Distribution of Virulence Genes in *Salmonella* Serovars isolated from poultry farms in Kwara State, Nigeria

Ahmed O. Akeem¹*, P. H Mamman², M. A. Raji¹, C. N. Kwanashie², I. A. Raifu¹ and A. Aremu³

¹Department of Veterinary Microbiology, University of Ilorin, Ilorin, Kwara State, Nigeria
²Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
³Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Ilorin, Kwara State, Nigeria

Received: 01/06/2017; Accepted: 04/10/2017

**Abstract:** This study was aimed at investigating the prevalence and detection of virulence genes in *Salmonella* isolated from poultry farms in Kwara State, Nigeria. Ten commercial layer farms located in three senatorial districts of the state were sampled. The presence of *Salmonella* was investigated using standard bacteriological techniques, the isolates obtained were serotyped and polymerase chain reaction (PCR) was used to detect presence of two genes (fimA and sefC) in the isolates. Of the 300 samples collected, 24 (8.0%) were positive for *Salmonella*; 4 different serovars were identified; *Salmonella* Larochelle, S. Muenster, *S. enterica* ser. 6, 7:d, *S. enterica* ser. 45:d:1,7 and *Salmonella* Typhimurium, the most frequent being *Salmonella* Typhimurium (3%). Varying distribution patterns of the studied genes were observed among the isolates. While, fimA was found in all the 24 (100%) isolates, sefC was found only among the *Salmonella enterica* ser. 45:d:1.7. The study indicates that the fimA gene is widely distributed among *Salmonella* irrespective of the serovars. However, the sefC gene appears to be serovar specific. The prevalence of *Salmonella* in chicken and poultry farms constitutes a major public health concern. Hence, further epidemiological studies are necessary.

**Keywords:** *Salmonella*, Kwara, Virulence, Poultry farms.

**INTRODUCTION**

Poultry industry is one of the major components of the Nigerian economy, serving as a source of income for farmers and a main source of high quality protein for the ever-growing population in Nigeria, due to their affordability and acceptability (Fagbamila et al., 2010; Bettridge et al., 2014). Poultry production plays an important role in the provision of animal protein, this account for about 25% of local meat production in Nigeria (Agbaje et al., 2010). The poultry industry in Nigeria has been expanding rapidly in past years despite facing many challenges like the global financial crisis, diseases outbreak such as Avian influenza, inadequate credit facilities, low egg production, poor chick quality, poor and low performing breeds, poor weight gain/ feed conversion, feeding and management issues (FAO, 2008; Agada et al., 2014a). The Nigerian poultry industry improved from 150,700 million chickens in 2005 to 192,313 million in 2010 (FAO, 2015). Across different regions of the country, the poultry sector is characterized by a low level of production and weak levels of specialization (FAO, 2008).

Salmonellosis is an important health problem and a major challenge affecting both humans and animals worldwide. *Salmonella* species are recognized as the most prevalent agent of food poisoning. Salmonellosis in human is often associated with gastroenteritis, which is usually self-limiting. In some cases, particularly in children, pregnant women, infants, the elderly, and immunocompromised patients, *Salmonella* infection can lead to invasive and focal infections that can be severe (Hald et al., 2007). The sources of nontyphoidal *Salmonella* are numerous, but most incidences of human foodborne illnesses have been traced to the consumption of poultry meat, eggs, and poultry-based food products (Rabsch et al., 2001; Andrews and Bäumler, 2005). The fact that these pathogens infect the host animals without apparent sickness contributes to high numbers of human infections (Patrick et al., 2004; Dawoud et al., 2011). Modes of transmission and carrier sources of nontyphoidal *Salmonella* are poorly understood in Africa due to the lack of

*Corresponding Author’s Email: ahmed.oa@unilorin.edu.ng

http://orcid.org/0000-0001-6430-8221
coordinated national epidemiological surveillance systems (Kagambèga et al., 2013).

In poultry, salmonellosis has become an important problem worldwide, causing great economic losses through substantial morbidity, mortality and drop in egg production in addition to huge amounts spent on vaccination and medication (Muhammed et al., 2010; Agada et al., 2014b). Poultry salmonellosis is related to host adapted serovars and remains a major constraint on poultry production in all parts of Nigeria (Garba et al., 2013 and Mamman et al., 2014). Salmonellosis in chicks is usually due to Salmonella Pullorum, the consequence of which is high mortality of both broilers and commercial layers (Idowu et al., 2017). It is characterized by nonspecific signs such as weakness, depression, dehydration, loss of appetite, drooping wings, huddling, somnolence and ruffled feathers. Labored breathing or gasping, as well as diarrhea and pasting of the vent feathers may also be seen (Nogrady et al., 2003). Most signs of salmonellosis in adult birds are thought to be due to Salmonella Gallinarum (Mamman et al., 2014). In addition, zoonotic serovars of Salmonella are harbored in the gastrointestinal tracts of poultry with no apparent signs of illness. Hence, these Salmonella can be excreted in faeces by apparently healthy animals and may be transferred to raw foods of animal origin through contamination during slaughtering and processing (Sanchez et al., 2002).

The virulence of Salmonella species is associated with a combination of chromosomal and plasmid factors (Oliveira et al., 2003). The pathogenicity of an organism in a host is a function of its virulence. Some genes participate in adhesion and invasion such as pef (plasmid encoded fimbriae), spv (Salmonella plasmid virulence) inv (invasion gene) or fim (fimbriae gene) (Alaa and Adnan, 2011). Others play pivotal roles in the survival of organism in the host system- mgtC (Magnesium transport C) (Blanc-Potard and Groisman 1997) or in the actual manifestation of pathogenic processes for example stn (Salmonella toxin), pip A, B, D (Marcus et al., 2000). Bacterial adherence (Kurkkonen et al., 1993) is considered to be a prerequisite for infection, and there are evidences that many bacteria have surface processes, such as fimbriae (Clegg et al., 1985; Aslanzadeh and Paulissen, 1992) or pili, that facilitate attachment to particular receptors on the epithelial cell surface, to urinary tract or intestinal mucus. Although certain strains of E. coli fimbriae are known to be important for virulence, only type 1 fimbriae (fimA) have been implicated in Salmonella pathogenicity.

Control of Salmonella infection will not only culminate into expansion of poultry industry in Nigeria but also lead to reduction in the threat of foodborne illness due to Salmonella in human. Effective prevention and control measures cannot be undertaken unless the status of the disease and epidemiology are well investigated. Therefore, the present study was undertaken to investigate the prevalence and detect the virulence genes in Salmonella serovars in selected layer farms in Kwara state.

MATERIALS AND METHODS

Study area

The study was carried out in Kwara State, Nigeria. Kwara State is located between latitudes (8° 30’N) and longitudes (5° 00’E). The state shares a common internal boundary with Niger State in the North, Kogi State in the East, Oyo, Ekiti and Osun States in the South and an international boundary with the Republic of Benin in the west. Kwara State is a summer rainfall area, with an annual rainfall range of 1,000 mm to 1,500 mm. The rainy season begins at the end of March and lasts until early September, while the dry season begins in early October and ends in early March. Temperature is uniformly high and ranges between 25 °C and 30 °C in the wet season throughout the season except in July – August when the clouding of the sky prevents direct insolation (heatstroke) while in the dry season it ranges between 33 °C to 34 °C (NBS, 2010).

Study strategy

The study involved sampling for Salmonella in layers farms that agreed to participate in the study. Two Local Governments from each of the three senatorial districts of the state were selected based on their commercial poultry production according to data obtained from the state veterinary services. The Local Governments were Ilorin South, Ilorin-West, Moro, Offa, Irepodun and Asa. Twelve farms were selected randomly by balloting for the study, two farms per Local Government; however, two farms declined.
Sample collection

Layer farms were visited across the selected Local Governments, and the following samples were collected: cloacal swabs, litters composed of faeces mixed with saw dust (from deep litters system), non-medicated water and poultry feed. Twenty-seven (27) cloacal swabs were taken per farm with one each from a bird, litters (n=1/farm), water (n=1/farm) and feed (n=1/farm) making a total of 300 samples. Cloacal swabs were collected using sterile swabs, 25 g of wet litters, approximately 50 g of feed and 100 ml of untreated water were collected per farm. All the samples were labelled properly and kept in a cool box containing ice packs and transported, within five (5) hours, to Veterinary microbiology laboratory, University of Ilorin for analysis.

Sample Processing

Samples were pre-enriched in buffered peptone water (Lab M, Lancashire, UK) at the ratio of 1:10 sample to broth incubated at 37 °C for 24 hours. 1 ml of the pre-enriched broth was inoculated in 9 ml of Selenite-F (Oxoid, Hampshire, UK) and Rappaport-Vassiliadis (Oxoid, Hampshire, UK) incubated for 24 hours at 37 °C and 42 °C, respectively (ISO, 2002; OIE, 2012).

Isolation, Identification, and Serotyping of Salmonella

The selective broths (Selenite F and Rappaport-Vassiliadis) were subcultured onto Salmonella-Shigella agar (Oxoid, Hampshire, UK) and Xylose Lysine Deoxycholate agar (Oxoid, Hampshire, UK) and were incubated aerobically at 37 °C for 24 hours. The presumptive Salmonella isolates on XLD (pink/red with/without central black spot due to hydrogen sulphide production) and SSA (transparent colonies with black centre) were then subjected to standard biochemical tests (Andrews, 1992; MacFaddin, 2002). Isolates biochemically identified as Salmonella were streaked on nutrient agar slants and shipped to the WHO National Salmonella and Shigella Center, Bangkok, Thailand, for serotyping according to the Kauffmann-White Scheme (Raufu et al., 2013; Issenhuth-Jeanjean et al., 2014).

Polymerase Chain Reaction (PCR) analysis of the isolates

DNA extraction

DNA extraction was carried out in accordance with manufacturer’s instruction using ZR fungal/bacterial DNA miniPrep™ (Zymo Research Corp. USA) at Department of Veterinary public health laboratory, University of Ilorin.

Primers

The sets of primer pairs used in the PCR assay are as shown in table 1. The primers were synthesized at Inqaba biotech (QN 201502628) South Africa.

Table 1: Primers sequence for PCR amplification.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence: F-(5’-3’)-R-(3’-5’)</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>fim A</td>
<td>F CCT TTC TCC ATC GTC CTG AA R CA CGA TCC GTC TAT TGT TGG</td>
<td>670</td>
<td>Huguette et al., 1996</td>
</tr>
<tr>
<td>Sef C</td>
<td>F GCGAAAACC AAT GCG ACT GTA R CCCACC AGA AAC ATT CAT CCC</td>
<td>1103</td>
<td>Murugkar et al., 2003</td>
</tr>
</tbody>
</table>
PCR assay

PCR analysis was carried out at Bioscience Laboratory unit of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The PCR reaction contained a final volume of 25 µL which included 12.5 µl master mix (Inqaba Biotech, S. Africa) containing (5 U/ul) Taq DNA polymerase, 2.5 mM each of dATP, dCTP, dTTP and dGTP, 50mM MgCl₂ and PCR buffer, 1.25 µl (1µM) each of forward and reverse primers and 2.5 µl of template DNA (100ng/µl DNA) and 7.5 µl of deionized water. The cocktail was used for each sample. The reaction was performed in a thermocycler (Perkin-Elmer, USA) in 40 cycles of initial denaturation (94 °C for 5 minutes), denaturation (94 °C for 30 seconds), primer annealing (55 °C for 45 seconds) and primer extension (72 °C for 45 seconds) followed by final extension at 72 °C for 7 minutes. Fifteen microliters (15 µl) of each PCR products were loaded on 2.0% agarose gel containing 0.5 µl/ml ethidium bromide (Pharmacia, Sweden). The gel picture was captured using Gel doc 2000 documentation system (Pharmacia, Sweden).

RESULTS

Isolation of Salmonella

Of the 300 samples collected, 24 (8.0 %) were positive for Salmonella. The different Salmonella serovars and their rates of isolation from different sources are as shown in table 2. Salmonella Typhimurium was the most frequently isolated serovar from 2 (20 %) of feed and water samples each and 5(1.9 %) of the cloacal swabs. Salmonella Muenster was only isolated from 2 water samples (20 %).

Detection of Salmonella genes using PCR

In this study, PCR assay was performed for the detection of two virulence genes. The fimA gene was detected in all the isolates by demonstrating the presence of 670 bp PCR product (Figure 1.0). The sefC gene was detected by the presence of 1103 bp PCR product (Figure 2.0) only in 20.8 % (5/24) of the isolates confirmed to be Salmonella enterica ser. 45:d:1,7 serovars isolates (Table 3).

Table 2: Different Salmonella serovars and rates of their isolation from different sources.

<table>
<thead>
<tr>
<th>Serovars</th>
<th>Sample Source</th>
<th>Cloacal swabs</th>
<th>Litters</th>
<th>Feeds</th>
<th>Water</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larochele</td>
<td></td>
<td>0(0)</td>
<td>1(10.0)</td>
<td>0(0)</td>
<td>1(10.0)</td>
<td>2(0.7)</td>
</tr>
<tr>
<td>Muenster</td>
<td></td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>2(20.0)</td>
<td>2(0.7)</td>
</tr>
<tr>
<td>S. enterica ser. 6,7 d</td>
<td></td>
<td>3(1.1)</td>
<td>0(0)</td>
<td>3(30.0)</td>
<td>0(0)</td>
<td>6(2.0)</td>
</tr>
<tr>
<td>S. enterica ser. 45,d 1,7</td>
<td></td>
<td>2(0.7)</td>
<td>1(10.0)</td>
<td>2(20.0)</td>
<td>0(0)</td>
<td>5(1.7)</td>
</tr>
<tr>
<td>Typhimurium</td>
<td></td>
<td>5(1.9)</td>
<td>0(0)</td>
<td>2(20.0)</td>
<td>2(20.0)</td>
<td>9(3.0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10(3.7)</td>
<td>2(20.0)</td>
<td>7(70.0)</td>
<td>5(50.0)</td>
<td>24(8.0)</td>
</tr>
<tr>
<td>No. of samples examined</td>
<td></td>
<td>270</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>300</td>
</tr>
</tbody>
</table>

Table 3: Distribution of fim A and sef C genes among Salmonella isolated from poultry farms.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. of isolates</th>
<th>No. positive for virulence genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fim A</td>
<td>sef C</td>
</tr>
<tr>
<td>S. Larochele</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>S. Muenster</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>S. enterica ser. 6,7 d</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>S. enterica ser. 45,d 1,7</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Total</td>
<td>24.0</td>
<td>24.0</td>
</tr>
</tbody>
</table>
Figure 1: Detection of fimA gene by PCR. Lane M-(50 bp) DNA ladder molecular weight marker, lane N-negative control (deionized water), 2 to 24-Test isolates.

Figure 2: Detection of sefC gene PCR. Lane M-(50 bp) DNA ladder molecular weight marker, lane N-negative control (deionized water), 1 to 24-Test isolates.
DISCUSSION

Salmonellosis is one of the major zoonotic foodborne diseases worldwide (Kagambèga et al., 2013) and it’s prevalence in animals poses a continuous threat to man (Muhammad et al., 2010). Several Salmonella serovars were isolated from all the sample sources examined in this study and many of these Salmonella serovars are known to be pathogenic to man. The isolation rate of Salmonella serovars in this study correlates with earlier study by Muhammad et al. (2010) which reported isolation rate of 9.0 % from farms in Jos, Plateau state, Nigeria. It is however higher than 2.8 % reported in Caribbean countries (Adesiyun et al. 2014)

Salmonella Typhimurium was the most frequent isolated serovar and this agree with previous work which also reported this serovar as one of the common serovars isolated from poultry in Nigeria (Orji et al., 2005). The isolation of invasive Salmonella such as serotype Typhimurium and other pathogenic salmonelae in this study highlights the risk of health hazards to consumers as contaminated poultry and poultry products may serve as source of infection to human food chain (Doaa, 2013).

Polymerase chain reaction (PCR) has become a potentially powerful alternative technique in microbiological diagnostics due to the fact that it is fast, simple, accurate, and reproducible (Pickup et al., 2003). In this present study, fimA gene was detected in all the 24 Salmonella isolates. These finding is in agreement with earlier reports by Huguette et al. (1996), Alaa and Adnan (2011) which both found that the fimA gene contains sequences unique to Salmonella isolates and demonstrated that this gene is suitable for PCR target for the detection of Salmonella strains. Bacterial adherence (Kurkkonen et al., 1993) is considered to be a baseline requirement for infection, and there is evidence that many bacteria have surface appendages, such as pili or fimbriae (Clegg et al., 1985; Aslanzadeh and Paulissen, 1992), that facilitate their binding to specific receptors on the epithelial cell surface, or intestinal mucus. Although certain types of Escherichia coli fimbriae are known to be important for virulence, only type I fimbriae (fimA) have been implicated in Salmonella pathogenicity.

Salmonella Enteritidis fimbrae 14 (SEF14) is encoded by the sef operon, which contains sefC gene. It contains 4 major protein subunits SefA, SefB, SefC, and SefD. SEF14 plays important role in the ability of Salmonella to colonize Peyer’s patches and in the adhesion and invasion of epithelial cells of the host intestine (Castilla et al., 2006). In the present study, the sefC gene was detected in 5(20.8 %) of the isolates which were confirmed to be Salmonella enterica ser. 45:d:1,7 by serotyping. The present finding does not agree with the findings of Rahman (1999) and Murugkar et al. (2003) which showed that except for strains of S. enteritidis and S. gallinarum, none of the other serotypes (S. Typhimurium, S. Newport, S. Kentucky, S. Weltevreden and S. Indiana) tested were found to contain sefC gene. These differences might be due to the fact that the serotype shown to contain this gene in our present study was not part of the serovars studied by Rahman (1999) and Murugkar et al. (2003) or might be due to evolution of new gene in this serovar. Future studies are needed to confirm this hypothesis.

CONCLUSION

Contamination of chicken farms with Salmonella indicate generally poor infrastructure and low biosecurity measures in farms in the state. The study reaffirmed the applicability of PCR amplification of fimA for identification of Salmonella species and reported for the first time the presence of SeFC gene in Salmonella ser. 45:d:1,7.

ACKNOWLEDGEMENT

We would like to thank all laboratory technologists at the Department of Veterinary Microbiology, University of Ilorin and Bioscience Unit, IITA, Ibadan for the technical assistance.

Conflicts of interest: none

REFERENCES


Aslanzadeh, J. and Paulissen, L. J. (1992). Role of the type 1 and type 3 fimbriae adherence and pathogenic of *Salmonella enteritidis* in mice. *Microbiology and Immunology, 36*: 351-359.


International Organization of Standardization (ISO) 6579 (2002). Microbiology general guidelines on methods for the detection of *Salmonella*. International organization of standardization, Geneva, Switzerland


