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Full Length Research Paper

Characterization of *Salmonella enterica* Ituri isolated from diseased poultry in Nigeria

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***Salmonella enterica* Ituri is an uncommon serotype associated with poultry disease. One of the serotype isolated from a poultry disease in Nigeria was characterized by serotyping and screening for the presence of *Salmonella* genomic island 1(SGI1) as a possible factor responsible for its involvement in a poultry disease outbreak. Despite the similarities in the antibiotic sensitivities patterns of the *Salmonella* Ituri serotypes with some SGI1-bearing serotypes, it does not bear the SGI1. This is the first report of association of this *Salmonella* serotype with avian paratyphoidal disease.**

Key words: *Salmonella enterica* Ituri, serotype, *Salmonella* genomic island 1 (SGI1).

INTRODUCTION

Salmonella is a member of the family *Enterobacteriaceae* (Wray and Davies, 2002). It is a facultative anaerobe, Gram-negative rod, 0.4 to 0.6 µm in size, most of which are motile by means of peritrichous flagella with the exception of the non motile serotypes: *S.* Gallinarum and *S.* Pullorum (Montville and Mattews, 2008). The genus consists of two species: *Salmonella enterica* and *Salmonella bongori* (Tindall et al., 2005). *S. enterica* contains six subspecies namely; "*arizonae",* "*diarizonae",* "*enterica", "houtenae", "indica", and* "*salame"* (Tindall et al., 2005). *S. enterica* subspecies *enterica* is the one mostly found in the intestinal tract of man and warm blooded animals (Grimount et al., 2000). One other species, *S. subterranean,* was subsequently proposed by the Judicial commission of the International committee of systemic bacteriology (Shelobolina et al., 2004), but the proposed species was later reported not to belong to the genus *Salmonella* by other workers (Grimont and Weil. 2007).

Salmonella is known to be a major public health problem all over the world. For instance in the year 2000, it was reported to have caused 41 800 cases of human food borne diseases in England and Wales (Adak et al., 2002). In the USA, about one million human salmonellosis cases are recorded per year, with 94% associated with a food borne origin (Scallan et al., 2011). Although, many *S. serotypes* have been associated with human salmonellosis, some are found more frequently in different regions of the world. For instance, in Belgium and most part of Europe, *S.* Enteritidis and *S.* Typhimurium are more frequently associated with human salmonellosis (Humphrey, 2000; Bertrand et al., 2007), where consumption of poultry meat is frequently identified as a source of human foodborne diseases (Humphrey, 2000; Adak et al., 2005). Apart from the known poultry-adapted non-motile *Salmonella* serotypes such as *Salmonella* Gallinarum and *Salmonella* Pullorum (Montville and Mattews. 2008), some other serotypes have been known to be commonly associated with poultry and poultry products. For example, according to the USDA *Salmonella* serotyping quarterly reports from January to June 2011, the following ten serotypes: Kentucky, Enteritidis, 4, 5, 12:i:-, Heidelberg, Brandenburg, Typhimurium, Berta, Montevideo, Thompson and S. Worthington predominated the list of

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identified serotypes from broilers(USDA,2011). *Salmonella* Ituri is one of the serotypes that are not usually associated with poultry and poultry products. This serotype was first discovered in 1953, when a group of scientists described three new *Salmonella* types from the Congo, where *Salmonella* Ituri was isolated from the feaces of an apparently normal duck (Kauffman and Fain, 1953).

According to Boqvist et al. (2003), *Salmonella* Ituri was isolated for the first time in Sweden in 1997. It was one of the 165 serotypes attributed to snake/lizard from the total of 555 isolated from animals during 1993 to 1997 study periods in a study carried out to identify the *Salmonella* serotypes associated with animals and feed production in Sweden (Boqvist et al., 2003). One *Salmonella* Ituri was also reported to have been isolated from a total of 6562 *Salmonella* , as an isolate from the category of other domestic animals/environment, being part of the clinical *Salmonella* isolates from non human sources to CDC and the National Veterinary Services Laboratories (NVSL) United States of America by serotype and sources in 2004 (Anonymous, 2004). This serotype is rarely associated with septicaemia in poultry.

The *Salmonella* Ituri characterized in this study was one of the 41 *S. enterica* isolated from poultry diseases outbreaks between April 2005 and August 2007 in Nigeria (Ogunleye et al., 2010). Due to the similarities in the antibiotic resistance patterns of the *S. serotypes* to a number of earlier reported SGI1-carrying *Salmonella* serotypes (Wall et al., 1994; Evans and Davies, 1996; Meunier et al., 2002; Doublet et al., 2004; Levings et al., 2005; Vo et al., 2006; Carlson et al., 2007; Ogunleye and Carlson 2012), we characterized the isolate by screening for the presence of the SGI1gene designated as SO13. This line of research was pursued since we recently reported an SGI1-bearing isolate of *S*. Kentucky as the etiologic agent of fowl paratyphoid (Ogunleye and Carlson, 2012).

MATERIALS AND METHODS

The *Salmonella enterica* isolate

The *Salmonella* isolate characterized in this study was recovered from one of the pooled organs of a septic poultry submitted for bacteriological examination in the Department of Veterinary Microbiology and Parasitology, University of Ibadan. The isolate was identified as *S. enterica* based on morphological, biochemical and serological typing with Polyvalent Salmonella Antisera (Difco^{m} Salmonella O Antiserum Poly A-I and V1) using standard methods (Edwards and Ewings, 1972; Barrow and Feltham, 1993; Ogunleye et al., 2010).

Serotyping of the isolate

The *S. enterica* isolates was sub-cultured into TSA agar and submitted to National Veterinary Service Laboratories in Ames, Iowa, USA for serotyping. Serotyping was performed as per the Kauffman-White Scheme.

Determination of the ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline MIC values

The isolate was grown aerobically in breakpoint concentrations of ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, or tetracycline (all obtained from SIGMA-ALDRICH). Resistance was ascribed if flocculent growth was observed after 16 h of aerobic growth at 37°C. *S. enterica* serovar Typhimurium definitive phage type DT104 and an SGI1 / SO13-carrying *S. enterica* Kentucky were used as positive controls (Ogunleye and Carlson, 2012).

SGI1 screening of the isolate

Since this *Salmonella* Ituri isolated caused avian disease in a manner similar to that recently reported for an SGI1-bearing isolate of *S*. Kentucky (Ogunleye and Carlson, 2012), we performed PCR-based studies to assess the presence of SGI1 elements such as SO13, *floR* (chloramphenicol/florfenicol resistance), *tetR* (regulator of tetracycline resistance), and *aadA2* (streptomycin/spectinomycin resistance) (Carlson et al., 2007). We used forward primer specific to *floR* and a reverse primer specific to *tetR* for the antibiotic resistance genes *floR* (chloramphenicol/florfenicol resistance), *tetR* (regulator of tetracycline resistance), and *aadA2* (streptomycin/spectinomycin resistance) in a PCR assay as earlier described (Carlson et al., 1999, 2007). *S. enterica* serovar Typhimurium definitive phage type DT104 and an SGI1 and SO13 bearing *S. enterica* Kentucky associated with hypervirulence due to hyperinvasion from septic poultry in Nigeria (Ogunleye and Carlson, 2012) were used as positive controls.

RESULTS AND DISCUSSION

Serotyping analysis revealed that the isolate is *S. enterica* serotype Ituri. As shown in Table 1, antibiotic susceptibility tests demonstrated that the strain is resistant to chloramphenicol, sulfamethoxazole, and tetracycline yet sensitive to ampicillin and streptomycin. PCR studies indicate that the *S. enterica* serotype Ituri does not bear any of the elements associated with SGI1 (Figures 1 and 2).

This study describes the characterization of *S. enterica* Ituri isolated from a poultry disease outbreak in Nigeria. Due to the similarities of the antibiotic resistance patterns of this serotype with the positive controls (DT104 and an SGI1-bearing *S enterica* Kentucky), the isolate was screened for the presence of SGI1 elements. *Salmonella* Ituri was resistant to chloramphenicol, sulfamethoxazole, and tetracycline yet was sensitive to ampicillin and streptomycin. *S. enterica* Kentucky exhibited the same antibiogram plus resistance to ampicillin while DT104 was resistance to all five antimicrobial agents. The *S. enterica* Ituri lacked the SGI1 elements.

SGI1-bearing *Salmonella* such as *S. Typhimurium*, *Agona*, and *Infantis* have been noted to be more virulent than other *Salmonella* strains in cattle (Rasmussen et al., 2005). We identified an SGI1-bearing *S. enterica* Kentucky isolated from a septicaemic poultry disease in Nigeria, although we cannot conclude that this isolate

Table 1. Antibiotic sensitivity patterns of the *S.* Ituri isolate, compared with the two positive controls.

Salmonella	**Antibiotic**				
	Ampicillin	**Chloramphenicol**	**Streptomycin**	**Sulfamethoxazole**	**Tetracycline**
S. ituri	Sensitive	Resistance	Sensitive	Resistance	Resistance
S. Kentucky	Resistance	Resistance	Sensitive	Resistance	Resistance
DT104	Resistance	Resistance	Resistance	Resistance	Resistance

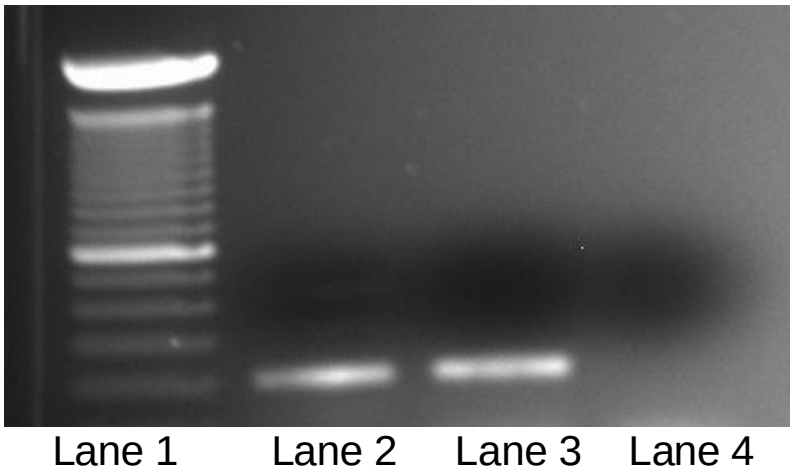

Figure 1. Presence of *aadA2* in *Salmonella* Kentucky (lane 2), DT104 (lane 3) and its absence in the *Salmonella* Ituri (lane 4). Lane 1 contains the 100bp DNA ladder. Expected amplicon size is 200 bp.

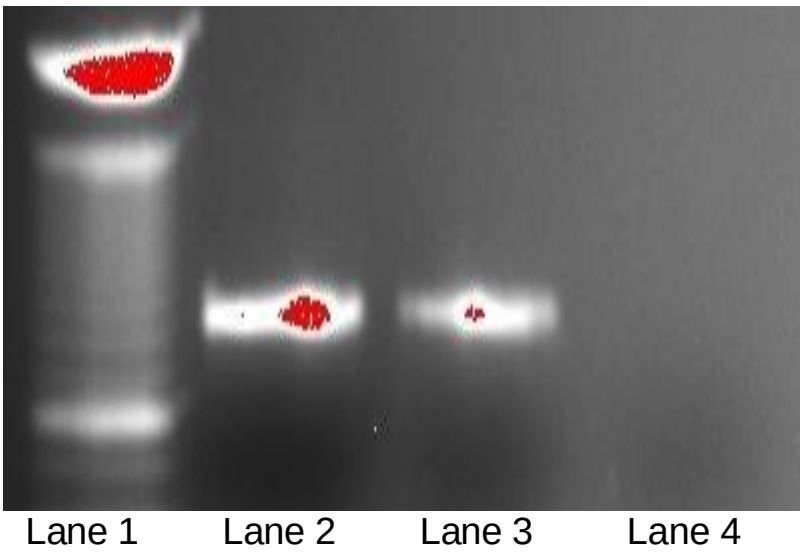

Figure 2. Presence of SO13 in *Salmonella* Kentucky (lane 2), DT104 (lane 3) and its absence in the *Salmonella* Ituri (lane 4). Lane 1 contains the 100 bp DNA ladder. Expected amplicon size is ~900 bp.

was hypervirulent because of the SGI1 (Ogunleye and Carlson, 2012). Nonetheless, we examined the possibility of a link between poultry disease and unusual *Salmonella* serotypes bearing SGI1. Our results indicate

that there is no link between the virulence attributes of the *S*. Ituri and the SGI1-bearing *S*. Kentucky. However, to the best of our knowledge, this is the first time this uncommon serotype has been associated with poultry disease.

REFERENCES

Adak GK, Long SM, O'Brien SJ (2002). Trends in indigenous foodborne disease and deaths, England and Wales 1992-2000. Gut 51:832-841.

Adak GK, Meakins SM, Yip H, Lopman BA, O'Brien S J(2005).Disease Risks from Foods, England and Wales, 1996-2000. Emerg. Infect Dis. 11:365-372.

Anonymous (2004).Salmonella annual Summary 2004: Clinical Salmonella isolates from non human sources. Reported to CDC and NVSL by serotype and sources, 2004.

Barrow GH, Feltham RKA (1993). Cowan and Steel's manual for identification of medical bacteria, 3rd Edn. Cambridge, UK, Cambridge University Press. 331 p.

Bertrand S, Baeyenns D, Decooman F, Steenhaut H, Lamranni F, Criselain J, van Meervenne E, Mairiaux E (2007). Rapport annuel: souches de salmonella et shigella isoles en Belgiquee en 2007. http://bacterio.iph.fgov.be/reporting/reportspdf/CNR%20salmonella%20rapportannuel%202007pdf.AccessedApril 17,2009.

Boqvist S, Hansson I, Nord Bjerselius U, Hamilton C, Wahlström H, Noll B, Tysen E, Engvall A (2003). Salmonella Isolated from Animals and Feeds Production in Sweden between 1993 and 1997. Acta Vet. Scand. 44:181-197.

Carlson SA, Sharma VK, McCuddin ZP, Rasmussen MA, Franklin SK (2007). Involvement of a *Salmonella* genomic island 1 gene in the rumen protozoan-mediated enhancement of invasion for multiple-antibiotic-resistant *Salmonella enterica* serovar Typhimurium. Infect Immun. 75:792-800.

Clinical and Laboratory Standards Institute (2009) M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. 8th ed. Wayne, PA.

Doublet B, Weill FX, Fabre L, Chaslus-Dancla E, Cloeckaert A (2004). Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster containing a novel 3´-*N* aminoglycoside acetyltransferase gene cassette, *aac*(3)-*Id*, in *Salmonella enterica* serovar Newport. Antimicrob. Agents Chemother. 48:585-591.

Edwards PR, Ewings WH (1972). The genus Salmonella. In: Identification of Enterobacteriaceae (3rd Edn. Burgess Publishing company, Minneapolis, USA pp. 208-337.

Evans S, Davies R (1996). Case control study of multiple-resistant *Salmonella typhimurium* DT104 infection of cattle in Great Britain. Vet Rec 139:557-558.

Grimont PAD, Grimont F, Bouvet P (2000). Taxonomy of the genus Salmonella. In: Salmonella in Domestic Animals. Eds C. Wray, A. Wray. CABI Publishing.

Grimont PAD, Weill FX (2007). Antigenic formulae of the Salmonella serovars. Ninth Edition, World Health Organization Collaborating centre for reference and research on Salmonella. Institute Pasteur, Paris.

Humphrey T (2000). Public health aspect of *Salmonella* infection. In Salmonella in Domestic Animals. Eds C. Wray, A. Wray. CABI Publishing pp. 245-263.

Kauffman F, Fain A (1953). Three new Salmonella types (S. ITURI, S. KASENYI and S.NIAREMBE) from Belgian Congo(Occurrence of Salmonella types in Ducks). Acta pathologica et microbiologica scandinavica. 32:513-515.

Levings RS, Lightfoot D, Partridge SR, Hall RM, Djordjevic SP (2005). The genomic island SGI1, containing the multiple antibiotic resistance region of *Salmonella enterica* serovar Typhimurium DT104 or variants of it, is widely distributed in other *S. enterica* serovars. J. Bacteriol. 187:4401-4409.

Meunier D, Boyd D, Mulvey MR, Baucheron S, Mammina C, Nastasi A, Chaslus-Dancla E, Cloeckaert A (2002). *Salmonella enterica* serotype Typhimurium DT 104 antibiotic resistance genomic island 1 in serotype Paratyphi B. Emerg. Infect Dis. 8:430-433.

Montville TJ, Mattews KR (2008). Food Microbiology: An Introduction (2nd ed).428p: United States of America. ASM Press, Washington DC. ISBN 9781-1-55581-396-3.

Ogunleye AO, Ajuwape ATP, Adetosoye AI (2010) Fluoroquinolone resistant *Salmonella enterica* of poultry origin from South Western States of Nigeria. Bull. Anim. Health Prod. Afr. 58:216-221.

Ogunleye AO, Carlson SA (2012). Emergence of an SGI1-bearing Salmonella enteric serotype Kentucky isolated from septic poultry in Nigeria. J. Infect. Dev. Ctries 6(6):483-448.

Rasmussen M, Carlson SA, Franklin SK, McCuddin ZP, Wu MT, Sharma VK (2005). Exposure to rumen protozoa leads to enhancement of pathogenicity of and invasion by multiple-antibiotic-resistant *Salmonella enterica* bearing SGI1. Infect Immun 73:4668–4675.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM (2011). Foodborne illness acquired in the United States- major pathogens. Emerg Infect Dis.17:7-15.

Shelobolina ES, Sullivan SA, O Neill KR, Nevin KP, Lovley DR, (2004). Isolation characterization and U(V1)-reducing potential of a facultatively anaerobic, acid resistant bacterium from low- pH nitrate- and U(V1)- contaminated subsurface sediment and description of Salmonella subterranean sp nov. Appl. Environ. Microbiol. 70:2959-2965.

Tindall BJ, Grimont PA, Garrity GM, Euzeby JP(2005). Nomenclature and taxonomy of the genus Salmonella. Int. J. Syst. Evol. Microbiol. 55:521-524.

USDA (2011). United States Department of Agriculture (USDA). Top Salmonella Serotypes isolated from Food safety and Inspection Services (FSIS) meat and poultry products January-June 2011 broilers.

Vo A, van Duijkeren E, Fluit A, Wannet W, Verbruggen A, Maas H, Gaastra W (2006). Antibiotic resistance, integrons and *Salmonella* genomic island 1 among non-typhoidal *Salmonella* serovars in The Netherlands. Int. J. Antimicrob. Agents 28:172-179.

Wall P, Morgan D, Lamden K, Ryan M, Griffin M, Threlfall E, Ward L, Rowe B (1994). A case control study of infection with an epidemic strain of multiresistant *Salmonella typhimurium* DT104 in England and Wales. Commun. Dis. Rep. 4:R130-R135.

Wray C, Davies RH (2002). Enterobacteriaceae. In Poultry Diseases.5th edition. EDs F Jordan, M. Pattison, D Alexander, T. Faragherw, B. Sanders. pp. 96-109.