this study will establish the virulence of *Salmonella* isolates from chicken source by amplification of the *invA* genes.

2. METHODS

Sample collection: A total of 400 (Donga,100; Wukari, 100; Ibi, 100 and Takum, 100) Chicken droppings were collected from 40 (Donga, 10; Wukari, 10; Ibi, 10 and Takum, 10) small-scale poultry farms, and 100 (Donga, 25; Wukari, 25; Ibi, 25 and Takum, 25) Chicken droppings were sampled from 20 (Donga, 5; Wukari, 5; Ibi, 5 and Takum 5) domestic chicken coops and analyzed. As documented in a previous prevalence study by [14], *Salmonella* was isolated from a total of 96 of the 500 samples examined and preserved as stock culture. A sterilized wire loop was used to inoculate from the stock culture into SSA incubated for overnight at 37°C to confirm the isolates. Isolates were further subjected to biochemical analysis.

Study Area: This research was conducted in Wukari, Donga, Ibi and Takum Local Government Areas located in Southern Taraba State, North-East Nigeria. Southern Taraba has a population of approximately 704,900 citizens. Southern Taraba is bordered by Gassol, Bali, Kurmi, Gashaka, and Ardo- Kola Local Government Areas to the North, Plateau and Nasarawa States to the Northwest, and to the Southeast by Benue State and the Republic of Cameroun. The population in this study are predominantly farmers and traders [14].

DNA extraction: The gene amplification process was carried out using Polymerase Chain Reaction (PCR). The genomic DNA of presumed *Salmonella* isolates was obtained using the conventional boiling method. Using a sterilized wire loop, colony from pure culture medium was inoculated into a microtube containing 300 µL basic buffer; TE (tris-ethylenedi aminetetraaceticacid). Following vortexing to mix contents, the microtube was placed in boiling water for approximately 20 minutes. Thereafter the microtube was centrifuged at 12,000 rpm for 10 minutes to separate the DNA from the contents of the microtubes. The obtained supernatant contained the DNA which was immediately transferred to a separate microtube.

DNA amplification: The *Salmonella invA* gene detection was carried out using the PCR. A final volume of 50µl of reagents used in the amplification are, sterile distilled water, 1 × GoTaq PCR reaction buffer (containing 1.5 mM MgCl₂), 5 units of GoTaq DNA polymerase (enzyme to amplify gene replication), 2.5 µl DNA template, 0.2 mM PCR nucleotide mix (reproducing a single DNA strand into two by unzipping the strand), and 0.5 µl each of forward and reverse DNA primers. Specific primers selected to target 284 bp segment of the *invA* gene of *Salmonella* are, forward (*invA*) '5GTG AAA TTA TCG CCA CGT TCG GGC AA3' and primers reverse (*invA*) '5TCA TCG CAC CGT CAA AGG AAC C3'. This is shown in Table 1 below. Final mixture of PCR reagent was placed in a thermo-cycler with an initial incubation at 94°C for 5 min, followed by 35 cycles consisting of 94°C for denaturation for 1 min, then annealing at 58.3°C for 30 s, and 72°C elongation for 1 min followed by a final extension for 5 mins at 72°C. A volume of the 3 µl of each amplified product was mixed with 3 µl of loading solution (Bromophenol blue and Xylen cyanol) until a homogenous mix was achieved. Negative control was solution without a DNA template.

Electrophoresis of PCR products and band visualization: A final volume of 6 µL of each amplification product were subjected to electrophoresis to verify amplicon sizes using 2% agarose gel prepared with tris-borate-ethylenediaminetetraacetic acid (TBE) and stained with 5 µg/ml ethidium bromide.

Electrophoresis procedure was conducted with a constant voltage of 100 v for 45 minutes. Results were viewed under ultraviolet light. Various DNA band patterns were compared against a 100bp DNA 100 bp marker to determine the size of amplicons.

3. RESULTS AND DISCUSSION

Table 1. Sequences, size and source of primers used in this study

SN	Target gene	Primer sequence	Amplicon size	Reference
1	<i>InvA</i>	GTG AAA TTA TCG CCA CGT TCG GGC AA TCA TCG CAC CGT CAA AGG AAC C"	284bp	[16]

Table 2. Prevalence of *InvA* gene detected among *Salmonella* isolates from Chicken source

SN	Location	No of NTS isolates	<i>invA</i>	Total <i>invA</i> (%)
1	Wukari	36	31	86.1
2	Donga	19	13	68.5
3	Takum	16	12	75
4	Ibi	27	23	85.2
	Total	98	79	80.6

Detection of *invA* gene in animal isolates:

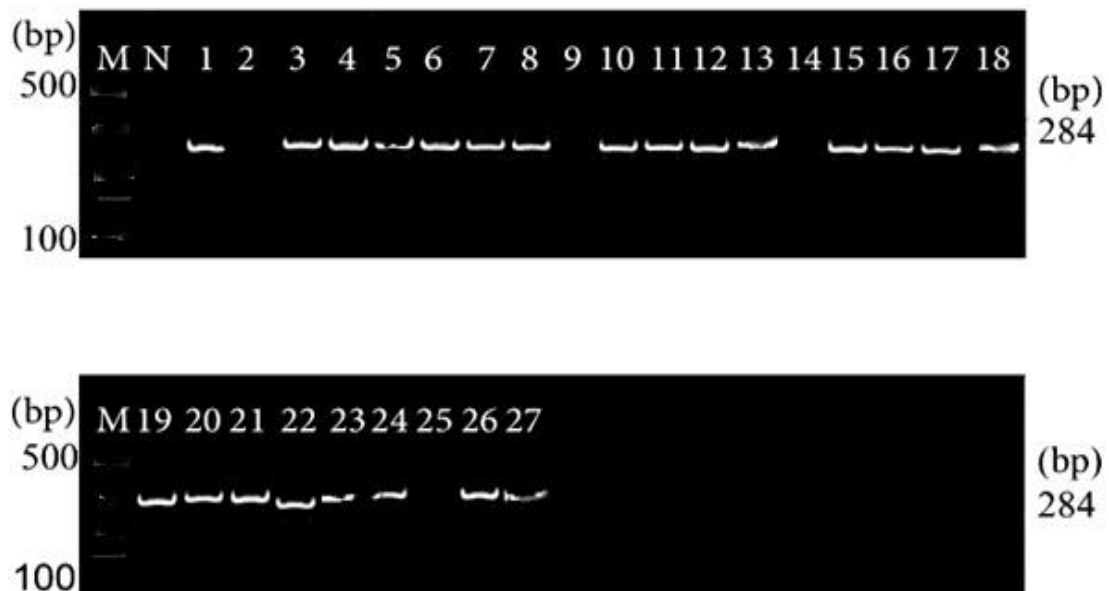


Fig. 1. Agarose-gel electrophoresis displaying PCR amplicons of 284bp *invA* gene in 23 *Salmonella* isolates from human sources in Ibi
Lane M, 100 bp marker; Lane 1–27, test samples (NTS); lane N, Negative control



Fig. 2. Agarose-gel electrophoresis displaying PCR amplicons of 284bp *invA* gene in 13 *Salmonella* isolates from human sources in Ibi
Lane M, 100 bp marker; Lane 1–27, test samples (NTS); lane N, Negative control

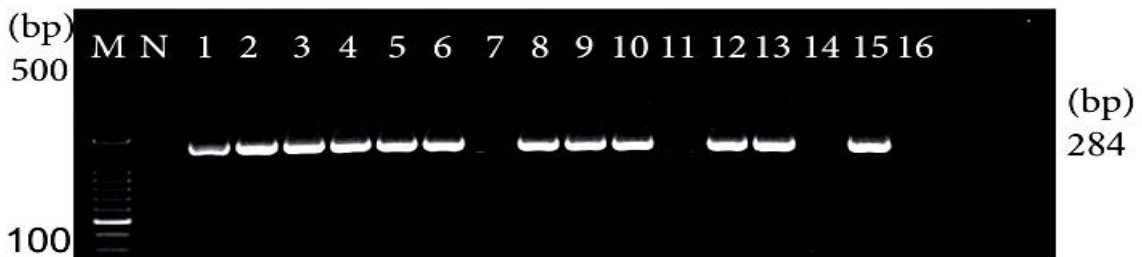


Fig. 3. Agarose-gel electrophoresis displaying PCR amplicons of 284bp *invA* gene in 12 *Salmonella* isolates from human sources in Ibi
Lane M, 100 bp marker; Lane 1–27, test samples (NTS); lane N, Negative control

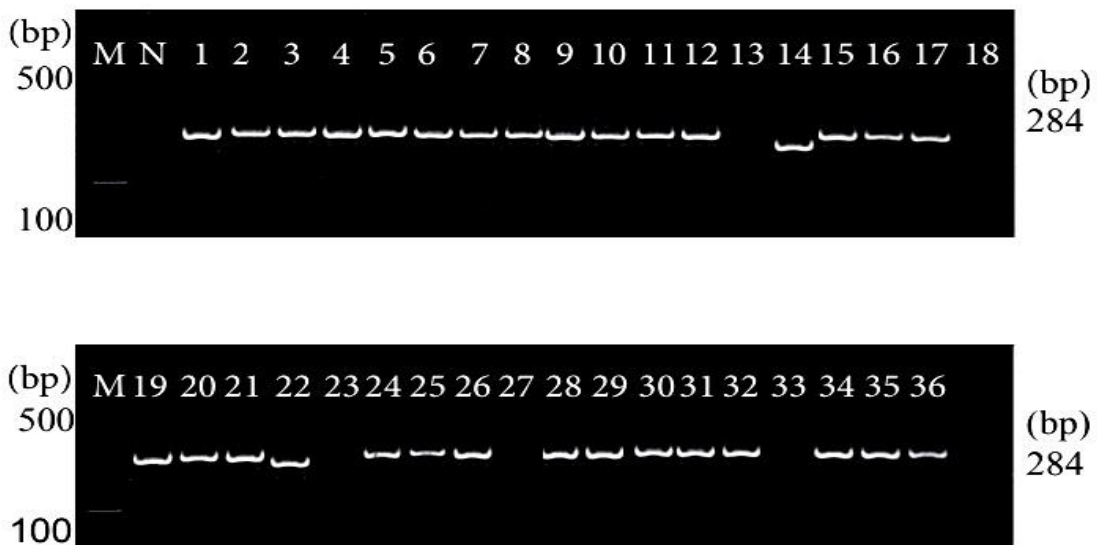


Fig. 4. Agarose-gel electrophoresis displaying PCR amplicons of 284bp *invA* gene in 31 *Salmonella* isolates from human sources in Ibi
Lane M, 100 bp marker; Lane 1–27, test samples (NTS); lane N, Negative control

Results from PCR analysis showed that *invA* gene was seen in 79 (80.8%) *Salmonella* isolates with an approximate length of 284bp. A total of 19 isolates did not show the target band implying that the isolates are not invasive, and even if they are, other invasive genes such as *misL* or *hilA* may be liable and not the *invA* gene. This finding is unswerving with the findings of [17] which detected “*invA* gene” in 106 of 195 *Salmonella* samples characterized. Other studies such as [18] identified the presence of the *invA* gene in 91% of NTS screened. Similarly, [9,11] discovered *invA* gene in 66% and 50% of respective isolates subjected to characterization. In a contrasting view, [19] detected the *invA* gene in all samples screened and argued that the *invA* gene is considered the main target gene for the detection of strains belonging to *Salmonella*. Supportably, [10] detected the invasive “*invA*” gene in all 37 *Salmonella* isolates screened and argued that the outcome was probable since the *invA* gene is conserved among all *Salmonella* serotypes particularly for pathogenetic purposes. Also, [20] had established previously that the amplification of *invA* gene is a molecular confirmatory test for *Salmonella* isolates.

Observably, identified studies disputing the absence of *invA* gene in *Salmonella* isolates have one or more similarities. From available evidence, *Salmonella invA* genes are strangely detected in all isolates subjected to characterization when several serovars from various locations are involved. In fact, only TS and NTS *Salmonella*, *Enteritidis*, *Salmonella Typhimurium* and a few other serovars have been studied based on their complicity in the invasion of intestinal macrophages and epithelial cells [21,22]. All 35 *Salmonella* species (13 *Enteritidis* and 24 *Typhimurium* 24) subjected to characterization by [10] were isolated from pigs in a particular abattoir environment and presented with invasive *invA* gene. Similarly, [19] amplified the *invA* gene successfully in all 126 *Salmonella Heidelberg* samples obtained from in a precise poultry slaughterhouse. Also, the study by [23] confirmed that the *invA* gene was not detected in some cultural and biochemical identified *Salmonella* isolates from clinical samples from diverse sources.

Available evidence has suggested that intracellular conjugation of bacteria is partially responsible for the evolution and spread of virulent and antibiotic resistance genes in pathogenic organisms. Intracellular virulent gene transfer between *Salmonella* is possible via

horizontally mobile elements (HMEs) which includes cis-acting signal sequences, integrons, conjugative plasmids and transposons collectively known as vectors [21,22]. It is possible for non- invasive form *Salmonella* to acquire virulent genes inside the mammalian cell from other resident pathogens via conjugative plasmid transfer, as many new virulence traits are plasmid borne [21,22]. According to [24], *Salmonella Enteritidis* and *Salmonella Typhimurium* are the most serovars harboring the invasive *invA* gene responsible for invasive gastroenteritis. When *Salmonella* specie with invasive potentials enters the cells, it can exchange plasmids containing virulent genes with other strains or even other bacterial species [22]. Since virulence is partially dependent upon invasion, the *invA* *Salmonella* probably dominates the human cells more, and can transmit *invA* genes to the non- invasive bacteria (De-Jong et al., 2012). It is therefore possible that the isolates which do not harbour the *invA* gene as observed from this study may not have been involved in a successful gene exchange with a donor or lacks antimicrobial resistance gene. Indiscriminate antibiotic use leading to antimicrobial resistance has been found to enhance bacterial gene exchange. This is because; intracellular gene exchange between pathogens is possible when antimicrobial resistant *Salmonella* translocate to the mesenteric lymph nodes and other systemic sites from the gut via the intestine [25]. Consequently, the detection of the *invA* gene in all human isolates as reported by several studies may be possible through intracellular conjugative virulent gene exchange with other *Salmonella* strains harboring the gene [26,27].

4. CONCLUSION

This current study has been able to substantiate that *Salmonella* may be or not invasive. However, the non-virulent ones have the potentials of receiving virulent genes via gene exchange. The detection of the *invA* invasive gene from *Salmonella* species from the fecal droppings of physically healthy chickens is really of great concern to public health. Chicken in commercial poultry farms and domestic coops have shown to harbor the invasive gene. This finding therefore implies that, if proper hygienic conditions are not maintained in the various aspects of food processing the NTS could be invasive opportunistically among immune compromised individuals and children. This research findings will ultimately be useful in

effective health promotion in the food production industry and *Salmonella* surveillance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the Research Ethics Committee of the Department of Microbiology, Federal University Wukari, Taraba State, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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