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Occurrence and Antibiogram of *Shigella* spp in Free Range and Intensively Reared Chickens in Nsukka, Enugu State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author IAC designed the study and corrected the manuscript. Author OOJ wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Both authors managed the analyses of the study and the literature search. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Shigellosis is considered a veterinary and public health problem of major importance. *Shigella* is implicated in food poisoning and bloody diarrhoea in humans and is an important cause of various diseases of livestock resulting in high morbidity and mortality. *Shigella* spp. is spread by direct contact with an infected host, or by eating contaminated food or drinking contaminated water. A cross-sectional survey was conducted to determine the occurrence of *Shigella* in free range and intensively reared chickens from markets and poultry farms in Nsukka, Enugu State, Nigeria, and to determine their antibiogram.
Materials and Methods: A total of 300 cloacal swabs from 150 free range and 150 intensively reared chickens, collected from 3 local markets and 3 farms respectively, were sampled. *Shigella* was isolated after passing the samples through pre-enrichment, selective enrichment and culture in a selective medium and identified using standard microbiological and biochemical methods.

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Antibiotic susceptibility test was performed by the disc diffusion method according to the CLSI method (Kirby-Bauer disk diffusion test) on Muller-Hinton agar.

Results: Out of the 300 samples, 10 (3.3%) were positive for shigellae. The occurrence in free-range chickens was 6.7%, while none was isolated in intensively reared chickens (0% occurrence). The *Shigella* spp. isolated were sensitive to ciprofloxacin (100%), moderately sensitive to ofloxacin (70%), gentamicin (70%), nalidixic acid (60%), cotrimoxazole (60%) and tetracycline (70%), and resistant to amoxicillin (100%) and augmentin (100%). The MIC ranges for cotrimoxazole, ciprofloxacin, gentamicin, and tetracycline, were 8-32, 0.015-0.25, 0.5-2.0, and 2.0-64.0 µg/ml, respectively. The MBC values obtained for ciprofloxacin, gentamicin, and tetracycline were 0.015-8, 2.0-8.0, and 128.0-512.0 µg/ml, were, respectively. The *Shigella* spp. isolates were classified as multidrug resistant. The difference in susceptibility patterns of the isolates from different sources was not significant ($p > 0.05$).

Conclusions: The findings of this study have shown that free-range chickens could serve as reservoirs/vehicles for the transmission of *Shigella* spp. in the study area.

Keywords: Shigella spp.; chickens; free range; intensively reared poultry; antibiogram; Nsukka; Nigeria.

1. INTRODUCTION

Shigellosis is a disease of primary health concern in developed and developing countries. It is a major public health concern in terms of its socio-economic impact [1]. It is implicated in food poisoning and bloody diarrhoea in humans and is an important cause of diseases associated with high morbidity and mortality in livestock [2]. In humans, *Shigella* spp. is estimated to be responsible for at least 80 million cases of bloody diarrhoea and 700,000 deaths annually [3]. Ninety-nine percent of infections caused by *Shigella* occur in developing countries, and the majority of cases (~70%), and deaths (~60%), occur among children less than five years of age.

Shigellosis is endemic in many developing countries and also occurs in epidemics causing considerable morbidity and mortality [3]. Shigellosis is primarily a disease of resource-poor, crowded communities that do not have adequate sanitation or safe water. *Shigella* spp. is spread by direct contact with an infected host, or by eating contaminated food or drinking contaminated water. Flies may also transmit the organism. The low infective dose, as few as 200 viable organisms, facilitates the easy spread of the organism [4].

The development of antimicrobial resistance among some of the microbial population has become a severe problem, especially the emergence of multi-drug resistant strains or variants. Some of these pathogens isolated from environmental sources have shown an increased resistance to antibiotics since they have developed a number of elaborate mechanisms for acquiring and disseminating plasmids, transposons, phages, and other genetic determinants [5]. There are no reports in the available literature on the occurrence and antibiogram of *Shigella* spp in free range and intensively reared chickens, hence the study. The objective of this study was, therefore, to evaluate the occurrence and antibiogram of *Shigella* spp in free range and intensively reared chickens in Nsukka, Enugu State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Nsukka zone of Enugu state comprising of Nsukka, Igbo-Eze South, Igbo-Eze North, Igbo-Etiti, Udenu, and Uzo-Uwani Local Government areas (L. G. A.). It is situated between latitude 6°45" N and longitude 7°12.5" E. The people of the zone are Igbo speaking tribe and are predominantly farmers. Nsukka town is the home of the first indigenous university in the country, University of Nigeria, Nsukka. The presence of the university has encouraged the establishment of medium and small-scale poultry farms within Nsukka town. So many local markets are found in the zone, which is used mainly for marketing of farm products and animals.

2.2 Sample Collection

A total of 300 cloacal swab samples were collected for the study. The 300 samples were made up of 150 collected from free range chickens at three different local markets (Markets 1, 2 & 3) and 150 from intensively reared

chickens collected from three farms (Farm A, B & C) randomly selected from the study area. The number of samples collected from Markets 1, 2 and 3 were 50, 76 and 24 respectively based on the availability of local chickens for sampling, while from each of the Farms A, B and C were collected 50 samples each. All the farms were located in Nsukka metropolis, while the three markets were selected from 3 different L. G. A. within the zone.

Samples were collected according to the recommendations of the International Office of Epizootics [6]. Cloacal swab samples were collected from live birds using sterile cotton-tipped swabs. The swab was inserted into the cloacae of each bird and rotated gently against the lining of the cloacae and immersed into a sterile tube containing 5 ml of buffered peptone water (Lab M, Lancashire, United Kingdom) at the site of collection. The collected samples were transported to the laboratory for further analysis within one hour from time of collection.

2.3 Isolation and Identification of *Shigella* spp

The cloacal swab samples inoculated into the buffered peptone water were pre-enriched by incubating aerobically overnight at 37°C. Subsequently, 0.1 ml of the overnight culture was transferred into 10 ml of Rappaport Vassiliadis (RV) medium (Oxoid, Hants, England) and incubated at 42°C for 24 h. Thereafter, a loop-full of broth culture was streaked on Salmonella-Shigella agar (SSA) (Titan Biotech, India) and incubated aerobically for 24 - 48 h at 37°C. Suspected colonies on the SSA were streaked on MacConkey agar (Micromedia Trading House, Torbagyi u, Hungary) for purity check and to obtain discrete colonies [7,8]. Presumptive colonies of *Shigella* were taken for further morphological and biochemical typing which included urease, triple sugar iron test, IMViC (Indole, Methyl red, Voges-Proskauer, Citrate), sugar fermentation tests, amino acid (lysine and arginine) decarboxylase, ONPG (ortho-nitrophenyl galactosidase), catalase, and motility.

2.4 Antibiotic Susceptibility Test

Antibiotic susceptibility test was performed by the disc diffusion method according to the CLSI method (Kirby-Bauer disk diffusion test) on Muller-Hinton agar (Titan Biotech, India). About 3 to 4 discrete colonies of each isolate was inoculated into Mueller-Hinton broth separately and incubated for 24 hours at 37°C. The broths were matched to 0.5 McFarland standard and streaked using sterile cotton swabs on Mueller-Hinton Agar plates. The antibiotics used were augmentin (30 µg), ofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofurantoin (200 µg), cotrimoxazole (25 µg), amoxicillin (25 µg) and tetracycline (25 µg) (Abtek Biologicals Ltd, Liverpool, United Kingdom). *Pseudomonas aeruginosa* ATCC 27853 served as control. Zones of inhibition were evaluated following the recommendations by CLSI [9]. The inhibition zone diameters were measured and reported in millimetres (mm), and the zones of inhibition generated by each antibiotic disc were grouped as susceptible, intermediate or resistant by comparing the measured diameter with the standard given in the interpretative chart [9,10].

2.5 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assay

The following antibiotic powders (ciprofloxacin, gentamicin, tetracycline and sulphamethoxazole/ trimethoprim) were dissolved in appropriate diluents to obtain a stock solution of 5120 µg/ml, respectively. Subsequent antibiotic dilutions were made in sterile Mueller Hinton broth and equal volume of the standardised inoculums was added to equal volume of an antibiotic concentration in test tubes. Antibiotic ranges were prepared one step higher than the final dilutions range required to compensate for the addition of an equal volume of inoculum [10,11]. The inoculated tubes were incubated at 37°C for 16-18 h. Inoculated/uninoculated tubes of antibiotic-free Mueller Hinton broths and *Pseudomonas aeruginosa* ATCC 27853 served as controls. The minimum inhibitory concentration (MIC) corresponds to the lowest concentration of antibiotic at which there is no visible growth of the organism. The minimum bactericidal concentration (MBC) was determined by plating out the tubes that showed no sign of growth on antibiotic free-Mueller Hinton agar plates and incubated at 37°C overnight [11,12]. The MBC corresponds to the lowest concentration of antibiotic that prevented the growth of the test isolates after subculture on the antibiotic free-Mueller Hinton agar plates.

2.6 Statistical Analysis

Test for differences in the occurrence of the *Shigella* spp. in the two groups of chickens was carried out using simple percentages. The mean susceptibility patterns of the *Shigella* spp. from

different sources were compared using student t-test. The confidence limit was set at 95%. All available data were analysed using SPSS version 16 (Chicago, IL, USA).

3. RESULTS

Out of the 300 cloacal swab samples analysed, bacteriological examination revealed 10 strains of *Shigella* spp. Overall; a 3.3% occurrence was obtained in the two groups of chickens. The occurrence in free-range chickens was 6.7% while that in intensively reared chickens was 0%. The highest isolation rate of 9.2% was observed for samples collected from Market 2, followed by samples collected from Market 1 which had an isolation rate of 6%. Samples collected from Market 3 and Farms A, B and C yielded no isolates (0% isolation rate). The antibiogram studies showed that the *Shigella* spp. isolates were 100% resistant to amoxicillin and augmentin, 70% resistant to tetracycline, 40% resistant to nitrofurantoin and cotrimoxazole, 30% resistant to nalidixic acid and gentamicin, and 10% resistant to ofloxacin (Fig. 1). Also, 10% of the isolates showed intermediate sensitivity to nalidixic acid and 20% intermediate sensitivity to nitrofurantoin and tetracycline.

The results of the MIC and MBC assay of the antibiotics on the *Shigella* spp. isolates showed that ciprofloxacin had both the lowest MIC and MBC of 0.0156 – 0.25 µg/ml and 0.0156 – 8.0 µg/ml, respectively, while tetracycline had the highest MIC and MBC of 2.0 – 64.0 µg/ml and 128 – 512 µg/ml respectively (Table 1). Trimethoprim/sulphamethoxazole had an MIC of 8.0 – 32.0 µg/ml but was not bactericidal against the isolates (Table 1).

A comparison of the mean susceptibility patterns of the *Shigella* spp. from the two markets that had isolation showed that *Shigella* spp. isolated from Market 1 were more sensitive to most of the antibiotics than those isolated from Market 2, except for gentamicin where the reverse was the case and nitrofurantoin where both were of equal sensitivity (Fig. 2). The differences were, however, not significant (P > 0.05).

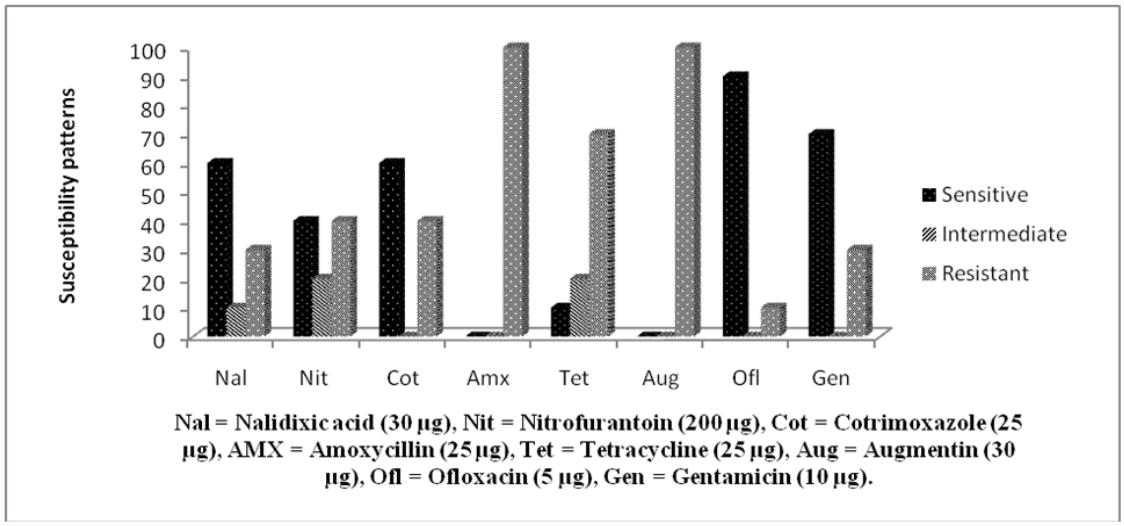

Fig. 1. The mean susceptibility patterns of the *Shigella* spp isolated from free-range chickens to the antibiotics

Table 1. The MIC/MBC values of the antibiotics tested against the *Shigella* spp. isolates obtained from free-range chickens in Nsukka

Antibiotics	MIC (µg/ml)	MBC (µg/ml)
Trimethoprim/Sulphamethoxazole	8.0-32.0	Nil
Ciprofloxacin	0.0156-0.25	0.0156-8.0
Gentamicin	0.5-2.0	2.0-8.0
Tetracycline	2.0-64.0	128-512

Nil = not bactericidal

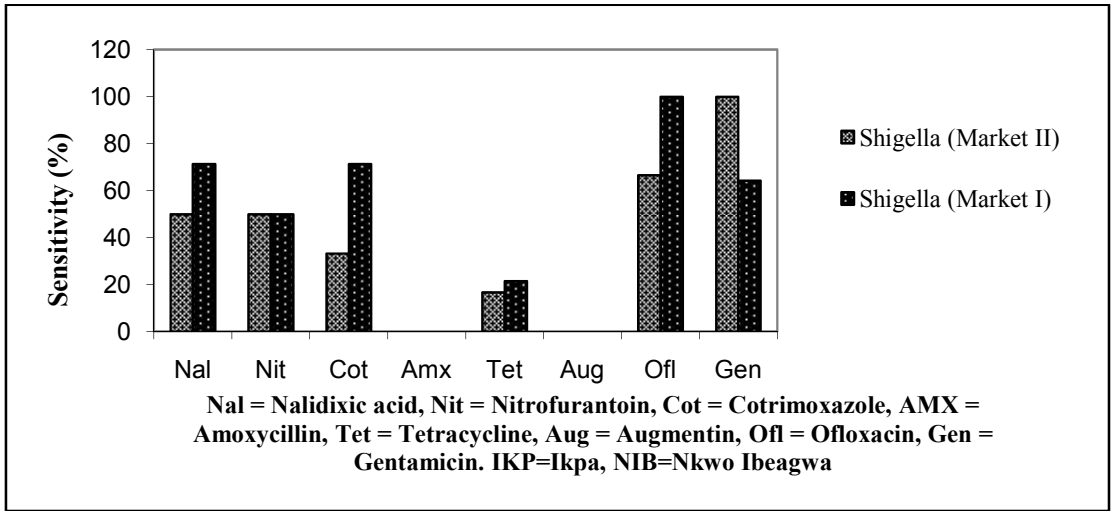

Fig. 2. The mean susceptibility patterns of the *Shigella* spp isolated from free-range chickens in Nsukka according to sample source (Markets 1 and 2)

4. DISCUSSION

The 6.7% isolation rate of *Shigella* spp from free range chickens in the study area could be attributed to the free-range method of rearing these birds that makes for them to scavenge for food at refuse and sewage dumps and possibly get contaminated from the faeces of humans and other animals in the environment. The isolation of *Shigella* spp from these free-range chickens is considered to be of immense public health concern because most families in Nsukka still raise free range chickens which are reared in family premises and compounds. The public health importance stems from the fact that *Shigella* spp have been associated with various infections in humans and animals, and all *Shigella* spp. are potentially pathogenic to humans with varying degrees of virulence depending on the strain, health status of the infected individual, age and infectious dose, with children, the elderly and immunocompromised patients being at great risk [2]. The nil isolation rates (0%) in intensively reared chickens could be attributed to improved hygienic conditions obtainable in the intensive poultry farm environment, and possibly, the incorporation of antimicrobials in poultry feeds. The non-isolation of *Shigella* spp. from samples obtained from free range chickens sampled at Market 3 could be due to geographic variation in occurrence, immunity of the chickens, proper disposal of refuse/sewage effluent by the community where the chickens were reared, and or non-shedding/intermittent shedding of the organisms by the birds sampled.

To the best of our knowledge, this is the first report of isolation of *Shigella* spp. from cloacal swabs of free-range chickens in this region of Nigeria. A similar report of shigellae from cloacal swabs of chickens has been documented in India by Shah and Qureshi [2]. Karaguzel et al. [13] reported the recovery of four isolates of *S. sonnei* from 616 faecal samples of gulls and suggested that the gulls acquired the organisms through feeding on refuse. Oakley et al. [14] reported the isolation of *Shigella* spp. from poultry wet litter in the United States, while Hemen et al. [15], reported an unprecedented 25% prevalence in poultry faecal samples in Northern Nigeria. Yong et al. [16] reported the presence of *Shigella flexneri* and *Shigella sonnei* in fresh faecal samples of large-billed crow (*Corvus* spp.) in Asia. In agreement with findings in this present study, Roscoe et al. [17] were unable to isolate *Shigella* spp. from five hundred flightless Canada geese captured at 16 locations in New Jersey. Also, Guarav et al. [18] failed to isolate the organisms from one hundred poultry faecal samples in India.

The findings in this present study that showed that the *Shigella* isolates were 100% resistant to amoxicillin and augmentin, 70% resistant to tetracycline, 40% resistant to nitrofurantoin and cotrimoxazole, 30% resistant to nalidixic acid and gentamicin, slightly resistant to ofloxacin (10%) and totally sensitive to ciprofloxacin brings to the fore the fact that antibiotic resistance in *Shigella* spp. in recent years has assumed alarming proportions worldwide [19]. In general, all the isolates were classified as multidrug-resistant

(MDR) being resistant to more than one antibiotic. Some of the *Shigella* spp. even showed increased resistant to the antibiotics (resistance to three or more unrelated antimicrobials). It is worthy to note that the MIC values obtained for trimethoprim/ sulphamethoxazole, ciprofloxacin, gentamicin, and tetracycline on some sensitive *Shigella* strains lie within the equivalent sensitive MIC breakpoint range and that the combination trimethoprim/ sulphamethoxazole was not bactericidal on the sensitive strains, whereas ciprofloxacin and gentamicin exhibited bactericidal activities. Tetracycline exhibited bactericidal activities at an elevated concentration of 512 µg/ml on few strains. The observed patterns of resistance could be a reflection of the consequence of drug use among human population in the study area as the birds could have acquired the organisms through scavenging on refuse/sewage dumps. The high level of resistance associated with other non fluoroquinolone antibiotics could be as a result of long overuse of the agents before the introduction of the fluoroquinolones. Moreover, the free-range chickens may have acquired the resistant bacteria by contact with other animal carriers or by ingestion of food and water that have been contaminated by faecal materials from other scavenging animals, which may have received veterinary care and treatment with antimicrobials [20,21]. The fact that the isolates from Market 2 showed higher sensitivity to more antibiotics tested including Nalidixic acid, Cotrimoxazole, Tetracycline and Ofloxacin and had lower sensitivity to only Gentamicin than the isolates from Market 1 (Fig. 2) could be a reflection of the usage and distributions of these antibiotics in the different communities served by these markets.

The susceptibility patterns to the antibiotics obtained in the present study for the *Shigella* spp. is in agreement with the report of Shah and Qureshi [2], that reported high susceptibility to the fluoroquinolone (ciprofloxacin) and moderately sensitive to gentamicin; but in variance with the report of Awad-Alla et al. [22], that documented 60%, 0%, 40%, and 20% sensitivity of the *Shigella* spp. isolated from wild Ibis (*Nipponia nippon*) to ciprofloxacin, cotrimoxazole (septrin), amoxicillin, and gentamicin respectively. The variation could be as a result of strain differences of the *Shigella* spp. between the two studies and geographical variations. Also, Hemen et al. [15] reported a high level of resistance to the newer quinolones including ciprofloxacin in *Shigella* isolates from poultry litter in Northern Nigeria. The differences in sensitivity pattern between the present study and the aforementioned study could be strain variation possibly due to differences in sample sources.

5. CONCLUSION

Based on the results of this study, it was concluded that there is a 6.7% occurrence of *Shigella* spp in free-range chickens in Nsukka, and that most of the isolates are multi-drug resistant. The implications of these findings are that free-range chickens could serve as reservoirs/vehicles for the transmission of *Shigella* spp., thus efforts should be made to educate the local free-range chicken raising populace on the public health implications.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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