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Antibiotic sucseptibility of *Salmonella Typhi* and *Klebsiella Pneumoniae* from poultry and local birds in Ado-Ekiti, Ekiti-State, Nigeria

[1]AJAYI, A. O. AND [2]EGBEBI, A. O.

[1]Department of Microbiology, University of Do-Ekiti, P.M B. 5363, Ado-Ekiti, Ekiti-State, Nigeria
[2]Department of Microbiology, Federal Polytechnic, Ado-Ekiti, Ekiti-State, Nigeria

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ABSTRACT

The emergence of antibiotic-resistant enteric bacteria, especially Salmonella typhi and Klebsiella pneumoniae in poultry has become a significant public health threat in Nigeria. This study was carried out to isolate S. typhi and K. pneumoniae from the faeces of poultry birds and local birds at selected locations in Ado-Ekiti, Nigeria. Sixty-four strains of S. typhi and 77 strain of K. pneumoniae were recovered from 120 poultry birds while 100 strains of S. typhi and 90 strains of K. pneumoniae were isolated 150 local birds. All the isolates were screened for their antibiotic susceptibility to the following antibiotics using the agar disk diffusion technique: augmentin (25µg), cotrimoxazole (25µg), ofloxacin ((25µg), gentamicin (10µg), nitrofurantoin (200µg), nalidixic-acid (30µg), amoxicillin (25µg) and tetracycline (25µg). The frequency of antibiotic-resistance from poultry birds ranged between 87.5% and 98.4% for S. typhi and 53.2% to 100% for K. pneumoniae. In addition, the frequency of antibiotic resistance among the isolates from local birds ranged between 39% and 100% for S. typhi and 28% to 88% among K. pneumoniae. Thirty-four multiple antibiotic-resistance phenotypes were observed among the isolates from poultry while 45 multiple antibiotic resistance phenotypes were observed among the isolates recovered from local birds. This study recommends that there should be a strict regulatory regimen for the use of antibiotics in animals to minimise the emergence and dissemination of antibiotic resistant bacteria.

INTRODUCTION

Salmonellosis is an endemic infection in Nigeria and has emerged as a global health concern being a leading cause of morbidity and mortality in humans [1]. The major causative agents responsible for the high endemicity of typhoid fever and other related infections are the *Salmonella* spp., mostly *Salmonella typhi*, which is responsible for most typhoidal infections; *Salmonella typhimurium* and *Salmonella enteritidis*, which have been attributed to most cases of non-typhoidal illnesses in Nigeria [2-4]. *Klebsiella pneumoniae* have been primarily implicated in nosocomial infections in intensive care units and also causative agent urinary tract infections,

skin abscess and some acute forms of diarrhoea [5] even though such infections occur less frequently compared to the high prevalence of infections caused by the *Salmonella* spp. These infections are mostly transmitted through consumption of contaminated foods and drinking water mostly in areas with poor hygienic standards [6]. *Salmonella* spp. and *Klebsiella pneumoniae* occur primarily as major components of the gut flora of domestic animals, making such animals a common medium of transmission of the infection with these organisms and facilitating cross-contamination of food and drinking water sources [7-8]. More specifically, *Salmonella* spp. is increasingly associated with poultry production and this has been a major factor for the upsurge of *Salmonella* infections over the years [9].

The sub-therapeutic use of antibiotics in poultry has become a popular practice and there is a growing body of scientific evidence to the effect that the increasing incidence of antibiotic resistant bacteria is closely associated with the heavy use of these antibiotics in poultry and other related agricultural practices [10]. This constitutes a significant public health risk due to possible cross-contamination with antibiotic resistant bacteria of food and drinking water meant for public consumption, which always culminates in human illnesses, mostly typhoid fever, non typhoidal illnesses, diarrhoea, with grave clinical consequences [11]. The growing incidence of multi-drug resistant Salmonella typhi has become a global phenomenon and antibiotic resistant bacteria are increasingly isolated form a wide array of sources in the clinical environments, poultry, cattle food, retail meat and drinking water sources. In different parts of Nigeria, there are some scientific evidence of the growing rate of recovery of antibiotic resistant *Salmonella typhi* and *Klebsiella pneumoniae* from poultry and local bird [12].

The city of Ado-Ekiti is an agrarian and bustling metropolis located about 200 kilometers from Lagos, South-Western Nigeria with an estimated population of about one million. Over the years, there has been an upsurge in poultry outlets within the city to cope with the increased consumption of poultry products. This has led to increased use of antibiotics in poultry is a cause for concern as it poses risks of emergence of antibiotic-resistant bacteria. In view of the imminent dangers posed by the use of antibiotics, this study was carried out to access the incidence of antibiotic- resistant *Salmonella typhi* and *Klebsiella pneumoniae* recovered form selected poultry outlets and the central local chicken pool at selected locations in Ado-Ekiti, South West Nigeria.

METHODOLOGY

Collection of sample and isolation of bacteria

With the use of deep cloacal swabs, samples were collected from 120 poultry birds at three different poultry outlets at the Ado-Ekiti metropolis and also from 150 local birds at the local chicken pool located in Ado-Ekiti. Pre-isolation enrichment of the faecal samples was carried out by inoculating each sample directly into tryptone soy broth (TSB) and incubated at 35˚C for 18-24 hours. Immediately after enrichment, the organisms were inoculated onto Salmonella-Shigella agar plates and MacConkey agar plates for the isolation of strains of *Salmonella typhi* and *Klebsiella pneumoniae* respectively. All plates were incubated at 35˚C for 24 hours and bacterial strains were examined for characteristic colonial morphology for *S. typhi* on the SS agar and *K. pneumoniae* on the MacConkey agar plates. Extensive biochemical tests were carried out to confirm the identity of all the isolates prior to antibiotic susceptibility tests [13].

Antibiotic susceptibility tests

All bacterial strains were tested for their antibiotic susceptibility against the following antibiotics using the agar disk diffusion tests: augmentin (25µg), cotrimoxazole (25µg), ofloxacin ((25µg),

gentamicin (10µg), nitrofurantoin (200µg), nalidixic-acid (30µg), amoxicillin (25µg) and tetracycline (25µg) (Abtek, UK). All susceptibility tests were carried out and interpreted using the criteria of the Clinical and Laboratory Science institute [14]. The *Escherichia coli* ATCC 25922 was used as the control strain for the antibiotic susceptibility tests.

Statistical analysis

The antibiotic susceptibility data obtained were subjected to statistical t-est to determine any significant difference among the prevalence of resistance demonstrated by *Salmonella typhi* and *Klebsiella pneumoniae* using the statistical package for social scientists (SPSS, version 13).

RESULTS

In this present study, 64 strains of *S. typhi* and 77 isolates of *K. pneumoniae* were recovered from the poultry birds, representing a frequency of 53.0% for *Salmonella typhi* and 64.0% for *Klebsiella pneumoniae*. Furthermore, 100 strains of Salmonella typhi and 90 strains of *Klebsiella pneumoniae* were recovered from the local birds, equivalent to a frequency of 67% and 60% for *S. typhi* and *K. pneumoniae* respectively among the poultry birds. The isolates showed varying degrees of prevalence of resistance to the antibiotics tested in this study. Among the poultry isolates, resistance against augmentin, nitrofurantoin and tetracycline were highest at prevalence rate of 98.4% for *Salmonella typhi* (Table 1) while the prevalence of resistance among isolates of *Klebsiella pneumoniae* ranged between 100% for tetracycline 53.2% for ofloxacin (Table 1). Among the strains recovered from the local birds, both *S. typhi* and *K. pneumoniae* showed the least susceptibility to tetracycline with 100% and 97.5% respectively (Table 1). In this study, multiple resistance was identified as reduced susceptibility to a minimum of two antibiotics and the organisms showed a wide array of multiple antibiotic resistance phenotypes. Among the poultry isolates, six different multiple antibiotic resistance phenotypes were observed among *S. typhi* while 32 different multiple antibiotic resistance phenotypes were observed among *K. pneumoniae* (Table 2). Twenty-one multiple antibiotic- resistance phenotypes were observed among *S. typhi* recovered from local birds while 39 multiple antibiotic resistance phenotypes occurred among *K. pneumoniae* isolates recovered from local birds (Table 3). The result of the statistical analysis showed that there is a significant difference (t=0.285) between the incidence of antibiotic resistance between *S. typhi* and *K. pneumoniae* isolated from poultry; and also between *S. typhi* and *K. pneumoniae* (t=0.492) from isolates recovered from local birds.

TABLE 1: Antibiotic susceptibility of *S. typhi* and *K. pneumoniae* among poultry and local birds to individual antibiotics

		Poultry		Local birds	
S/N	**Antibiotics**	***S. typhi* (n=64)**	***K. pneumonia* (n=77)**	***S. typhi* (n=100)**	***K. pneumonia* (n=90)**
1	Aug	63 (98.4)	66 (85.7)	93 (93.0)	68 (75.6)
2	Ofl	59 (92.1)	41 (53.2)	39 (39.0)	28 (31.1)
3	Gen	58(87.5)	56 (72.7)	42 (42.0)	55 (61.1)
4	Nal	59 (92.1)	59 (76.6)	79 (79.0)	41 (45.6)
5	Nit	63 (98.4)	50 (64.9)	95 (95.0)	57 (63.3)
6	Cot	61 (95.3)	73 (94.8)	95 (95.0)	72 (80.0)
7	Amx	61(95.3)	73 (94.8)	98 (98.0)	84 (93.3)
8	Tet	63 (98.4)	77(100)	100 (100.0)	88 (97.8)

The prevalence in percentage are indicated on brackets.

The data obtained were also tested to evaluate the significant difference in the incidence of antibiotic resistance between the poultry and local birds isolates and it revealed that there was a

significant different in antibiotic resistance among *S. typhi* between poultry and local birds (t=7.17). The same trend was observed between antibiotic resistance among *K. pneumoniae* observed in poultry and local birds (t=0.562). The statistical analysis was carried out at 95% confidence interval.

TABLE 2: Multiple antibiotic resistance phenotypes among *S. typhi* and *K. pneumoniae* from poultry.

S/N	Resistance phenotypes	*S. typhi*	*K. pneumoniae*
	Three antibiotics		
1.	Aug-Nit-Tet	1	-
	Four antibiotics		
2	Aug-Cot-Amx-Tet	-	4
3	Gen-Cot-Amx-Tet	-	2
4	Nal-Cot-Amx-Tet	-	1
5	Nit-Cot-Amx-Tet	-	1
	Five antibiotics		
6	Aug-Nit-Cot-Amx-Tet	1	3
7.	Ofl-Gen-Nal-Nit-Tet	1	-
8	Gen-Nal-Cot-Amx-Tet	-	2
9.	Aug-Ofl-Cot-Amx-Tet	-	1
10	Aug-Nal-Cot-Amx-Tet	-	1
11	Aug-Ofl-Gen-Cot-Tet	-	1
12	Aug-Ofl-Nal-Cot-Tet	-	1
13	Aug-Gen-Nal-Nit-Cot	-	1
14	Aug-Gen0cot-Amx-Tet	-	2
15	Ofl-Nal-Cot-Amx-Tet	-	1
16	Aug-Nal0nit-Amx-Tet	-	1
	Six antibiotics		
17	Aug-Gen-Nit-Cot-Amx-Tet	1	2
18	Aug-Ofl-Gen-Nal-Amx-Tet	-	1
19	Aug-Gen-Nal-Cot-Amx-Tet	-	11
20	Aug-Ofl-Gen-Cot-Amx-Tet	-	1
21	Aug-Nal-Nit-Cot-Amx-Tet	-	1
22	Aug-Gen-Nal-Nit-Cot-Tet	-	1
23	Aug-Ofl-Nal-Cot-Amx-Tet	-	3
24	Ofl-Nal-Nit-Cot-Amx-Tet	-	1
25	Aug-Nal-Nit-Cot-Amx-Tet	-	3
26	Aug-Ofl-Gen-Nit-Cot-Tet	-	1
27	Aug-Nal-Nit-Cot-Amx-Tet	-	1
28	Aug-Gen-Nal-Nit-Amx-Tet	-	1
	Seven antibiotics		
29	Aug-Gen-Nal-Nit-Cot-Amx-Tet	1	7
30	Aug-Ofl-Gen-Nit-Cot-Amx-Tet	1	1
31	Aug-Ofl-Gen-Nal- Cot-Amx-Tet	-	4
32	Aug-Ofl-Gen-Nal-Nit-Amx-Tet	-	1
33	Ofl-Gen-Nal-Nit-Cot-Amx-Tet	-	3
	Eight antibiotics		
34	Aug-Ofl-Gen-Nal-Nit-Cot-Amx-Tet	57	20

LEGEND: *Aug- augmentin, Ofl- Ofloxacin, Gen- Gentamicin, Nal- Nalidixic acid, Nit- nitrofurantoin, Cot- Cotrimoxazole, Amx- amoxicillin, Tet- tetracycline*

TABLE 3: Multiple antibiotic resistance phenotypes among *S. typhi* and *K. pneumoniae* from local birds

S/N	Resistance phenotypes	*S. typhi*	*K. pneumoniae*
	Two antibiotics		
1.	Gen-Tet	-	1
2	Cot-Tet	-	1
	Three antibiotics		
3	Cot-Amx-Tet	-	3
4	Nit-Amx-Tet	-	1
5	Nit-Cot-Tet	-	1
6	Aug-Nal-Tet	-	1
7	Aug-Gen-Nal	-	1
	Four antibiotics		
8	Aug-Cot-Amx-Tet	-	4
9.	Aug-Ofl-Amx-Tet	-	1
10	Aug-Gen-Nal-Amx	-	1
11	Gen-Nit- Amx-Tet	-	2
12	Nit-Cot-Amx-Tet	2	2
13	Aug-Ofl-Gen-Nal	-	1
14	Gen-Cot-Amx-Tet	-	1
15	Nal-Nit-Cot-Tet	1	-
16	Aug-Nit-Cot-Tet	1	-
	Five antibiotics		
17	Ofl-Nal-Cot-Amx-Tet	-	1
18	Gen-Nit-Cot-Amx-Tet	-	1
19	Aug-Gen-Cot-Amx-Tet	1	7
20	Aug-Nit-Cot-Amx-Tet	7	8
21	Aug-Nal-Cot-Amx-Tet	2	1
22	Nal-Nit-Cot-Amx-Tet	1	1
23	Gen-Nal-Nit-Amx-Tet	-	1
24	Ofl-Gen-Nit-Amx-Tet	-	1
25	Ofl-Gen-Nal-Amx-Tet	-	1
26	Aug-Nal-Nit-Amx-Tet	3	1
27	Aug-Gen-Nit-Amx-Tet	1	-
28	Ofl-Nal-Nit-Amx-Tet	1	-
	Six antibiotics		
29	Aug-Nal-Nit-Cot-Amx-Tet	14	2
30	Aug-Gen-Nit-Cot-Amx-Tet	7	8
31	Aug-Gen-Nal-Nit-Amx-Tet	1	-
32	Aug-Gen-Nal-Cot-Amx-Tet	1	1
33	Ofl-Nal-Nit-Cot-Amx-Tet	1	-
34	Aug-Ofl-Nit-Cot-Amx-Tet	-	1
35	Aug-Ofl-Nal-Cot-Amx-Tet	-	1
36	Aug-Ofl-Gen-Nit-Amx-Tet	-	1
37	Aug-Gen-Nal-Nit-Cot-Tet	-	1
38	Ofl-Gen-Nal-Cot-Amx-Tet	-	1
	Seven antibiotics		
39	Aug-Gen-Nal-Nit-Cot-Amx-Tet	18	2
40	Aug-Ofl-Nal-Nit-Cot-Amx-Tet	7	1
41	Ofl-Gen-Nal-Nit-Cot-Amx-Tet	2	-
42	Aug-Ofl-Gen-Nal-Cot-Amx-Tet	1	4
43	Aug-Ofl-Gen-Nit-Cot-Amx-Tet	1	2
44	Aug-Ofl-Gen-Nal-Nit-Amx-Tet	-	1
	Eight antibiotics		
45	Aug-Ofl-Gen-Nal-Nit-Cot-Amx-Tet	26	9

LEGEND: Aug- augmentin, Ofl- Ofloxacin, Gen- Gentamicin, Nal- Nalidixic acid, Nit- nitrofurantoin, Cot- Cotrimoxazole, Amx- amoxicillin, Tet- tetracycline

DISCUSSION

In this current study, *S. typhi* and *K. pneumoniae* were recovered from poultry birds and local birds at selected locations in Ado-Ekiti, Nigeria. The detection of these organisms in this study agrees with the fact that the bacteria are part of the enteric flora of the birds. However, it was observed from results obtained that there is a slight variation in the carriage of the organisms in both poultry birds and local birds. This could be due to a host of factors that are beyond the scope of this study but such variations may be due to the environmental settings in which the birds are raised, the nutritional status of the birds, and so on. Furthermore, the probiotic and physiological state of the gut of animals has been described as one of the factors that could influence the distribution, and ultimately the recovery rate of organisms from the gut of animals [15].

More importantly, all the organisms were tested for their susceptibility to selected antibiotics and a high incidence of resistance against individual antibiotics were confirmed. This finding is in consonance with other studies that have also confirmed the high incidence of antibiotic-resistance among bacteria recovered from poultry birds [16-18]. As observed in this present study, the isolates were particularly resistant tetracycline, amoxicillin and augmentin – which are confirmed to be the most commonly used antibiotics in the study area. Gentamicin is another commonly used antibiotic in the study environment but resistance against the antibiotic is one of the lowest along with ofloxacin and nalidixic-acid. The low resistance against gentamicin despite its heavy use could definitely be attributed to intrinsic factors while nalidixic-acid and ofloxacin are not commonly used antibiotics in the study location. This may explain the low resistance of the bacteria against these antibiotics. The high incidence or prevalence of resistance could be attributed to the heavy dependence of these antibiotics for therapeutic and sub-therapeutic uses in the animals which creates a selective pressure for the emergence of antibiotic-resistant bacteria in the gut of the poultry and local birds that were sampled. There is a huge body of scientific evidence that the drastic upsurge in the prevalence of antibiotic resistance among animals, particularly poultry, is increasingly linked to the heavy reliance on antibiotics in animals to treat infections, prevent infections an enhance weight gain [10-20].

The rate of multiple antibiotic resistance observed in this present study was extremely high (Tables 2 and 3). Of a particular note is that 77 isolates from poultry and 35 isolates form local birds showed resistance to all the antibiotics that were tested. In addition, the prevalence of multiple antibiotic-resistance among the bacteria among poultry appeared to be higher that the bacteria recovered from local birds. This could be explained in view of the heavier dosing of the poultry birds for therapeutic and sub-therapeutic purposes than in local birds that are rarely given antibiotics and are oftentimes left to graze around in search for foods. On the other hand, the poultry birds are mostly confined in spaces, making them easier to manage in terms of feeding and dosing with antibiotics. It has also been confirmed that antibiotic resistant *Salmonella typhi* could also be transmitted without selective pressure and more intricate mechanisms may be involved in the emergence of resistance among such bacteria [20].

The presence of antibiotic resistant *S. typhi* and *K. pneumoniae* among poultry and local chicken in Ado-Ekiti presents serious implications in view of the public health significance of the presence of antibiotic resistant bacteria among poultry and local birds in Ado-Ekiti, Nigeria. Over the years, the consumption of turkey and poultry meat have increased and this carries with it the risk of infection of humans through direct contact with the poultry and local birds and the possibility of cross-contamination with *S. typhi* and *K. pneumoniae* of food and drinking water sources with antibiotic resistant bacteria which could be faecal shed directly into the

environment [21-22]. This may epidemiologically increase the prevalence of the typhoid fever and other clinical illness associated with *S. typhi* and *K. pneumoniae* [23]. There should be strict regulation of the use of antibiotics in animals to minimise the emergence of resistant bacteria in animals which may further aggravate the public health problem associated with dissemination of antibiotic resistant bacteria into the environment.

REFERENCES

[1] S. L. Foley, A. M. Lynne and R. Nayak. (**2008**). *Jour. Anim. Science.* 86: E149-E162.
[2] K. O. Akinyemi, B. S. Bamiro and A. O. Coker (**2007**). *Jour. Health. Pop. Nutrition.* 3:351-358.
[3] I. O.Okonko, F. A. Solaye, O. D. Eyarefe, T. A. Amusan, M. J. Abubakar, A. O. Adeyi, O. M. Ojezele and A. Fadeyi (**2010**). *Brit. Jour. Pharm. Toxicol.* 1:6-14.
[4] E. A. Eze, B. N. Ukwah, P. C. Okafor, and K. O. Ugwuh (**2011**). *Afr. Jour. Biotech.* 10:2135-2143.
[5] G. S. Babini and D. M. Livermore (**1999**). *Jour. Antimicrob. Chemother.* 45: 183-189.
[6] P. S. Mead, L. Slutsker, L. F. McCraig, J. S. Bresee, C. Shapiro, P. M. Girffin, R. V. Tauxe and V. Dietz (**1999**). *Emer. Infect. Dis.* 5: 607-625.
[7] S. H. Kim, C. I. Wei, Y. M. Tzou and H. An (**2008**). *Jour. Food Protec*. 68: 2022-2029.
[8] C. L. Gyles (**2008**). *Animal Health Research Reviews*. 9: 149–158.
[9] R. Bada-Alambedji, A. Fofana, M. Seydi and A. J. Akakpo (**2006**). *Br. Jour. Microb*. 37: 510-515.
[10] A. Kilozo-Nthenge, S. N. Nahashon, F. Chen and N. Adefope (**2008**). *Poultry Sci.* 87: 1841-1848.
[11] A. B. Sutiono, A. Qiantori, H. Suwa and T. Ohta (**2010**). *BMC Research Notes*. 106-115.
[12] S. A. Enabulele, P. O. Amune, W. T. Aborisade (**2010**). *Agric. Biol. J. N. Am*., 1(6): 1287-1290.
[13] P. O. Olutiola, O. Famurewa and H, G. Sonntag (**2001**). Biochemical reactions of organisms. In: *An introduction to general microbiology*: *a practical approach.* . Bolabay Publications. pp 144-153.
[14] Clinical Laboratory Science Institute (**2008**). Performance standards for antibiotic susceptibility tests. Document M100-517, Wayne, PA.
[15] R. Fuller (**2006**). *Biologia.* 61: 751-754.
[16] A. Kobe, B. Eggerdin, B. Skubitch and R. Fries (**1995**). *Berl Munch Tierarztl Wochenschr.* 108: 412-417.
[17] M. A. Akond, S. Alam,S. M. R. Hassam and M. Shrin (**2009**). *Int. Jour. Food Safety.* 11: 19-23.
[18] M. Wouafo, A. Nzouakeu, J. A. Kinfack,F. C. Fonkoua, G. Ejenguele, G. Ninje and A. Ngandjio (**2010**). *Microb*. *Drug*. *Res*. 16:171-176.
[19] I. Phillips,M. Casewell, T. Cox, B. De-Groot, C. Friis, R. Jones,C. Nightingale,R. Preston and J. Walldell (**2004**). *Jour. Antimicrob. Chemother.* 115: 1048-1057.
[20] J. Bauer-Garland, J. G. Frye,M. E. Berrange, M. A. Harrisson. and P. J. Fedorka-Cray (**2006**). *Jour. Appl. Microbiol.* 101: 1301-1308.
[21] M. S. Al-Ghamdi, F. El-Morsey, Z. H. Al-Mustafa, R. Al-Ramadan and F. Hanif (**1999**). *Trop. Med. Int. Health.* 4:278-283.
[22] S. Zhao, P. F. McDermont, S. Friedman, S. Qaiyumi, J. Abott, C. Kieddling, S. Ayers, R. Singh, S. Hubert, J.Sofos and D. G. White (**2006**). *Jour. Food Prot.* 69:500-507.
[23] S. Barrow (**2000**). *Rev. Sci. Tech. off. Int. Epiz.*, 2000, 19 (2), 351-375.