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Research Article

Phenotypic Identification and Antimicrobial Susceptibility Profile of *Salmonella* from Local and Exotic Chicken in Maiduguri, Nigeria

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Abstract

Background and Objectives: *Salmonella* has been regarded as one of the most common causes of food borne diarrhoeal diseases, as well as important zoonoses worldwide. It is transmitted through contaminated food such as; meat, poultry eggs or egg products. The objectives of this study were to isolate, phenotypically identify and determine the antimicrobial susceptibility profile of *Salmonella* associated with faeces and cloaca of local and exotic chicken in Maiduguri, Borno state, Nigeria. **Materials and Methods:** A total of 160 faecal and cloacal swab samples of local and exotic chickens in Maiduguri were analyzed. Eighty samples each from male and female local and exotic chicken were collected from Monday market and University of Maiduguri, respectively by using purposive sampling technique. Samples were processed according to standard bacteriological procedures. **Results:** Overall percentages of *Salmonella* from Maiduguri were found to be 1.88% and 3.13% for males and females, respectively. *Salmonella* from local chicken had the highest level of sensitivity to Nitrofurantoin (33.30%) and lowest to Ofloxacin (11.10%) and Ciprofloxacin (11.10%). The highest resistance of 100.00% was associated with Ceftazidime, Cefuroxime, Gentamicin, Cefixime and Augmentin, while 66.60% for Nitrofurantoin was found to be the lowest level of resistance. **Conclusion:** The study has demonstrated the presence of *Salmonella* in faeces and cloacal swabs of local and exotic chicken and their antimicrobial susceptibility profile in Maiduguri, Borno state, Nigeria.

Key words: *Salmonella*, chicken, antimicrobial, susceptibility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salmonella are Gram-negative bacteria, facultative anaerobes, catalase positive, oxidase negative and are motile with peritrichous flagella, with the exception of *Salmonella* Gallinarum and *Salmonella* Pullorum that are non-motile. The occurrence of infection with *Salmonella* is primarily via faecal-oral route and human infections with non-typhoidal *Salmonella* are commonly caused by ingestion of food that has been contaminated with animal faeces¹. Salmonellosis is diagnosed by following the isolation of the causative agent from faecal culture, blood, urine and food samples^{2,3}.

Salmonella has been labeled as the most common and important zoonoses since 1950 according to World Health Organization (WHO) and the Food and Agriculture Organization (FAO). This has led to its inclusion in the terrestrial animal health code of the World Animal Health Organization⁴.

Salmonella is one of the most common causes of food borne diarrhoeal diseases worldwide and can be transmitted through contaminated food such as; meat, poultry eggs or egg products⁵. Avian host specific salmonellae include *Salmonella* Gallinarum and *Salmonella* Pullorum and are associated with fowl typhoid and pullorum, respectively⁶. Salmonellosis is an important disease of chicken caused by the organism in question and is found all over the world. Therefore, consumption of raw or undercooked contaminated poultry products with *Salmonella* can induce acute gastroenteritis in humans resulting in public health concerns even in Nigeria⁷.

The purpose of the present study was to determine the presence of *Salmonella* in local and exotic chicken and their antimicrobial susceptibility profile in Maiduguri, Borno state, Nigeria.

MATERIALS AND METHODS

Study area: The study area is Maiduguri, Borno state which is located in the North-Eastern, arid zone of Nigeria. It lies within latitude 11°50'46"N and longitude 13°08'29"E. Maiduguri is the capital of Borno state and is centrally located in the map of the state⁸.

The study was carried out in Veterinary Microbiology Laboratory, Bacteriology Unit of the Department of Veterinary Microbiology, University of Maiduguri from April-September, 2019.

Sampling and sample collection: A total of 160 samples were collected from both male and female chickens in Monday

market and exotic chicken from University of Maiduguri poultry farm by using purposive sampling technique⁹. Eighty cloacal swab samples were collected from University of Maiduguri poultry farm, while on the other hand, 80 faecal samples were obtained from chicken at slaughter slabs in Monday market. All samples were collected aseptically and placed into a sterile container, then labeled properly and kept in a cool box containing ice blocks and then transported to the Veterinary Microbiology Laboratory, University of Maiduguri for processing.

Isolation and Identification of *Salmonella*: Samples of faecal and cloacal swabs were processed based on standard protocols¹⁰. Faecal samples and cloacal swabs were inoculated into 5 mL of prepared selenite F broth and incubated at 37°C for 18-24 h. The growths in peptone water were sub-cultured onto *Salmonella-shigella* agar (SSA) followed by incubation at 37°C for 24-48 h. The isolates were further sub-cultured onto nutrient agar slants incubated at 37°C for 18 h and were stored at 4°C until required.

Biochemical tests: This was done based on standard techniques in which all isolates that gave reactions typical of *Salmonella* were inoculated onto various biochemical test media such as; triple sugar iron agar, simon citrate agar and urease agar slants and were incubated at 37°C for 24 h. Pure colonies on SSA that indicated the characteristic features of *Salmonella* were subjected to Gram's staining and examined under the microscope at ×100 magnification.

Antimicrobial susceptibility profile: The antimicrobial susceptibility test was carried *in vitro* with Gram-negative multi-susceptibility discs to determine the antimicrobial susceptibility of *Salmonella* isolates to Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Ofloxacin (OFL), Cefixime (CXM), Augmentin (AUG), Nitrofurantoin (NIT) and Ciprofloxacin (CPR). Two milliliters of peptone water containing the isolates were spread on the nutrient agar plate and antimicrobial discs were placed at the centre of the media and gently pressed down onto the agar with sterile forceps to ensure complete contact with the agar surface and then incubated at 37°C for 24 h. The zone of inhibition was measured and recorded¹¹.

RESULTS

Occurrence of *Salmonella* isolates in faeces and cloacal swabs of chicken in Maiduguri: The occurrence of *Salmonella* isolates in faeces and cloacal swabs of chickens in Maiduguri

Table 1: Occurrence of *Salmonella* isolates in faeces and cloacal swabs of local and exotic chickens in Maiduguri

Location	Total no. of collected	Identity of positive samples	No. of positive (%)	
			Selenite F	SSA
Monday market	80	LCM 7, 8, 20-40 LCF 3, 4, 6, 8, 20-40	50 (62.50)	8 (10.00)
University of Maiduguri	80	ECM 26, 27, 30-36 ECF	9 (11.25)	0 (0.00)
Total	160		59 (36.88)	8 (5.00)

LCM: Local chicken male, LCF: Local chicken female, ECM: Exotic chicken male

Table 2: Occurrence of *Salmonella* isolates according to sex of chickens in Maiduguri

Location	Total no. of collected	No. of positive males (%)	No. of positive females (%)
Monday market	80 local chickens	3 (3.75)	5 (6.25)
University of Maiduguri	80 exotic chickens	0 (0.00)	0 (0.00)
Total	160	3 (1.88)	5 (3.13)

n = 5.00% (8/160)

Table 3: Phenotypic identification of *Salmonella* by using biochemical tests

Location	Total no. of collected	Sample identity	No. of positive males (%)			No. of positive females (%)		
			TSI	Ure	Cit	TSI	Ure	Cit
Monday market	80	LCM 7	1	1	-	-	-	-
		LCM 8	1	-	-	-	-	-
University of Maiduguri	80	LCF 3	-	-	-	-	1	1
		LCF 4	-	-	-	1	1	1
		LCF 6	-	-	-	1	1	-
		LCF 7	-	-	-	1	1	-
Total	160		2 (1.25)	1 (0.63)	0 (0.00)	3 (1.88)	4 (2.50)	2 (1.25)

TSI: Triple sugar iron, Ure: Urease, Cit: Citrate

Table 4: Antimicrobial susceptibility profile of *Salmonella* isolates from chickens in Maiduguri

Antimicrobials	Concentration (μ g)	No. of sensitive (%)	No. of resistant (%)
CAZ	30	0 (0.00)	9 (100.00)
CRX	30	0 (0.00)	9 (100.00)
GEN	10	0 (0.00)	9 (100.00)
CXM	5	0 (0.00)	9 (100.00)
OFL	5	1 (11.10)	8 (88.80)
AUG	30	0 (0.00)	9 (100.00)
NIT	30	3 (33.30)	6 (66.60)
CPR	5	1 (11.10)	8 (88.80)

CAZ: Ceftazidime, CRX: Cefuroxime, GEN: Gentamicin, OFL: Ofloxacin, CXM: Cefixime, AUG: Augmentin, NIT: Nitrofurantoin, CPR: Ciprofloxacin

was analyzed. The occurrence of *Salmonella* in selenite F broth and on *Salmonella-shigella* agar was found to be 50 (62.50%) and 8 (10.00%), respectively from 80 faecal samples collected from Monday market. Similarly, 9 (11.25%) from University of Maiduguri poultry farm were found to grow on selenite F broth, while no isolate was recovered on SSA. A total occurrence of *Salmonella* in selenite F broth and SSA was found to be 59 (36.88%) and 8 (5.00%), respectively (Table 1).

Occurrence of *Salmonella* isolates according to sex of chickens in Maiduguri: The occurrence of *Salmonella* isolates according to sex of chicken in Maiduguri has shown that the number of positive samples for *Salmonella* from Monday market was 3 (3.75%) for male chicken and 5 (6.25%) for their

female counter part. No positive *Salmonella* isolates were found to be associated with cloacal swabs from University of Maiduguri poultry farm. The overall percentage of positive *Salmonella* isolates from both locations with respect to individual sex were 3 (1.88%) for males and 5 (3.13%) for females. Additionally the overall percentage for *Salmonella* was 5.00% (8/160) (Table 2).

Phenotypic identification of *Salmonella* with biochemical tests: The percentages of positive values for triple iron agar (TSI) test, urease and citrate for isolates of *Salmonella* indicated that most of the isolates from local female chicken sampled from Monday market were positive for urease (2.50%) followed by TSI test (1.88%) and then citrate (1.25%).

Conversely, in local male chicken from the same location, the highest percentage was obtained with TSI test (1.25%) followed by urease (0.63%) while no positive isolates were found to give positive reaction with citrate. On the other hand, none of the *Salmonella* isolates gave positive reaction with all the 3 types of biochemical tests employed (Table 3).

Antimicrobial susceptibility profile of *Salmonella* isolates from chickens in Maiduguri:

The resistance and sensitivity profile were displayed by 9 *Salmonella* isolates to eight different antimicrobial agents. One *Salmonella* isolate from local chicken was sensitive to Nitrofurantoin, 3 (33.30%), followed by Ofloxacin, 1 (11.10%) and Ciprofloxacin, 1 (11.10%).

The highest resistance of 9 (100.00%) was displayed by Ceftazidime, Cefuroxime, Gentamicin, Cefixime and Augmentin, while the lowest percentage of resistance of 6 (66.60%) was observed with Nitrofurantoin (Table 4).

DISCUSSION

The occurrence of presumptive *Salmonella* on *Salmonella-Shigella* agar from 80 faecal samples collected from Monday market was found to be 58 (10.00%). However, no single isolate from faecal sample was recovered on SSA. The total occurrence of *Salmonella* on SSA was found to be 8 (5.00%). The pathogenic *Salmonella* has been a major concern to public health all over the world¹². It is one of the major causes of bacterial food-borne gastroenteritis of humans associated with bacterial infection in poultry. A total of 160 *Salmonella* isolates from faeces and cloacal swabs of chickens in Maiduguri was analyzed¹³. The isolation and detection of *Salmonella* followed standard protocols have previously been reported by Jajere *et al.*¹⁴, Fagbamila *et al.*¹⁵ and Raufu *et al.*¹⁶. Although, the laboratory media used for isolation in this study differ from those previously used. However, the media used such as; Rappaport vassiliadis and Xylose lysine deoxycholate agar were equally selective as *Salmonella-shigella* agar and growth of *Salmonella* were shown on each of these media. The percentage isolation rate associated with environmental samples, where *Salmonella* was detected in 5.14% (9/175) of water was in consonance with the findings of this study, but lower than 7.14% (5/70) percentage of detection from feed¹⁴. Although, the samples were of different types, the obtained findings indicated that *Salmonella* is associated with bacterial infection in the birds and the environment.

The overall percentage of *Salmonella* in Monday market and University of Maiduguri poultry farm in this present study was found to be 8/160 (5.00%). No *Salmonella* was isolated from University of Maiduguri poultry farm. This disagreed with the previous findings, where the overall prevalence of *Salmonella* in poultry samples from Teaching and Research Poultry Farm, University of Maiduguri indicated higher prevalence of 31.67%⁸. Understand that, this is the same location where higher percentage of *Salmonella* was reported. The reason for this improvement may not be unconnected with improvement in the management practices or biosecurity and possibly, careful use of antimicrobial agents on the farm.

Of all the nine *Salmonella* isolates subjected to eight antimicrobial agents, the highest resistance of 9 (100.00%) was displayed by ceftazidime, cefuroxime, gentamicin, cefixime and augmentin. The finding of this study was closely related to 80.0% resistance of *Salmonella* isolates to gentamicin, as earlier documented¹⁷. On the contrary, it was lower than 6.38% and 2.13% for gentamicin and augmentin which was earlier reported by Musa *et al.*⁸. Additionally, 7.14% resistance was also reported for *Salmonella* from poultry¹⁸. Antimicrobial resistance may be associated with subtherapeutic doses and frequent usage both in livestock and public health as relatively cheaper and commonly available agents. The reason for the infection by pathogens as observed in this study may be due to feco-oral or due to the contamination of poultry housing, equipment like; drinkers and feeders, which may also be associated with poor sanitary condition around the poultry house. As such, extensive system of poultry rearing needs to be discouraged. It was concluded that the recovery rate may be present in food safety problems if not adequately handled in terms of hygienic and cooking of poultry meat or their products. Finally, Nitrofurantoin, Ofloxacin and Ciprofloxacin may be the drugs of choice that may be recommended for treating salmonellosis in poultry. A connection has been established between the increase in the prevalence of *Salmonella* in poultry and the cases of human salmonellosis. Consequently, if contamination of poultry is minimized, it would further reduce human salmonellosis¹⁹.

CONCLUSION

This study has demonstrated that there was less *Salmonella* infection in exotic chicken vis-à-vis the local chicken. This may likely be as a result of proper hygienic practices in the environment where exotic chickens were kept. Similarly, good and proper personnel and environmental sanitary practices needs to be maintained in poultry houses,

farms and back yards poultry. This will further minimize the risk associated with zoonotic salmonellosis that may be transmitted to humans.

SIGNIFICANCE STATEMENT

This study discovered that *Salmonella* were only present in local chickens, but absent in exotic species indicating that the exotic chickens were well managed by ensuring adequate biosecurity and proper use of antimicrobial agents. This study will help the researchers to explore other areas of bacterial infection in chickens with respect to indigenous domesticated and wild chickens and possibly areas that other researchers were not able to explore. Thus, further research in this area may likely come up with interesting findings.

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