ANTIMICROBIAL RESISTANCE AND MOLECULAR TYPING OF *Salmonella* spp. ISOLATED FROM POULTRY IN KHARTOUM STATE, SUDAN

Hassan Elsiddig Hag Elsafi*[1], Mohamed Amin Mohamed Siddig[2], Anne[3] Muckle, Jaime Martinez-Urtaza[4]

Address (es):
[1] Ahfad Centre for Science and Technology, Ahfad University for Women, Omdurman, Sudan.
[2] Department of Botany, Faculty of Science, University of Khartoum, Khartoum, Sudan.
[3] Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada.
[4] Department of Genetics and Microbiology, Facultat de Biociències - Edifici C, Universitat Autònoma de Barcelona (UAB), Barcelona · Spain

*Corresponding author: hassanelsafi@gmail.com

ABSTRACT

Salmonella is considered one of the main foodborne pathogens. In this study a total of 38 *Salmonella* isolates were recovered from 679 (5.6 %) specimens collected within Khartoum State. The specimens included, intestinal contents 11.4% (14/160) egg 7% (7/99), liver 5% (5/100) skin 5% (10/200), feed 0% (0/40%), water 0% (0/100).. Serotyping revealed the presence of eight serovars: Kentucky 11 (28.9%), Stanleyville 8 (21.05%), Virchow 6 (16%), Alachua 5 (13.16%), Blockley 4 (10.53%), Hadar 2 (5.26%), Typhimurium 1 (2.63%) and Havana 1 (2.63%). Antibiotic resistance profile, using disc diffusion method, indicated that all isolates were sensitive to apramycin, chloramphenicol, cefoperazone and cefotaxime. The 38 isolates were found to be resistant to tetracycline (52.6%), nalidixic acid (50.0%), compound sulfonamide (44.7%), sulfamethoxazole-trimethoprim (31.6%), streptomycin (26.3%), gentamycin (15.8%), neomycin (15.8%), furazolidone (7.9%), ampicillin (5.3%), ciprofloxacin (5.3%), amikacin (2.6%), amoxicillin-clavulanic acid (2.6%), ceftazidime (2.6%), and colistin (2.6%) in decreasing order. Only 20 isolates (52.6%) demonstrated multiple drug resistance. Four of the *Salmonella* isolates were untypable by *Xba*I Pulsed Field Gel Electrophoresis (PFGE) technique however the 33 typed isolates were differentiated into 22 PFGE patterns. This study aimed to determine the prevalence, antimicrobial resistance and molecular typing of *Salmonella* spp. form poultry

Keywords: *Salmonella*, Antimicrobial resistance, Poultry, PFGE, PCR, Khartoum State, Sudan

INTRODUCTION

Salmonella sp. is one of the main sources of foodborne illnesses worldwide **(Fung et al., 2018)**. The main sources of *Salmonella* infections are associated with consumption of meats, milk and eggs **(Jajere, 2019)**. *Salmonella* comprises more than 2600 serovars **(Shi et al., 2015)**. Generally there are two groups of *Salmonella*, typhoidal and non-typhoidal *Salmonella*. While typhoidal *Salmonella* can result in systemic infections with high fatal capabilities, non-typhoidal *Salmonella* infections are generally self-limiting (Su and Chiu, 2007; Gal-Mor *et al*., 2014).

The widespread use of antibiotics poses problems for antimicrobial resistance, which leads to an increase in treatment costs and even to therapy failure **(Hur et al., 2011)**. Multiple drug resistance (MDR) among *Salmonella* is prevalent. The selective pressure caused by the application of antimicrobials in poultry production and veterinary practice for growth promotion and prophylaxis has resulted in an increase in antibiotic resistance and an increase in the presence of genes conferring antimicrobial resistance to *Salmonella* **(Zishiri et al., 2016)**. Strains that have been detected are resistant to some clinical first line antibiotics used in the treatment of severe *Salmonella* infections **(Tadee et al., 2015).** As antibiotic resistant bacteria can be directly transmitted through the food chain or transfer their antimicrobial resistance to human pathogens by mobile genetic elements, it is important to monitor antibiotic resistance among *Salmonella* isolates and control the risk.

The purpose of this study was to determine the prevalence, antimicrobial resistance and molecular typing of *Salmonella* isolates obtained from poultry in Khartoum State, Sudan.

MATERIALS AND METHODS

Sample collection and *Salmonella* isolation

A total of 679 samples of livers, intestinal contents, skin swabs and egg specimens were collected in Khartoum State between October 2004 to May 2005. Samples were selectively enriched in Selenite Cystine broth (Merck KGaA, Darmstadt, Germany) incubated at 37°C for 24 h. then were streaked onto Xylose Lysine Desoxycholate agar (Oxoid, Basingstoke, Hampshire, UK) and incubated at 37°C for 24 h. Suspected *Salmonella* isolates were identified using API20E test strips (BioMerieux, France) and confirmed by polymerase chain reaction (PCR) amplification using *invA* as the target gene **(Malorny et al., 2003).**

Serotyping

The *Salmonella* isolates obtained in this study were submitted to the Office International des Épizotties (OIÉ) Reference laboratory for Salmonellosis, Public Health Agency of Canada (PHAC), Guelph, Ontario, Canada for serotyping. Serological characterization was performed at the O (somatic) and H (Flagellar) antigens levels. The tests for serological identification were carried out by the slide agglutination using a micro-technique that employs microtitre plates. Naming of the identified serovars was made according to the antigenic formulae proposed by **Popoff (2001).**

Antimicrobial susceptipility testing

Salmonella isolates were tested by the disc diffusion method for antimicrobial susceptibility following the guidelines of the National Committee for Clinical Laboratory Standards National Committee for Clinical Laboratory Standards, **(NCCLS, 2000).** The antimicrobial drugs tested were amikacin, amoxicillin-clavulanic acid, ampicillin, apramycin, chloramphenicol, cefoperazone, ceftazidime, cefotaxime, colistin, furazolidone, gentamicin, nalidixic acid, ciprofloxacin, neomycin, streptomycin, sulfamethoxazole-trimethoprim, compound sulfonamide and tetracycline.

Pulsed filed gel electrophoresis (PFGE)

PFGE was performed according to the protocol of the Centers for Disease Control and Prevention (CDC) PulseNet protocol **(CDC, 2002).** Agarose-embedded DNA was digested with 50 U of the enzyme *Xba*I (Promega, Southampton, UK). DNA restriction fragments were separated by PFGE on 1% SeaKem Gold agarose (Cambrex, Bio Science Rockland Inc., USA) using 0.5X Tris-Borate-EDTA extended-range buffer (Bio-Rad, Hercules, USA) with recirculation at 14 WC in a CHEF DRIII system (Bio-Rad, Hercules, USA). DNA from *Salmonella* Braenderup H9812 restricted with *Xba*I was used as a size marker. Pulse times were ramped from 2.2 to 63.8 s over 18 h with an angle of 120W at 6.0 V cm_1. Genomic-DNA profiles or 'fingerprints' were analyzed using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium).

RESULTS

Presence of *Salmonella* spp.

During this study 5.6% (38/679) *Salmonella* isolates were isolated. Intestinal contents 11.4% (14/160) egg 7% (7/99), liver 5% (5/100) skin 5% (10/200), feed 0% (0/40%), water 0% (0/100).

Serotyping

The results obtained led to recognition of eight serovars within the 38 isolates. The detected serovars were: Kentucky, 11 (29.0%), Stanleyville, 8 (21.1%), Virchow, 6 (16.0%), Alachua, 5 (13.2%), Blockley, 4 (10.5%), Hadar, 2 (5.3%), Typhimurium, 1 (2.6%) and Havana, 1 (2.6%).

Antimicrobial susceptipility of *Salmonella* isolates

The 38 isolates were found to be resistant to tetracycline (52.6%), nalidixic acid (50.0%), compound sulfonamide (44.7%), sulfamethoxazole-trimethoprim (31.6%), streptomycin (26.3%), gentamycin (15.8%), neomycin (15.8%), furazolidone (7.9%), ampicillin (5.3%), ciprofloxacin (5.3%), amikacin (2.6%), amoxicillin-clavulanic acid (2.6%), ceftazidime (2.6%), and colistin (2.6%) in decreasing order (Fig 1).
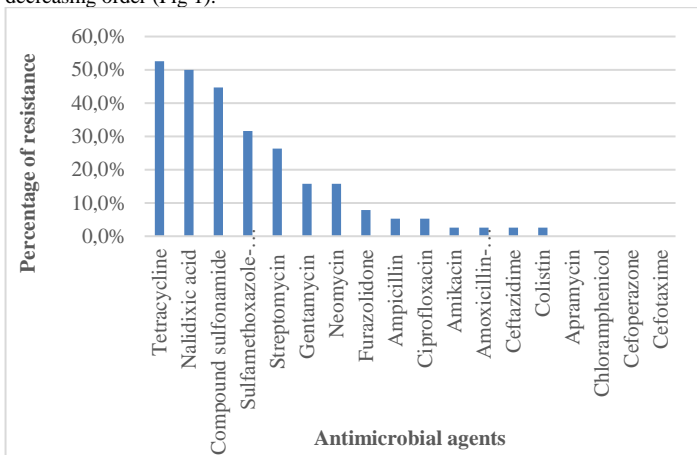

Figure 1 A histogram showing percent resistance of *Salmonella* isolates to 18 different antimicrobial agents.

Table 1 Multiple drug resistance pattern of the *Salmonella* isolates.

Isolate No.	Serovar	Pattern of resistance
S.2	Kentucky	TE,CN,NA,S
S.5	Virchow	TE,NA,FR,SXT,CS
S.6	Virchow	TE,NA,FR,SXT,CS
S.7	Virchow	TE,NA,FR,SXT,CS
S.8	Alachua	CAZ, CT, AMP
S.9	Blockley	TE, N, NA, ST
S.10	Stanleyville	TE, SXT, CS
S.12	Stanleyville	N, SXT, CS
S.13	Kentucky	TE, CN, NA, ST, CS
S.14	Kentucky	TE, CN, NA, ST, CS
S.16	Virchow	TE, NA, SXT, CS
S.17	Virchow	TE, NA, SXT, CS
S.22	Stanleyville	N, ST, AMP
S.23	Stanleyville	TE, NA, SXT, CS
S.25	Stanleyville	TE, SXT, CS
S.27	Kentucky	TE, CN, NA, ST, SXT, CS
S.31	Kentucky	TE, CN, NA, ST, CS
S.34	Kentucky	TE, CN, NA, ST, CS
S.37	Virchow	TE, NA, SXT, CS
S.38	Havana	TE, SXT, CS

TE,Tetracycline; N, Neomycin; CN, Gentamicin; NA, Nalidixic acid; FR, Furazolidone; ST, Streptomycin; SXT, Sulfamethoxazole-trimethoprim;; CAZ, Ceftazidime; CT, Colistin; AMP, Ampicillin.

None of the isolates was found to be resistant to all of the antimicrobial agents used in this study while five isolates; were susceptible to all the 18 different antimicrobial agents used. All of the *Salmonella* isolates were susceptible to chloramphenicol, cefoperazone, cefotaxime and apramycin.

Table 1 shows the multiple drug resistance profile for some of the *Salmonella* isolates. Multiple drug resistance was recorded whenever an isolate was found to be resistant to three or more antibiotics. Among the 38 isolates tested for antibiotic susceptibility, 20 isolates (52.6%) demonstrated multiple drug resistance. Multi-drug resistance was predominantly seen among the most prevalent serovars mainly Virchow (15.8%), Kentucky (15.8%) and Stanleyville (10.5%). Only one isolate (2.6%) of each of the serovars Blockley, Alachua and Havana showed multidrug resistance.

PFGE analysis

The 38 *Salmonella* isolates collected in this study were subjected to PFGE analysis. The fingerprints generated by PFGE using restriction enzyme *Xba*I. Four isolates did not produce any restriction bands even with repeated trials with other isolates and the standard control. In isolates S.1, S.6 and S.11 apparently, there is a problem with plug preparation while in S.7 the plug was nicely prepared, however, no restriction bands were observed

Fig.2 is a dendrogram produced by BioNumerics soft ware. Analysis of *Xba*I restricted DNA of all the *Salmonella* isolates in this study. Twenty two PFGE patterns (X1 to X22) were observed among the 34 typable Strains. *Xba*I restriction bands of the isolates were typically composed of 11 to 16 fragments; the most common patterns are X1 and X4 each comprised of 3 strains. Considerable genetic diversity clearly exists among the strains. Fig 2 shows that the percentage of similarity between different patterns ranged between 58.9 to 100%. Nine profiles with 100% similarity are composed of isolates of the same serotype. Moreover, PFGE showed consistency of PFGE profiles with serotyping as no isolates within the same PFGE pattern were of different serotypes.
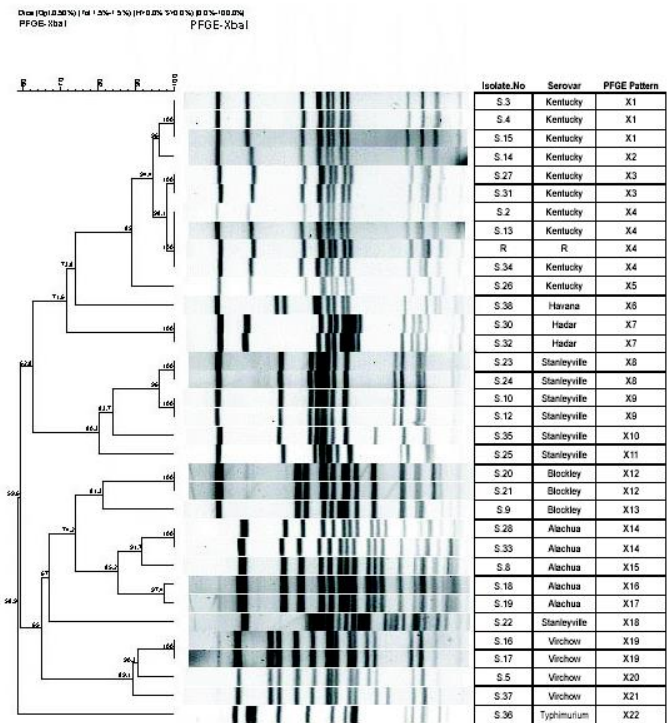

Figure 2 Represantative *Xba*I PFGE patterns and clustering analysis of *Salmonella* isolates recovered from poultry. Isolate. No, Serovars identified and PFGE pattern are indicated at the right. Dendrogram was generated by BioNumerics software using Dice coefficient and unweighted pair group method with arithmetic averages (UPGMA). Numbers at dendrogram roots show similarity value (similarity percentage) among identified clusters

DISCUSSION

Prevalence of *Salmonella* in poultry, 5.6% in this study, is comparably higher than most earlier reported figures for prevalence in Sudan. While **Yagoub (1986)** reported a 3.9% prevalence, **Fadlalla (2003)** reported prevalence within the range of 0.4% of the examined specimens. However, prevalence of *Salmonella* in this study is lower than the 7.3% prevalence reported by **Abu Elbashar (1996).**

Apparently, intestinal contents are the highest *Salmonella* harboring parts demonstrating incidence of *Salmonella* spp. up to 11.4 %. This finding is in agreement with earlier reports that *Salmonella* concentrate in the gastrointestinal tract **(Rasschaert *et al*., 2007; Van Schothorst and Notermans, 1980).**

Eggs were second in rating of prevalence with a 7% incidence. Eggs are known as the main source of transmission of *Salmonella* to humans **(Baumler *et al*., 2000).** Liver and skin demonstrated similar rates of contamination with a value of 5% prevalence. Ten (5%) of skin specimens were found to be contaminated by *Salmonella*. Isolation of *Salmonella* spp. from poultry livers in this study is in agreement with reports of **Procura et al. (2019).**

The high rates of resistance reported in this study can be partially attributed to the widespread use of antibiotics agents given to poultry. The massive use of antimicrobial agents for medical and veterinary purposes creates a selective pressure which leads to an increasing development of antibiotic resistance worldwide **(Mateu and Martin, 2001).**

In this study resistance to Tetracycline was the most common resistance. Tetracycline has been one of the most commonly used antibiotics for animal production **(Carraminana *et al*., 2004).** Resistance to β-lactams antibiotics was manifested by one isolate showed resistance to amoxicillin-clavulanic-acid and ceftazidime.

Seventeen isolates (44.7%) were found to be resistant to sulfonamides; compound sulfonamide and sulfamethoxazole-trimethoprim. The sulfonamides are broad-spectrum antimicrobial agents, but their antimicrobial activity is significantly limited by the resistance that has developed after more than 50 years of use **(Prescott, 2000).**

CONCLUSION

This study showed that presence of *Salmonella* spp. in poultry and detection of eight serovars which are related to poultry. The high rates of resistance reported in this study can be partially attributed to the widespread use of antibiotics agents given to poultry. The massive use of antimicrobial agents for medical and veterinary purposes creates a selective pressure which leads to an increasing development of antibiotic resistance worldwide. The PFGE analysis showed different patterns.

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