Vol. 12(2), pp. 85-90, April-June 2020
DOI: 10.5897/JVMAH2020.0837
Article Number: 2045E3564003
ISSN: 2141-2529

http://www.academicjournals.org/JVMAH

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Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Occurrence and antimicrobial resistance of *Salmonella* spp. in broiler chicken neck skin from slaughterhouses in Zambia

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Received 8 April, 2020; Accepted 26 May, 2020

Salmonella **is an important zoonotic foodborne pathogen and poultry meat is considered as one of its major sources. This study evaluated** ***Salmonella*** **spp. detected in broiler chicken carcasses in Zambia. A total of 440 broiler neck skin samples were collected from 6 slaughter houses along the process line after evisceration and tested for** ***Salmonella*** **spp. Eleven samples (2.5%) were positive for** ***Salmonella*** **spp. The suspected isolates were serotyped according to White- Kauffmann-Le Minor scheme and tested for antimicrobial susceptibility using the Sensititre broth microdilution method. Eight serovars of** ***Salmonella enterica*** **were confirmed namely;** ***S.*** **Bolton (2),** ***S.*** **Enteritidis (1),** ***S.*** **Texas (1),** ***S.*** **Liverpool (1),** ***S.*** **Chomeday (1),** ***S.*** **Mbandaka (1),** ***S.*** **Vellore (1),** ***S.*** **Montevideo (1). Two isolates were not typed completely giving results as** ***S. enterica*** **subsp.** ***enterica*** **O:4:Z and** ***Salmonella enterica*** **subsp.** ***enterica*** **O:3,10:Y. Antimicrobial susceptibility showed a 20% multidrug resistance in which** ***S. Vellore*** **and** ***S. Mbandaka*** **were resistant to 5 antimicrobials namely Ampicillin, Ciprofloxacin, Gentamicin, Tetracycline, Trimethoprim.** ***S. Enteritidis*****,** ***S. Bolton*** **and** ***Salmonella enterica*** **subsp.** ***enterica*** **O:3, 10:Y were resistant to the antimicrobial Colistin. 50% of the strains were susceptible to the antimicrobials tested. This study reported** ***Salmonella*** **spp. in broiler chickens that have not been reported before in Zambia and showed the presence of antimicrobial resistant strains.**

Key words: Salmonella serovars, foodborne disease, broiler chicken, antimicrobial resistance.

INTRODUCTION

Salmonella, a genus of bacterium, is one of the common and important zoonotic foodborne pathogens responsible for foodborne disease outbreaks and illnesses in humans worldwide (Mylrea et al.,, 2010; Cassini et al., 2016). It is widely known for causing non-typhoidal foodborne infections and enteric, typhoid fever in humans. It can be

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severe leading to hospitalization and death in some cases (Jones et al., 2008).

Poultry meat is considered as one of the major sources of *Salmonella* spp. in foodborne disease outbreaks (Daum et al., 2002; Barua et al., 2014) which acts as an important source in transmission of various zoonotically important serotypes of *Salmonella* spp. through food to humans (Barua et al., 2014). Chicken meat might provide the main source of human infection by *Salmonella*, especially with the increasing consumers' demand and production for this food item in many countries including Zambia.

Contamination of chicken meat by *Salmonella* can occur via several means such as cross-contamination of the carcases with faeces, water, instruments and workers' hands during the slaughtering and dressing processes (Sanchez et al., 2002; Magwedere et al., 2015). There are over 2500 serovars of *Salmonella* that have been identified worldwide according to the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). Majority of the serovars belonging to *Salmonella* enterica subspecies enterica (*Salmonella enterica subsp. enterica)* have been reported in food producing animals (Ishihara et al., 2009; Mathole et al., 2017; Gelaw et al., 2018). Of the serovars, *S. enteritidis* and *S. typhimurium* are the most commonly reported serovars (Johnson et al., 2011; Olobatoke and Mulugeta, 2015).

In the poultry industry, however, the two, host specific, poultry *Salmonella* pathogens causing high mortality and economic losses are *Salmonella enterica* subsp. *Enterica serovar Gallinarum biovar* gallinarum (*Salmonella* Gallinarum) known to cause fowl typhoid, and *Salmonella* enterica subsp. *Enterica serovar Gallinarum biovar* pullorum (*Salmonella* Pullorum), that causes pullorum disease. Many developed countries have eradicated these pathogens from commercial flocks through implementations of poultry improvement plans (Barrow and Freitas, 2011). The pathogens continue to affect poultry in many other developing countries leading to big economic losses due to destruction of bird flocks (Barrow and Freitas, 2011; Pulido-Landínez et al., 2014; Sannat et al., 2017).

Previous studies have reported *Salmonella* species and various *Salmonella* serovars in chickens in Zambia, possibly more than 20 (Isogai et al., 2005; Mpundu et al., 2019). Serovars *S. enteritidis*, *S. typhimurium* and *S. infantis* have been reported as important non-typhoidal causes of human salmonellosis in Zambia associated with consumption of contaminated food (Chiyangi et al., 2017).

Antimicrobial resistance (AMR) of *Salmonella* spp.is a global concern and studies have shown that *Salmonella* serotypes are resistant to several antibiotics (Mir et al., 2015; Nair et al., 2018). The AMR of *Salmonella* spp. is associated with the use of antibiotics in animals raised for food. Antibiotics are extensively used in the animal production systems to promote growth, prevent, treat, and control infectious diseases; and indiscriminate use of antimicrobials, administration of sub-therapeutic dose and self-medication could have contributed to the development of drug-resistant bacteria (McEwen and Fedorka-Cray, 2002). Resistant bacteria can be transmitted to humans through foods of animal origin. A case of antimicrobial resistant *Salmonella* involving *S*. *sefteinberg*, leading to death was reported in Zambia (Hendriksen et al., 2013).

Monitoring of *Salmonella* in livestock and livestock products is absent or poor in most resource-limited countries including Zambia, making people more vulnerable to various non-typhoidal *Salmonella*-contaminated food. This study focused on detection, characterization and antimicrobial susceptibility testing of *Salmonella* spp isolated from chickens meant for human consumption from slaughter houses in Zambia. The knowledge gained can be used to aid in suggesting proper, effective therapeutic measures and providing a baseline data that could be used in the development of effective strategies for control of *Salmonella* spp along the entire food chain.

MATERIALS AND METHODS:

Collection of samples

Samples consisted of chicken neck skin of broiler carcasses meant for human consumption as meat. A total number of 440 samples were collected from the 6 main chicken slaughter houses in Zambia from June 2018 to January 2019. In each slaughter house, chicken neck skins were collected from freshly dressed broiler carcasses along the process line, after evisceration, both before and after the hot wash. The neck skins were collected at random, and immediately placed into sterile polyethylene bags. They were numbered, stored in a cool box with ice between 4-8°C and transported to the laboratory at Central Veterinary Research Institute (CVRI).

Isolation of *Salmonella* spp

Samples were prepared for *Salmonella* testing within 24 h after sampling, using the method described by OIE (2018) with minor alterations. Colonies presumptive of *Salmonella* spp. were selected and sub-cultured on nutrient agar (HI-Media, Mumbai, India) between 34 and 38°C for 24 h. The colonies were subjected to biochemical tests using Triple sugar/iron (TSI) agar (Titan Biotech Ltd, Rajasthan, India), slants to observe the triple sugar iron reaction, lysine decarboxylase (Oxford lab chem, Navghar, India), Urea agars (Sigma, St Loius, USA) slants and a Liofilchem®EnteroPluri-Test. The reactions were observed for typical *Salmonella* characteristics after incubation at 36°C± 1 for 24 h. The presumptive *Salmonella* isolates were stored in micro bank vials until further processing.

Serotyping

Serological typing of the isolates for the O and H *Salmonella* antigen was carried out from colonies on nutrient agar and TSI slants using the White-Kauffmann-Le Minor scheme slide agglutination method that generates a formula to differentiate the serovars (Grimont and Weill, 2007). A loopful of normal saline was placed on a clean glass slide, followed by addition and mixing with a colony from nutrient agar and TSI slant until a smooth opaque suspension was formed. A drop

or two of anti-salmonella A-61+viomnivalent (Sifin) serum was added to the suspension and mixed for a few seconds. Bacterial suspensions that remained homogenous were considered negative and those that agglutinated were considered positive reactions confirming the presence of *Salmonella* spp. The strains with positive reactions were then typed with polyvalent O antisera (OMNT + Poly A-E+vi + 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 19, 20, 27, 46) followed by individual monovalent O and H antisera pools to obtain the identification of the serovar.

Antimicrobial susceptibility testing

Confirmed strains of *Salmonella* spp. were tested for resistance and susceptibility to 12 antimicrobials (ampicillin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, colistin, gentamycin, meropenem, nalidixic acid, tetracycline, tigecycline and trimethoprim) using the Sensititre broth microdilution method (TREK® Diagnostic systems). Briefly, using a sterile swab, about 2 to 3 colonies were emulsified in demineralised sterile water and adjusted to 0.5 McFarland standard using a Sensititre® nephelometer. About 10 ul of the bacterial suspension was transferred into a tube of cation adjusted Mueller-Hinton broth of 11 ml to give an inoculum of approximately $1x10^5$ cfu/ml and mixed. The tubes were closed with sensititre® single use dose heads. 50 ul was transferred into each well of Sensititre™EUVSEC plates (Piastraantibiogramma EUVSEC per *Salmonella* spp. and E.coli) using a Sensititre®autoInoculator®. After inoculation, the wells were carefully covered using adhesive seal tape. The plates were incubated at 37°C for 24 h. The plates were read and interpreted using the Vision® machine. Growth appeared as a deposit of cells at the bottom of the wells. The positive growth control wells were read first. Any plates with no growth in the positive growth wells were considered as invalid.

Data management

Original research data were captured in a dedicated Microsoft® Excel spreadsheet for subsequent analysis. Descriptive statistics was employed to obtain values of proportions and percentiles.

RESULTS

Isolation of *Salmonella* species

From a total of 440 samples collected, 11 (2.5%) samples were positive for *Salmonella* spp. from 3 of the 6 slaughter houses. The isolates had typical pink, round colonies with a black centre and surrounding transparent zone on XLD agar; pinkish cream colonies on BGA and on Rambach agar revealed reddish-pink colonies. Other bacteria with similar characters to *Salmonella* spp. were also observed and a Vitek 2 analysis revealed presence of *Proteus*, *Citrobacter* and *Pseudomonas* species in the carcases. Further analysis of these bacteria was not done as it was not part of the study.

The confirmed isolates on biochemical tests showed typical characteristics of *Salmonella* spp. that included an alkaline slant, acidic butt with blackish discolouration of varying degrees on TSI. All were lactose and urea negative. All were positive for lysine decarboxylation and arabinose except *S*. Liverpool which was negative for both tests.

Serotyping

The antigenic typing of *Salmonella* using the White-Kauffmann-Le Minor scheme identified 10 different serovars isolated from the slaughter houses summarised in Table 1. Two isolates could not be completely serotyped to serovar level and are reported as *Salmonella enterica* subsp. *enterica* 4: Z and *Salmonella enterica* subsp. *enterica* 3,10: Y based on the antigen detected.

Antimicrobial susceptibility testing

The results of antimicrobial susceptibility of the *Salmonella* serovars from the slaughter houses are summarised in Table 2. The serovars *S*. Vellore and *S.* Mbandaka were found to be resistant to 5 out of the 12 antimicrobials tested. These included ampicillin, ciprofloxacin, gentamicin tetracycline and trimethoprim. The serovars *S*. Bolton, *S*. Enteritidis and *S*. *enterica* subsp *enterica*3,10:y were resistant to colistin. There was no antimicrobial resistance detected against cefotaxime, ceftazidime, chloramphenicol, meropenem, nalidixic acid and tigecycline.

DISCUSSION

In this study, *Salmonella* spp. were detected in 11(2.5%) of the 440 samples of chicken neck skins collected from the major poultry slaughter houses in Zambia. Of the 11 isolates, 10 different serovars of *Salmonella* spp. were identified. The findings of this study are similar to that of Mpundu et al. (2019) who found a 2.5% prevalence of *Salmonella* spp. in 2 poultry slaughter houses in Zambia.

Despite the low proportion of *Salmonella* detected in this study, these findings highlight the role that these food processing facilities may play in the spread of this bacterium and may be one of the major contributors to diarrhoea diseases in humans (Kagambèga et al., 2013). Higher levels have been reported in chicken carcases in Cameroon (60%) (Nzouankeu et al., 2010), Egypt (80%) (Hassan et al., 2016) and Ethiopia (17.9%) (Tibaijuka et al., 2003). Contamination of chickens in the slaughter houses could be attributed to several factors such as cross-contamination of the carcases with faeces during evisceration, water, instruments and workers' hands during the slaughtering and dressing processes.

This study reports a diversity of 10 *Salmonella* serovars, from the 11 isolates detected. Detection of a diversity of *Salmonella* serovars is not uncommon and has been similarly reported in earlier studies. Nigeria reported 82 serovars from 370 *Salmonella* isolates detected on poultry commercial farms (Fagbamila et al., 2017), 13 different

Table 1. A summary of the slaughter houses and the serovars detected.

Sampling site	*Salmonella* serovar	Antigenic formular
Slaughter house 1	*Salmonella enterica* subsp. *enterica* serovar Bolton(*S*. Bolton)	3, 10: y : e, n, z_{15}
	Salmonella enterica subsp. *enterica* serovar Enteritidis (*S*. Enteritidis)	1, 9, 12: g, m
Slaughter house 2	*Salmonella enterica* subsp. *enterica* serovar Chomedy (*S*. Chomeday)	8, 20: z10, e, n, z_{15}
	Salmonella enterica subsp. *enterica*	4: z
	Salmonella enterica subsp. *enterica* serovar Montevideo (*S*. Montevideo)	6, 7, 14: g, m, s
	Salmonella enterica subsp. *enterica* serovar Texas (*S*. Texas)	4: k: e, n, z_{15}
Slaughter house 3	None	None
Slaughter house 4	*Salmonella enterica* subsp. *enterica* serovar Bolton (*S*. Bolton)	03:10: y: e, n, z_{15}
	Salmonella enterica subsp. *enterica*	3, 10: y
	Salmonella enterica subsp. *enterica* serovar Liverpool (*S*. Liverpool)	1,3,19: d: e, n, z_{15}
	Salmonella enterica subsp. *enterica* serovar Mbandaka (*S*. Mbandaka)	6, 7, 14: z_{10}: e, n, z_{15}
	Salmonella enterica subsp. *enterica* serovar Vellore (*S*.Vellore)	1, 4, 12, 27: z_{10}: z_{35}
Slaughter house 5	None	None
Slaughter house 6	None	None

Table 2. Antimicrobial resistance of *Salmonella* serovars isolated from the slaughter houses in Zambia.

Serovar	AMP 1-64	CHL 8-128	CIP 0.03-8	COL 1-16	NAL 4-128	FOT 0.25-4	TET 2-64	TGC 0.25-8	TAZ 0.5-8	MERO 0.06-16	TMP 0.25-32	GEN 0.5-32
S. Enteriditis	S	S	S	**R**	S	S	S	S	S	S	S	S
S. Bolton	S	S	S	**R**	S	S	S	S	S	S	S	S
S. Chomeday	S	S	S	S	S	S	S	S	S	S	S	S
S. Montevideo	S	S	S	S	S	S	S	S	S	S	S	S
S. Enterica 4:z	S	S	S	S	S	S	S	S	S	S	S	S
S. Texas	S	S	S	S	S	S	S	S	S	S	S	S
S.Enterica3,10:Y	S	S	S	**R**	S	S	S	S	S	S	S	S
S. Vellore	**R**	S	**R**	S	S	S	**R**	S	S	S	**R**	**R**
S. Mbandaka	**R**	S	**R**	S	S	S	**R**	S	S	S	**R**	**R**
S. Liverpool	S	S	S	S	S	S	S	S	S	S	S	S

R= Resistant, S= Susceptible; AMP= Ampicillin; CHL= Chloramphenicol; CIP= Ciprofloxacin; COL= Colistin; FOT= Cefotaxime; GEN= Gentamicin; MERO= Meropenem; NAL= Nalidixic Acid; TAZ= Ceftazidime; TET= Tetracycline; TGC= Tigecycline; TMP= Trimethoprim.

serovars detected from 32 isolates in France poultry slaughter houses (Hue et al., 2011) and 14 different serovars detected from 32 isolates in an Indian study from poultry species (Mir et al., 2015). The serovars isolated in this study, with the exception of *S.* Enteritidis and *S.* Mbandaka, have not been reported in chickens in Zambia to the best of the authors' knowledge.

The isolation of *S. enteritidis* in chicken carcases in this study raises great public health concern as it is a well-recognized pathogen that causes food poisoning in man. Infections caused by *S. enteritidis* have been one of the major causes of non-thyphoid food-borne *Salmonellosis*, alongside the serovars *S. typhimurium* with several reports world-wide (Braden, 2006; Niehaus et al., 2011; Muvhali et al., 2017).

Other serovars reported in this study have also been implicated to cause non-typhoid salmonellosis. *S*. Mbandaka was reported to infect several people in a case in Australia and three people were hospitalised (Scheil et al., 1998). The source of the pathogen was traced to jars of peanut butter. *S*. Montevideo was reported in *Salmonella* outbreaks in Australia and New Zealand with the pathogen

traced to a sesame seed based food (Unicomb et al., 2005). In the United States of America, S. Montevideo has also commonly been associated with human infections over the recent years (Foley et al., 2008). The recovery of pathogenic serovars in food products shows the need to implement strict hygiene along the production line.

Results in this study show AMR of *Salmonella* spp. to ampicillin, ciprofloxacin, colistin, gentamicin, tetracycline and trimethoprim. Two (20%) of the serovars (*S.* Mbandaka and *S*. Vellore) were resistant to 5 of the antimicrobials namely ampicillin, ciprofloxacin, gentamicin, tetracycline and trimethoprim. Three (30%) serovars *S*. Bolton, *S*. Enteritidis and *S*.enterica sub. spp. enterica 3,10:y were resistant to the antimicrobial colistin, while five (50%) were susceptible to all the antimicrobials they were subjected to.

Resistance of *Salmonella* spp. to β-lactam antibiotics such as ampicillin has similarly been reported in other studies (Diarra et al., 2014; Yoon et al., 2017), with as high as 43% of the isolates being resistant .β-lactam antibiotics are among the commonly prescribed drugs in humans hence the isolation of pathogens with such characteristics causes worry to the community.

Resistance of *Salmonella* spp. to gentamycin, tetracyclines, trimethoprim and colistin has also been reported previously (Cardoso et al., 2006; Quesada et al., 2016; Liljebjelke et al., 2017). The reports also show a multi drug resistant pattern ranging between 28-43%. This is in line with the current study which has demonstrated a 20% pattern of multidrug resistance.

Results of the current study have shown that *S. enteritidis* was resistant to colistinonly. In contrast, *S. enteritidis* serovars have been reported to be resistant to several other antimicrobials that include ampicillin, chloramphenicol and tetracycline in other studies (Cardoso et al., 2006; Diarra et al., 2014; Yoon et al., 2017). The low resistance in our study could be attributed to the low number of the serovars isolated and assessed. This study shows a serious need of continuous monitoring, surveillance and quality inspection programs for the prevalence of *Salmonella* spp. and its resistance in the food chain supply because of the public health implications of a potential spread of resistant microorganisms. Efforts should be made to educate producers, retailers, and consumers on the proper handling and cooking of chicken meat to reduce salmonella infections.

Conclusions

This study demonstrated the presence of *Salmonella* spp. in broiler chicken carcases slaughtered for human consumption in abattoirs in Zambia and the presence of antimicrobial resistance *Salmonella* serovars. Continuous surveillance and monitoring of *Salmonella* not only in livestock but throughout the food chain needs to be enhanced together with laboratory diagnosis of *Salmonella*.There is need to extend this research to other small scale slaughter houses as well as other districts.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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