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RESEARVOIR OF ANTIMICROBIAL RESISTANT SALMONELLAE AMONG POULRY IN A LOCAL GOVERNMENT AREA IN OGUN STATE, NIGERIA

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ABSTRACT

A survey was carried out to determine the reservoir of antimicrobial resistant *Salmonella* among poultry in a local government in Ogun State, Nigeria. Isolation and identification of Salmonella strains mainly from faecal samples of nonclinical birds and blood samples from few clinical cases were carried out. The resistance profile of the *Salmonella* isolates was determined by disc-diffusion method. The results indicated relative large number of resistant *Salmonella* from birds. Young birds, ducks, exotic hens and intensively reared birds are useful characteristics for identifying birds likely to carry resistant *Salmonella*.

Resistance to ampicillin, cephalexin and cefuroxine are highest among the tested drugs. In all, 22 resistant phenotypes were identified. Thirty-two out of 38 isolates were multi-resistant.

Keywords: Reservoir, Antimicrobial, Resistant Salmonella, Poultry.

INTRODUCTION

Salmonellae are gram negative aerobic rods that are pathogenic to man and animals and are usually acquired through the oral route. Members of this important group of pathogens are usually isolated from faeces or faecal matter, blood and urine of infected animals. In the poultry industry, Salmonella infection causes severe economic losses through mortalities, unthriftness and reduced production. Often the most suitable method of treating Salmonella infection in poultry is to medicate with an appropriate antimicrobial drug.

Many years of antimicrobial drug usage have brought great benefit to man and animals in terms of saving of lives and the relief of suffering when infected with the clinically important microorganisms including *Salmonella.* Substantial amounts of antimicrobials have also been used for prophylaxis and growth promotion in animal husbandry. The non-therapeutic uses of antimicrobials drugs have played a significant economic role in controlling losses due to infectious diseases and in helping to meet the growing demands for animal protein food.

These benefits, however, have not been without disadvantages. Independent surveys of both animals and man have shown that bacteria have the ability to develop resistance to these drugs. It has also been shown that about 70% of these bacterial strains are capable of transferring the whole or part of their infectious drug resistance to drug sensitive bacteria. (Adetosoye and Rotilu 1985). The emergence of this multiple drug resistance has been recognized in many parts of the world especially where there is widespread use of oral antimicrobial agents for prophylaxis and therapy. (Munoc *et al.,* 1993)

The implication of the emergence of drug resistance microorganisms is the decline in

the usefulness of antimicrobial agents in the treatment of infectious diseases in both man and animals. Consequently, the treatment of choice on the basis of the most favourable drug both from the pharmacological point of view and cost may have to be modified to meet the resistance properties of the infecting organisms.

Although the problem has been recognised since the advent of the drugs, the consequences of the emergence were controlled by the availability of effective alternative agents (Neu.. 1992). Unfortunately at the moment, the slow pace in the development of new antimicrobial agents has aggravated the problem (Neu, 1992), and an increasing number of diseases are resisting treatment due to the spread of drug resistance (Gold and Moellering. 1996). Anderson (1997) alerted that unless some urgent action was taken, we would have widespread occurrence of multi drug resistant strains of very serious kind.

As part of measures to prevent the spread of antimicrobial resistance, Van den Bogaard (1993) suggested a systematic registration and analysis of patterns of resistance in pathogenic and non-pathogenic faecal bacteria flora. Anderson (1997) and John and Fishman (1997) also suggested routine susceptibility testing and surveillance programmes as essential measures to assess the prevalence of many of the drug resistant strains of bacteria.

This investigation was therefore undertaken to provide information on the presence of antimicrobial resistant *Salmonella* strains among poultry in Ijebu-North Local Government Area of Ogun State. Nigeria. This is part of the global effort to control the emergence and spread of resistant bacterial strains.

MATERIALS AND METHODS

Sample collection

Markets and poultry farms in Ijebu-North Local Government Area of Ogun State were visited for sample collection (Table 1). Faecal samples from non-clinical cases were collected from not less than 5% of the total number of birds present at the time of visit. The samples were collected with the aid of clean polythene sheet spread under the cages in such a way as to collect freshly voided faeces from six birds at a time. Faecal samples collected from six birds were pooled. Where the birds were held in unconventional (local) cages, cloaca faecal samples were collected into polythene sheet with the aid of sterile swabs.

After the collection, the polythene sheet containing the faecal samples were properly folded and kept overnight in the refrigerator at 4°C before bacterial isolation. 1 milliliter of blood was collected from each sick bird by cardiac puncture with sterile needle and syringe (Table 1).

Bacterial isolation

The sample in each polythene sheet consisting faeces from six birds was thoroughly mixed. Two grammes from this was suspended in 15.0 ml sterile saline in sterile universal bottle. The faecal suspension was thoroughly shaken and allowed to settle for about 30 minutes. With the aid of a sterile dropping pipette, 1.0 ml from the faecal suspension was dropped in 9.0 ml of selenite F broth (Oxoid®) in sterile test tube plugged with cotton wool. The inoculated broth was incubated aerobically for 48 hrs at 37°C. Loopful from the selenite broth culture was then streaked on to deoxycholate citrate agar

Table 1. Distribution of resistant *Salmonella* among birds from farms and markets

Source	Nature of sample	Bird type	Age	Age in lay (weeks)	No. of birds in flock	No. of birds sampled	No. of samp1es collected	No. of samp l es with resistant *Salmonella*	Percentage of sample with resistant *Salmonella*
Farm	Faeces	Exotic Chicken (Layers)	152	26	200	72	12	4	33.3
Farm	Faeces	Exotic Chicken (Layers)	50	26	80	24	4	0	0
Farm	Faeces	Exotic Chicken (Layers)	50	27	260	78	13	10	76.9
Farm	Faeces	Exotic Chicken (Layers)	50	[1]26	320	96	16	10	62.5
Market	Faeces	Indigenous Chicken (Hen)	>52	Not Known	100	30	5	1	20
Market	Faeces	Indigenous Chicken (Cock)		Not Applicable	60	18			
		Exotic Chicken (Cockrels)	4	Not Applicable	100	30		4	80
	Blood	Exotic Chicken (Cockrels)		Not Applicable	600	*4			100
Market	Faeces	Duck	>52	Not Known	100	30			80
Market	Faeces	Guinea Fowl	>52	Not Known	40	12			
Market	Faeces	Pigeon	>52	< Not Known	40	12	2		
TOTAL					1,900	406	71	38	53.5

(Biotest®) plate containing $25\mu g$ of ampicillin (Sigma"") per ml of the agar. After incubation at 37°C for 48 hrs, non lactose fermenting colonies with morphologic characteristics of *Salmonella* were picked from the elevated central surface with a straight platinum wire and inoculated into sterile peptone water in bijou bottle. The inoculated peptone water was incubated at 37°C for 24 hrs and was subsequently kept at room temperature for further test.

At the time of examination, suspected *Salmonella* cultures in peptone water were subcultured onto MacConkey agar (Oxoid®) plates and incubated at 37°C for 24 hrs.

All cultures that failed to hydrolyse urea but showed positive methyl red reaction, positive lysine decarboxylase test and reduced nitrate to nitrite were further tested for agglutination in polyvalent `O' antiserum. (Wellcome).

Cultures suspected to be *Salmonella* from these tests were inoculated into Kligler iron agar tubes by stabbing the center of the butt and streaking the slanted surface of the medium. The inoculated tubes were then incubated at 37°C for 48 hrs. Kligler cultures giving reactions suggestive of *Salmonella* were further examined in motility, indole and urea (MIU) medium and in peptone water sugar medium with Andrade indicator. The test sugars include glucose, lactose, inositol and trehalose. The results obtained from these tests enabled us to identify the isolates as *Salmonella.*

Antimicrobial susceptibility test

The isolates identified to be *Salmonella* were tested for their antimicrobial sensitivity by disc-diffusion method (Barry and Thornberry, 1991) using diagnostic sensitivity test (DST) agar (Oxoid®). Resistance to a specific antimicrobial was based on reference zonal diameter interpretative standards (Barry and Thornberry, 1991). An isolate was considered to be resistant for its ability to grow on DCA-ampicillin medium, and if it was resistant to l - 3 of the antimicrobial tested. It is also considered to be of multiple resistance if resistance was shown towards 4 or more antimicrobial agents.

RESULTS

A total of 71 (67 faecal and 4 blood) samples were collected from 406 birds. The 406 birds comprised of 402 non-clinical birds (birds not showing signs of *Salmonella* infection) and 4 clinical birds (birds showing signs of *Salmonella* infection). The non-clinical birds were randomly selected from 1,300 birds while the clinical ones were selected from 600 sick birds (Table 1).

The *Salmonella* isolates showed resistance predominantly to ampicillin, cephalexin and cefuroxine antimicrobials (Table 2). Among the non-clinical birds, resistant *Salmonella* strains were highest among the indigenous ducks than any other bird type (Table 3). Higher percentage of the exotic than the indigenous chicken carried resistant *Salmonella* strains. Resistant *Salmonella* strains occurred among the exotic cockerels than the old indigenous cock, the intensively reared than the extensively reared l ns and the old exotic than the old indigenous layers. Resistant *Salmonella* strains were higher in farm than in market poultry and in clinical than in non-clinical chicken. In this study, pigeon and guinea fowl were not found to carry any resistant *Salmonella* strains.

Table 2. Frequency of antimicrobial resistance among *Salmonella* organisms isolated from poultry

Antimicrobial agent	No. of isolates tested	No. of resistant isolates	Percentage
Ampicillin (25μg)	38	35	92.11
Cephalexin (25μg)	38	35	92.11
Cefuroxine (30μg)	8	35	92.11
Gentamicin (10μg)	38	10	26.32
Floxacin (10μg)	8	0	0
Nitrofurantoin (200μg)	38	14	36.84
Co-trimoxazole (50μg)	38	20	52.63
Tetracycline (50μg)	38	21	55.26
Chloramphenicol (30μg)	38	07	18.41

From the 38 resistant *Salmonella* isolates that were obtained in this study, 32 were multi-resistant (resistance to 4 or more antimicrobials) while 22 resistant phenotypes (R-phenotypes) were demonstrated (Table 4). The spread of the R-phenotypes among the birds are shown on Table 4.

DISCUSSION

An attempt to assess the potential threat posed by resistant *Salmonella* strains must take into account the meat animals involved. In this study, we have examined poultry (Table 1) and the results showed that a large number of the *Salmonella* isolates from birds were resistant to the clinically important antimicrobial agents such as ampicillin, chloramphenicol, gentamicin and co-trimoxazole. The recommendations of the Swann Committee restricting the clinically useful antimicrobials as growth promoters (Swann Report, 1969) were based on the assumption that resistant bacteria from animal sources pose definite risk to the treatment of human diseases because patterns of antimicrobial resistance have been related to the usage of a particular drug (Linton, 1977).

For example, in 1983 in the U.S.A., eighteen people infected with a multi drugresistant strain of *Salmonella newport* were reported to have eaten hamburger from beef cattle fed sub-therapeutic dose of chlortetracycline for growth promotion (Spika *et al.,* 1987). Lee *et al.* (1993) also reported isolation of resistant *Salmonella* species from broiler chicken after slaughter.

It has been shown that exposure to an antimicrobial agent a month before *Salmonella* illness is a risk factor for having a resistant infection (Lee *et al.,* 1994). In this study, information was not available on the exposure of the birds to antimicrobial agents in the 30 days before collection of the faecal samples. But, results of previous study by Oyekunle and Owonikoko (2002) had shown that massive quantities of antimicrobial drugs were used for poultry production in the study area. The high rate of resistance to ampicillin in

Table 3. Frequency of carriage of resistant *Salmonella* by bird's characteristics

Characteristics	No of birds sampled	No of samples collected	No of bird's with Salmonella	Percent resistant
Bird type				
Chicken	352	62	34	54.83
Duck	30	05	04	80
Guinea Fowl	12	02	0	0
	12	X 02	0	
Bird variety				
Exotic chicken	304	54	32	59.26
Indigenous chicken	48	08	02	25
Indigenous duck	30	05	04	80
Indigenous guinea fowl	12	02	0	0
Indigenouspigeon	12	02	0	0
Sex/use				
Exotic laying hen	270	45	24	53.33
Indigenous breeding hen	30	05	01	20
Indigenous breeding/meat cock	18	03	01	33.33
Exotic meat cockrel	34	09	08	88.89
Indigenous breeding duck	30	05	04	80
Management				
Hen (intensive)	270	45	24	33.33
Hen (extensive)	30	05	01	20
Cock (ectensive)	18	03	01	33.33
Cockrel (intensive)	34	09	08	88.89
Duck (extensive)	30	05	04	80
Age (weeks)				
Duck (>52)	30	05	04	80
Chicken (>52)	30	05	01	20
Chicken (50 - 52)	270	45	24	53.33
Cock (>52)	18	03	01	33.33
Cockrel (4 - 5)	34	09	08	88.89
Source				
Farm	304	54	32	59.26
Market	102	17	06	35.29
Health status				
Non-Clinical	402	67	34	50.07
Clinical	04	04	04	100

this study (Table 1) is of particular concern since the drug is useful in the treatment of a wide range of bacterial infections.

Similar results to ours were previously reported (Adetosoye and Rotilu, 1985; Rowe and Threlfall, 1984) suggesting that strains of *Salmonella* resistant to ampicillin

phenotypes of *Salmonella* isolated from poultry

Resistance group*	Phenotype	No. of strains	Sources					
			Chicken (exotic layers)	Chicken (indigenous breeder)	Chicken (indigenous cock)	Chicken (exotic cockrels)	Chicken (exotic cockrels Clinical	Duck (indigenous)
R1	Amp.cxm.Te							
R2	Amp.cx.cxm.Te	7					1	
R3	Amp.cx.gen.f.cxm.Te							
R4	Amp cx.cot.gen.cxm.Te							
R5	Amp cx.cot.gen.cxm.Te		1					
R6	t Amp.cx.cot.gen.cxm_Te							
R7	Amp.cx.cot.							
R8		1						
R9	Cx	1						
R10	Amp.cx							
R11	Amp.cccot.cxm.te.e							
R12	Cxm.te							
R13	Amp.cx.cxm.te.e							
R14	Amp.cx.cot.cxm.							
R15	Cx.f.cxm.te.							
R16	Amp Amp.cot.gen.cxm.te							
R17	Amp.cx.cxm.te.c							
R18	Amp.cx.cot.cxm.te.c.							
R19	Amp.cx.cot.gen.cxm.							
R20	Amp.cx.cxm.c.							
R21	Amp.cx.cot.gen.f.cxm.							1
R22	Amp.cx.gen.f.cxm.							

Amp. - Ampicillin
te. - Tetracycline
gen. - Gentamicin
C. - Chloramphenicol
cxm. - Cefuroxine
cx. - Cephaloxin
cot. - Cotrimoxazole
f. - Nitrofuratoin

and other drugs are becoming more frequent. It is documented (Lee *et al., 1994)* that outbreak of ampicillin resistant *Salmonella* may occur when animals have taken a penicillin-derivative a week before acquiring *Salmonella* organism. Other workers (Murray. *1986;* Lopardo *et al., 1991; Rossi et al., 1995)* also reported *Salmonella* strains that were resistant to co trimoxazole, ampicillin, tetracycline, chloramphenicol and gentamicin as obtained in this study.

However, the levels of resistance of *Salmonella* strains to these drugs as reported

by the various authors differ from our results (Table *2).* Out of *92 Salmonella* strains that were examined by Lopardo *et al. (1991) 14%* were resistant to ampicillin while 6% were resistant to co-trimoxazole.

Rossi *el al.* (1995) examined 127 *Salmonella* strains and found 72% to be resistant to ampicillin, 70% to gentamicin and 44% to co-trimoxazole.

Although an assessment of trends in the level of antimicrobial resistant *Salmonella* strains was not part of this study, reports from other authors indicated an increase. Ward *el al.* (1990) reported an increase from 5% in 1981 to 12% in 1988 among human *Salmonella* strains while Threlfall et *al.* (1992, 1993) reported an increase from 15% in 1981 to 66% in 1990 among animal *Salmonella* strains. The increase in the trend as reported above may explain the high percentages of resistance of our *Salmonella* isolates to some of the clinically useful antimicrobial agents that were tested in this study.

The quinolone such as floxacin is recommended as the therapeutic agent of choice for invasive salmonellosis (Gupta. 1995; Ivanoff, 1995). This recommendation lends credence to our finding in this study that none of our *Salmonella* isolates was resistant to floxacin. Lee *et al.* (1994) reported similar finding to ours while Wang *et al.* (1989) reported a resistance level of 0.003% for floxacin. On the other hand, our result differs from that of Frost e/ *al.* (1995) who reported that 40% of the *Salmonella* strains isolated from poultry were resistant to floxacin. According to Frost *et al.* (1995) the licensing of floxacin for veterinary use might have been responsible for his observation. It is important to note however, that floxacin is expensive and may impose a substancial burden on the health budget of developing countries like Nigeria.

Of all the mechanisms by which bacteria.. R-plasmid infection constitutes the most important. Transferable drug resistance occurs *in vivo* both in animals and man but the frequency at which it occurs is debatable. It is also speculated that *in vivo* transfer of drug resistance cannot be the only factor responsible for the current prevalence of anti microbial

antimicrobial resistance may arise in resistance. Two additional factors which are sequel to medical, veterinary and agricultural uses of antimicrobials have been suggested (Linton, 1997).

These are

1. The survival of resistant cells at the expense of sensitive ones under antimicrobial selection pressure such that they grow and become the dominant component of the flora.

2. Such dominant bacteria may penetrate into the sensitive gut flora of other animals and possibly man by cross infection.

Exotic birds, farm birds, clinical birds, chicks and ducks are useful characteristics for identifying birds likely to carry resistant *Salmonella* (Table 4).

The high level of resistant *Salmonella* among these bird groups would be due in part to the differences in the distribution of the serotypes being carried (Lee *et al.,* 1994) and on the continuing use of antimicrobials which have favoured selective development of resistance (Linton, 1977; Oyekunle and Owonikoko, 2002). AntimicrobiaIs in proprietary feeds could serve as source of continuing administration of the drugs to exotic chicken and other birds (Oyekunle and Owonikoko, 2002). Ducks, because of their association with damp, dirty and muddy places, can carry high loads of *Salmonella*

serotypes (Thear and Fraser, 1986) that may be drug resistant.

The development of resistance to antimicrobials is a dynamic and complex process and reducing the relative contributions made by the use of these drugs in human and animals will require a collaborative effort by everyone involved. The potential risk associated with misuse of antimicrobials can be quantified by good surveillance and epidemiological studies. Several studies have indicated the stability of the plasmid profiles of *Salmonella* species and thus plasmid analysis has been suggested as a useful method for epidemiological investigation. The importance of epidemiological research in antimicrobial resistance was emphasized by Swann Committee (Swann Report, 1969) and if the committee's recommendations had been fully implemented, the problem could have been better solved than is currently the case.

Bax (1997) opioned that cooperation was the key to tackling the problem of antimicrobial resistance. Thus, in Nigeria, there is a need to educate the general public on the potential hazards associated with irrational and indiscriminate uses of antimicrobials in human and animals. According to Bax (1997), if you are not part of the solution, you are actually part of the problem.

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