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Antimicrobial Susceptibility Patterns of Salmonella Species from Sources in Poultry Production Settings in Calabar, Cross River State, Nigeria

Nchawa Yangkam Yhiler[1], Bassey Enya Bassey[1, 2, *]

[1]Department of Medical Laboratory Science, Faculty of Allied Medical Science, University of Calabar, Calabar, Nigeria
[2]World Health Organization, UN House, Central Area District, FCT, Abuja, Nigeria

Email address:
yankgam@yahoo.com (N. Y. Yhiler), bassey69@yahoo.com (B. E. Bassey)

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Abstract: Globalisation and technology especially in the area of antimicrobial chemotherapy have greatly improved the quality of health care in both the veterinary and the public settings. Nonetheless, irrational use of antibiotics has considerably encouraged the development of antimicrobial resistance. This present study was aimed at determining the sources within the poultry production setting that are significantly responsible in the development and spread of antibiotic resistant strains of Salmonella. A total of 374 samples were collected from poultry environment sources (170), from poultry bird themselves (136) and from poultry personnel sources (68) in Calabar within the period of August 2013 and May 2014. The isolation of Salmonella was in accordance with the ISO 6579:2002 and the antibiotic susceptibility testing was carried out based on the CLSI by means of the Kirby-Bauer disk diffusion method involving 12 antibiotic disks (HDx). The paired sample t-test was used to determine the significant differences in the resistance of Salmonella species from the poultry sources against the antibiotics used in this study at 95% confidence level. The Salmonella isolates exhibited the highest rate of resistance against Ampicilin-10 µg (72.9%) and the least against Gentamicin-10 µg (1.4%). All the Salmonella isolates were sensitive to Chloramphenicol-30, Ciprofloxacine-5 µg and Imipenem-10 µg and hence the most sensitive. Majority (68.8%) exhibited resistance against more than one type of antibiotics hence revealing a high rate of multidrug resistant Salmonella strains. The results of the paired sample t-test revealed that the development and spread of resistance against antibiotics was significantly dependent on the poultry environment sources and therefore should serve as important targets for prevention measures.

Keywords: *Salmonella*, Antibiotic Susceptibility, Multidrug Resistance

1. Introduction

Salmonella serotypes have been extensively incriminated as the most important zoonotic pathogens in several countries worldwide [1]. They are responsible for the significant morbidity and mortality in both humans and animals [1]. Each year, an estimated 1.3 billion cases resulting to about 3 million deaths occur worldwide due to salmonellosis alone [2,3]. In spite of the importance of poultry as the major element in the human food chain, it has been frequently labelled as one of the most important sources of food poisoning due to *Salmonella* serovars causing the majority of food born outbreaks worldwide [4,3,5,6,7,8,9].

The use of antibiotics together with the improvement of sanitation and hygiene as well as immunization and proper nutrition has provided major benefits in human life expectancy [10]. However, the increased utilization of antibiotics in both public and veterinary settings has led to the emergence of antibiotic resistance and as a consequence poses a serious threat to public health safety [10]. In general, the persistent use of drugs initiates selective pressure that encourages the development of antibiotic resistant pathogens. One of the most important factors necessary for the development of antibiotic resistant strains of microorganisms is the irrational use of such antibiotics in the animal production settings, which has brought about the emergence of bacteria strains that were otherwise infective only to animals to now become infective to humans as well due to the acquisition of the antibiotic resistance traits [3].

The drastic and continuous increase in the development of

drug resistant *Salmonella* strains in recent years has been frequently reported and is of major concern in both the developed and the developing countries [11, 12]. Animals that have become infected with antibiotic resistant strains of *Salmonella* are important sources of resistant determinants that give room for the *Salmonella* serovars to become infective to humans [5]. Antibiotic resistant strains of *Salmonella* have been frequently recovered from food of animal origin of which poultry is of major concern [13, 14]. In veterinary medicine, the use of antimicrobial agents is exploited in animal feeds as prophylaxis and as growth promoters in order to enhance growth and to improve the health of the animals. However, antibiotic incorporated into animal feeds are at sub-therapeutic concentrations which in consequence creates a favourable ground for the development of antibiotic resistance within the animal production setting [15].

The frequency of isolation of *Salmonella* strains resistant to several antimicrobial agents has increased in several countries worldwide [16, 17] and the association between the veterinary use of antibiotics and the development of resistant strains of microorganisms against these antibiotics in human infection is now an established fact [18]. Multidrug resistance against commonly used antibiotics in both the veterinary and the public setting have been found to be exhibited by almost all *Salmonella* serotypes [11].

In spite of the relatively considerable information of antimicrobial susceptibility of *Salmonella* serotypes in human infections and the contamination of foods of animal origin in many parts of the world, there is however a dearth of such information in most parts of Africa including Nigeria [19]. Therefore, it is of importance to monitor antimicrobial resistance of *Salmonella* serovars from the production setting of food animals of which poultry is a major concern. This study therefore attempts to analyse the antibiotic resistance of *Salmonella* serovars recovered from three major sources (the poultry environment, the poultry birds themselves and the poultry personnel) in the poultry production setting in Calabar.

2. Materials and Methods

2.1. Study Design and Sample Collection

This study was carried out within the periods of August 2013 and May 2014 in the Calabar metropolis of Nigeria. Three hundred and seventy four (374) specimens were randomly collected from three different sources within the poultry production settings in Calabar; out of which 170 were obtained from the poultry environmental sources which included poultry feeds, poultry drinking water, poultry litter, poultry abattoir reins and dust from poultry house; 136 were obtained from poultry birds themselves which included guts, cloacal swabs, poultry meat and eggs; and 68 were obtained from the poultry personnel which included the poultry personnel stool and hand washings.

Samples were collected based on the method as described by [20]. The samples were immediately placed in an insulating box containing ice packs in order to maintain the temperature at 4-6^{0}C and transported to the laboratory within four hours.

2.2. Isolation and Identification of Salmonella Species

The isolation of *Salmonella* species from the poultry production setting in Calabar was based on the ISO 6579:2002 involving the non-selective pre-enrichment stage using 10% buffered Peptone Water, the selective enrichment stage using Modified Semisolid Rapapport-Vassiliadis and Muller-Kauffmann Tetrathionate-nivobiocin broths, and finally the selective plating stage using Brilliant Green and Xylulose Lysine Deoxycholate agars. Typical *Salmonella* colonies on Brilliant Green and Xylulose Lysine Deoxycholate agars were purified by further sub-culturing on Nutrient agar prior to confirmation.

2.3. Confirmation of the Salmonella Isolates

The presumptive *Salmonella* isolates were confirmed following their morphological, biochemical and serological characteristics. The morphological characteristics included their colonial appearance on Brilliant Green and Xylulose Lysine Deoxycholate agar plates as well as their Gram reaction when observed under the 100X objective of a light microscope. The biochemical reaction included their characteristics on Triple Sugar Iron agar slants, Christensen agar slats, urea, Lysine decarboxylase test, β-galactosidase test, Acetone production test, indole test and methyl red test (Hardy Diagnostics, CA, USA). Finally, confirmation was supplemented by serology using commercially available polyvalent *Salmonella* serum (Denka Sieken Co. Ltd. Japan) specific against all group and type factor *Salmonella* antigens. Those colonies that agglutinated within two minutes were confirmed as belonging to the genus *Salmonella*. They were then stored on Nutrient agar slants overlaid with paraffin oil at 4^{0}C prior to antibiotic susceptibility testing.

2.4. Antibiotic Susceptibility Testing

The antimicrobial susceptibility testing was carried out based on the Clinical Laboratory Standard Institute (CLSI) method by means of the Kirby-Bauer Disk Diffusion test using Mueller-Hinton agar (Hardy Diagnostics CA, USA) [21].

Four to five colonies of each isolate were transferred into a test tube containing 2.5 ml of sterile normal saline by means of a sterile wire loop. The suspension was vortexed and the turbidity compared with that of Barium Chloride (0.5 McFarland Turbidity Standard: 1.0×10^8 CFU/µl). The optical density of the standard was regularly monitored by means of a spectrophotometer at $\lambda = 625$ nm and 1 cm light path (ODλ = 0.08-0.1) [22]. 100 µl of the bacterial suspension was then inoculated on the iso-sensitivity test agar plates, and the excess was siphoned by means of sterile Pasteur pipettes. The plates were allowed to dry at room temperature in a laminar flow. The pre-determined antibiotic

disks were then dispensed into the bacterial lawn by means of a sterile pair of forceps and gently pressed to ensure complete contact with the agar. The discs were positioned 15 mm way from the edge of the plate and 25 mm away from each other. The plates were incubated at 35-37^{0}C for 18 to 24 hrs. The diameters of the zones of inhibition were read and interpreted in accordance with [21]. The antibiotic disks used in this study included 12 commercially available drug disks (Hardy Disks, HDx): cefotaxime-30 µg (CTX), ceftazidime - 30 µg (CAZ), chloramphenicol - 30 µg (CH), ciprofloxacin -5 µg (CIP), carbenicillin 100 µg (CB), amoxicillin/clavulanic acid-30 µg(AmC), ampicillin 10 µg (AM), amikacin -30 µg(AN), tetracycline-30 µg (TE), sufamethaxole/trimethoprime - 25 µg (SXT), imipenem - 10 µg (IMP) and gentamicin - 10 µg (GE).

2.5. Statistical Analysis

The data generated in this study were analysed using the predictive analytic software (PASW) 18.0 (IBM, USA). The paired sample student t-test was used to determine significant differences in the resistance of *Salmonella* species isolated from the poultry environmental, bird, and personnel sources in the poultry production system against the antibiotics used in this study. P values of less than 0.05 ($P < 0.05$) was considered statistically significant.

3. Results

Out of the 374 specimens obtained from the poultry production setting in Calabar, 221 (59.1%) were found to be positive for *Salmonella* species as determined by the morphological, biochemical and serological confirmation characteristics. *Salmonella* species were recovered from all the three sources in the poultry production setting. Samples from the poultry environmental sources revealed 58.8% rate of recovery of *Salmonella* species, 55.1% from poultry bird source and 67.6% from poultry personnel sources.

The antibiotic susceptibility testing revealed that the *Salmonella* isolates exhibited the highest resistance against ampicillin - 10 µg (72.9%) and the least resistance against gentamicin - 10 µg (1.4%). However SXT, CTX, TE, AmC, CB, CAZ, and AN exhibited 46.2%, 44.4%, 38.0%, 29.4%, 20.4%, 19.5% and 5.0% resistance respectively. All the *Salmonella* isolates exhibited zero resistance (sensitive) to chloramphenicol - 30 µg, ciprofloxacin - 5 µg and imipenem - 10 µg.

With respect to the resistance of the *Salmonella* isolates against the different antibiotics used in this study, the *Salmonella* isolates were grouped thus: those that exhibited zero resistance (were sensitive) to all the antibiotics used (S-types), those that exhibited resistance against only one type of antibiotic disk (Single R-type), those that exhibited resistance against more than one type of antibiotic disk (multiple R-type).

The majority of all the *Salmonella* isolates were of the multiple R-type (68.8%), with varied resistance patterns. Only a very few of the *Salmonella* isolates 9.5%were of the S-types while 21.7% of the *Salmonella* isolates were of the single R-type.

The *Salmonella* isolates recovered from the poultry environmental sources showed the highest percentage of multiple R-types (78.0%) involving 9 antibiotic disc types out of 12 (AM, SXT, CTX, TE, AmC, CB, CAZ, AN and GE) followed by the *Salmonella* isolates from the poultry bird sources (65.3%) involving 7 out of 12 antibiotic disk types (AM, SXT, CTX, TE, AmC, CB and CAZ) and then the *Salmonella* isolates from the poultry personnel sources (54.3%) involving 8 out of 12 antibiotic disc types (AM, SXT, CTX, TE, AmC, CB, CAZ and AN). However, the *Salmonella* isolates from all three sources were resistant to at least 7 out of 12 antibiotics (AM, SXT, CTX, TE, AmC, CB and CAZ).

Table 1. *Frequency of Salmonella species recovered from sources in the poultry production settings.*

Poultry Source	Number collected	Number Positive	Percentage Positive (%)
Environment	170	100	58.8
Bird	136	75	55.1
Personnel	68	46	67.6
Total	374	221	59.1

Table 2. *Resistance pattern of Salmonella species isolated from poultry sources against different antibiotics.*

Antibiotic disks	Resistance of *Salmonella* isolates from sources			Total
	Environmental (%). (n = 100)	Bird (%). (n = 75)	Personnel (%). (n = 46)	
Ampicillin 10 µg (AM)	74 (74.0)	54 (72.0)	33 (71.7)	161 (72.9)
Sufamethaxole/ trimethoprime - 25 µg (SXT)	51 (51.0)	33 (44.0)	18 (39.1)	102 (46.2)
Cefotaxime – 30 µg (CTX)	49 (49.0)	24 (32.0)	25 (54.3)	98 (44.4)
Tetracycline - 30 µg (TE)	37 (37.0)	23 (30.7)	24 (52.2)	84 (38.0)
Amoxicillin/clavulanic acid - 30 µg (AmC)	34 (34.0)	18 (24.0)	13 (28.3)	65 (29.4)
Carbenicillin 100 µg (CB)	24 (24.0)	10 (13.0)	11 (23.9)	45 (20.4)
Ceftazidime - 30 µg (CAZ)	16 (16.0)	13 (17.3)	14 (30.4)	43 (19.5)
Amikacin 30 µg (AN)	6 (6.0)	0 (0.0)	5 (10.9)	11 (5.0)
Gentamicin - 10 µg (GE)	3 (3.3)	0 (0.0)	0 (0.0)	3 (1.4)
Ciprofloxacine - 5 µg (CIP)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Imipenem - 10 µg (IMP)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chloramphenicole - 30 µg (Ch)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 3. *Antibiotic disk types involved in Salmonella resistance in the poultry production setting.*

Source of *Salmonella* isolates	Total number of *Salmonella* isolates	Number of S-type (%)	Number of single R-type	Number of multiple R-type	Antibiotic disks involved in resistance
Poultry environmental sources	100	5 (5.0)	17 (17.0)	78 (78.0)	AM, SXT, CTX, TE, AmC, CB, CAZ, AN and GE
Poultry bird sources	75	9 (12.0)	17 (22.7)	49 (65.3)	AM, SXT, CTX, TE, AmC, CB and CAZ
Poultry personnel sources	46	7 (15.2)	14 (30.4)	25 (54.3)	AM, SXT, CTX, TE, AmC, CB, CAZ and AN
Total	221	21 (9.5)	48 (21.7)	152 (68.8)	AM, SXT, CTX, TE, AmC, CB and CAZ

S-type: *Salmonella* isolates that exhibited zero resistance (sensitive) to all the antibiotics used
Single R-type: *Salmonella* isolates that exhibited resistance to a single type of antibiotic disk type
Multiple R-type: *Salmonella* isolates that exhibited resistance to more than one antibiotic disk type

Table 4. *Statistical comparison of the mean resistance of Salmonella isolates from the poultry personnel, bird and environmental sources in the poultry production setting against different antibiotics.*

Pairs	Standard deviation	t	df	P - value	Remark
Bird sources vs. Personnel sources	7.5959	1.216	11	0.249	P > 0.05: Not statistically significant
Environmental sources vs. Personnel sources	14.2667	3.055	11	0.011	P < 0.05: Statistically significant
Environmental sources vs. Bird sources	8.8980	4.2632	11	0.003	P < 0.05: Statistically significant

There was no statistical significant difference in resistance of *Salmonella* isolates from the poultry personnel sources when compared with the mean resistance of those from the poultry bird sources against the antibiotics used (P=0.249). There was a statistical significant difference in the resistance of the *Salmonella* isolates from the poultry personnel sources against the antibiotics used compared with the mean resistance of those from the poultry environmental sources against the antibiotics used (P = 0.011). Also, there was a statistical significant difference in the resistance of *Salmonella* isolates from the poultry bird sources compared with the mean resistance of those from the poultry environmental sources against the antibiotics used (P = 0.003).

4. Discussion

Recently, there has been a marked increase of in the development of antimicrobial resistant strains of Salmonella from foods of animal origin especially poultry. Antimicrobial resistant strains of Salmonella species from food animals are important in causing human infections which previously could not.

Non-typhoidal *Salmonella* species have been recognized as the major cause of food borne diseases posing major public health problems worldwide [23, 24]. In humans, the disease is usually self-limiting when confined within the intestines, even though sometimes it may result to significant discomforts. In some cases however, the infection may cross the intestinal barrier to reach other vital organs resulting in serious complications in which case require significant antimicrobial interventions [17]. This therefore calls for the surveillance and sharing of antimicrobial susceptibility data especially in the poultry production setting in order to ensure the effective implementation of public health control programs.

Multidrug resistance is phenomenal when resistance is observed against classical first line antimicrobial agents like ampicillin and sulfamethaxole/timethoprime [25] and this implies that the *Salmonella* isolates in the poultry production setting in Calabar exhibited multidrug resistance. The results of this study corroborates [6] who demonstrated the resistance of *Salmonella* isolates against commonly used antimicrobials in the poultry production setting in Edo-Ekiti, Nigeria. Some workers demonstrated a high resistance of *Salmonella* against ampicillin (90.91%) within the poultry production setting in the United Arab Emirates [26], and this is in close agreement with the result of this study.

The *Salmonella* isolates from the poultry production setting in Calabar exhibited resistance against 9 out of the 12 antibiotics used in this study. This reveals a wide range of antibiotics which the *Salmonella* species have become resistant against. Such a wide range of antibiotic resistance can be attributed to the gross indiscriminate use of antimicrobial agents in the poultry production setting [27, 28]. Furthermore, the incorporation of antibiotics in the diet of animals at sub therapeutic concentrations as prophylaxes and as growth promoters has invariably contributed to the development of antibiotic resistant strains of *Salmonella* [29]

This current study also revealed that all the *Salmonella* isolates recovered from the poultry production setting in Calabar were sensitive to Imipenem - 10 µg, Chloramphenicol - 30 µg and Ciprofloxacin - 5 µg, implying these drugs to be the most active drugs against *Salmonella*

species in the poultry production setting in Calabar and therefore could serve as the drugs of choice in the control of salmonellosis in the poultry production setting in Calabar. This closely agrees with the work carried out in Zaria, Nigeria [30], but is however contradicted by the work carried out in Cameroon [5]. Such differences could result from the difference in the geographical location as well as the habitual drug administration standards which differ with different regions [5].

Out of the 221 *Salmonella* isolates recovered from the poultry production setting, only 21 (9.5%) were completely sensitive (no resistance) to all the 12 antibiotics used in this study whereas the rest 21.7% and 68.8% (90.5%) exhibited resistance against only a single antibiotic disk type and more than one antibiotic disc types respectively.

Based on the objective of this study, the null hypothesis stated that there is no significant difference in the resistance of *Salmonella* isolates from the different sources within the poultry production setting against all the antibiotics used in this study. However, when the resistance of the *Salmonella* species from the poultry personnel sources against the antibiotics used were compared with the resistance of those from the poultry bird sources against the antibiotics used, there was no statistical significant difference ($P = 0.249$). This implies that the resistance of the *Salmonella* species against all the antibiotics used was not dependent on either the poultry personnel sources or the poultry bird sources.

However, on the other hand, when the resistance of the *Salmonella* species isolated from the poultry environmental sources against the antibiotics used was compared with the resistance of those from the poultry personnel sources and the poultry bird sources against the antibiotics used, there was statistical significant difference in each of the cases ($P = 0.011$ and $P = 0.003$ respectively). This implies that the resistance of *Salmonella* species against the antibiotics used depended on the poultry environmental sources. Hence, the poultry environmental sources (poultry feeds, water, abattoir reins, litter from floor and dust from wall as used in this study) are important sources in the development of antibiotic resistant strains of *Salmonella* serving as a means of spread to the poultry birds, and the poultry personnel as well as the human population.

5. Conclusion

In conclusion, bacterial resistance against antibiotics greatly interferes with the effectiveness of control strategies against infectious diseases [31]. Therefore, good hygiene practices and the controlled use of antibiotics is necessary to prevent the spread of antibiotic resistant strains of *Salmonella*. This current study has revealed poultry environment sources as important sources of antibiotic resistant strains of *Salmonella*. This implies that in order for control strategies to be more effective in minimizing the development and spread of antibiotic resistant *Salmonella* strains, they should be geared towards preventing environmental contamination within the poultry production setting.

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