Nigerian Veterinary Journal
VOL:33 (2) 483-491
ARTICLE

Quinolone Resistance in Bacterial Isolates from Chicken Carcasses in Abeokuta, Nigeria: A Retrospective Study from 2005-2011.

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SUMMARY

Quinolone resistance in bacteria from food animals is now globally recognized as a serious veterinary and public health problem. To determine the rate of quinolone resistance in pathogenic bacteria isolated from samples from dead chickens submitted for microbiological examination, a six-year retrospective study (April, 2005 – March, 2011) was carried out. Data from records of bacteriological investigations at a Veterinary Teaching Hospital in Nigeria were evaluated. Two hundred bacterial isolates including *Escherichia coli* (95; 47.5%), *Salmonella* serotypes (78; 38.0%), *Klebsiella* (17; 8.5%) and *Staphylococcus aureus* (12; 6.0%) were isolated from chicken carcasses within the six-year period. On the overall, the isolates were resistant to ciprofloxacin (40.5%), enrofloxacin (21.0%), nalidixic acid (9.5%) and norfloxacin (44.0%). Overall, resistance to quinolones (except nalidixic acid) was highest in *S. aureus* (ciprofloxacin, 58.3%; enrofloxacin, 33.3%; and norfloxacin, 83.3%) followed by *Klebsiella spp* (ciprofloxacin, 41.2%; enrofloxacin, 29.4%; and norfloxacin, 64.7%), *E. coli* (ciprofloxacin, 40.0%; enrofloxacin, 23.2%; and norfloxacin, 41.1%) and least in *Salmonella* (ciprofloxacin, 38.6%; enrofloxacin,14.5%; and norfloxacin, 36.8%). However, resistance to nalidixic acid was highest in *Klebsiella spp* (23.5%) followed by *S. aureus* (16.7%), *E. coli* (9.5%) and least in *Salmonella* (5.3%). There was a general decline in quinolone resistance in the last three years (2009-2011) of this investigation. Quinolone resistance in avian pathogenic bacteria could lead to increase in economic loss from bacterial infection and refractory to treatment. Their possible transmission to humans is of public health significance.

KEY WORDS: Bacterial isolates, Commercial poultry chickens,Quinoloneresistance.

INTRODUCTION

In Nigeria, the poultry industry has good potentials for the attainment of food security, poverty eradication and foreign exchange earnings (Bourn *et al.* **1992)**. However, the realization of these potentials is hampered by many obstacles among which is the morbidity and mortality caused by infectious diseases resulting in high economic losses (Pritchett *et al.* 2005). Infections caused by *Escherichia coli*, *Salmonella* serotypes and other members of the enterobacteriaceae are among the common bacterial diseases of poultry birds (Kabir, 2010).

In most cases, bacterial diseases are treated with antimicrobial agents. These

agents are also administered in feed and water as prophylactic measure to forestall outbreak of infections. However, over dependence on antimicrobials has placed selection pressure on pathogenic bacteria leading to the emergence and spread of antimicrobial resistance as commonly encountered in the treatment of bacterial diseases (WHO, 1998). In the poultry industry, it has been observed that more and more bacterial diseases of poultry are becoming resistant to commonly used antimicrobial (Gross, 1994; Kilonzo-Nthenge *et al.,* 2008; Ogunleye *et al.,* 2008).

The emergence of antimicrobial resistance in pathogenic bacteria constitutes a serious problem in the control of infectious diseases. Many infections which were hitherto successfully treated based mainly on the clinician's past experience are increasingly becoming more refractory to traditional therapy (OIE, 2008). This may necessitate a longer duration of therapy, an increase in dose or a change of drug. The consequences of these are reduction in the level of production, increase in the cost of production and a threat to availability of animal protein to human population. Antimicrobial resistance therefore could be a major limitation to the growth of the poultry industry.

The increasing incidence of resistance to the first-line antimicrobial has led clinicians and farmers to resort to using newer generation antimicrobials such as quinolones (especially the fluoroquinolones) which are considered more effective in combating the resistant bacteria (Alo and Ojo, 2007). Quinolones are now widely being used in the treatment of all kinds of bacterial infection both in humans and in animals (Piddock, 1996). However, there has been emergence of resistance to the quinolones among bacteria following their introduction for use in the treatment of infectious diseases (Conly, 2002). This trend is on the increase (Ogunleye *et al.,* 2008; Overdevest *et al.,* 2011).

In Nigeria, only few reports are available on the prevalence and patterns of quinolone resistance in common bacteria pathogens of avian origin (Ogunleye *et al.,* 2008). This study investigated the occurrence of quinolones resistance in common bacterial pathogens isolated from cases of chicken mortality from commercial poultry farms in Ogun State, Nigeria; over a period of six years (April, 2005 to March, 2011)

MATERIALS AND METHODS

Data were extracted from the laboratory records of bacteriological investigations carried out on samples taken from chicken carcasses submitted to the Microbiology laboratory of the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta from April, 2005 to March, 2011. Information provided in the records included date of sample collection, source of sample, nature of sample, media for bacterial isolation, bacterial isolates identified, antimicrobial susceptibility testing method and result of the susceptibility test. Records showed that the samples included liver, spleen, lung, heart and ovaries which were aseptically collected during post mortem examination.

Bacterial isolation and identification:

Bacterial isolation was by inoculation of samples onto both MacConkey and 5% blood agars incubated aerobically at 37 ^{o}C for 18 – 24 hours. Isolates were identified by cultural characteristics, microscopy, biochemical tests (oxidase, catalase, substrates utilization) coagulase tests (*Staphylococcus aureus*) as described by Barrow and Feltham (1993).

Antimicrobial susceptibility test:
Antimicrobial susceptibility testing was by disk diffusion method and interpretation of breakpoint was based on the guidelines provided by Clinical and Laboratory Standards Institute (2000). For the purpose of the present study, only susceptibility of bacteria isolates to the quinolones, namely: ciprofloxacin (10 µg), enrofloxacin (5 µg), nalidixic acid (30 µg) and norfloxacin (10 µg) manufactured by Oxoid® (Basingstoke, UK) were considered.

Statistical analysis
Prevalence rates were expressed in percentages and represented in bar chart.

RESULT
Within the six years under review, 200 bacterial isolates were recovered from samples of dead chickens submitted for microbiological examination. These comprised of *E. coli*, 95 (47.5%); *Salmonella*, 78 (38.0%); *Klebsiella spp*, 17 (8.5%); and *Staphylococcus aureus*, 12 (6.0%) (Table 1). *Escherichia coli* and *Salmonella* were detected in each year of the study period while no *Klebsiella spp* was detected in the fifth and sixth year respectively (Table I). *Staphylococcus aureus* was encountered only in first and second years (Table I).

Of the 200 isolates, 81 (40.5%) were resistant to ciprofloxacin, 42 (21.0%) to enrofloxacin, 19 (9.5%) to nalidixic acid and 88 (44.0%) were resistant to norfloxacin (Table II). On the overall, resistance to quinolones (except nalidixic acid) was highest in *S. aureus* (ciprofloxacin, 58.3%; enrofloxacin, 33.3%; and norfloxacin, 83.3%) followed by *Klebsiella spp* (ciprofloxacin, 41.2%; enrofloxacin, 29.4%; and norfloxacin, 64.7%), *E. coli* (ciprofloxacin, 40.0%; enrofloxacin, 23.2%; and norfloxacin, 41.1%) and least in *Salmonella* (ciprofloxacin, 38.6%; enrofloxacin, 14.5%; and norfloxacin, 36.8%) (Figure 1). However, resistance to nalidixic acid was highest in *Klebsiella spp* (23.5%) followed by *S. aureus* (16.7%), *E. coli* (9.5%) and least in *Salmonella* (5.3%).

DISCUSSION
This study revealed a high incidence of the enterobacteriaceae especially *E. coli* and *Salmonella* in cases of mortalities in chickens. *Escherichia coli* and *Salmonella* were consistently detected throughout the six-year period under review. In each year, *E. coli* accounted for over 40% of all isolates while *Salmonella* accounted for between 25% and 49% of the isolates. Other bacterial pathogens such as *Klebsiella* and *Staphylococcus aureus* were also detected. All over the world, diseases caused by *E. coli* and *Salmonella* serotypes are commonly reported as responsible for morbidity and mortality in poultry chickens (Bajuwa *et al.,*1992; Kilonzo-Nthenge *et al.,* 2008; Kabir, 2010). Previous studies have shown that *E. coli* and *Salmonella* are common causes of morbidity and mortality in the study area (Ogunleye *et al.,* 2008; Agbaje *et al.*, 2010). In the present study, the detection of other bacterial pathogens such as *Klebsiella* and *Staphylococcus aureus* varied within the six-year period. *Klebsiella* was detected consecutively for four years and accounted for about 3.3% to 15.7% of the total isolates. *Staphylococcus aureus* was only detected in the first and third year. Previous studies (Turtura *et al.,* 1990; Kilonzo-Nthenge *et al.,* 2008) have identified *Klebsiella species* in chickens. *Klebsiella species* have been recognized as secondary bacterial agents responsible for complications in other diseases (Bleich *et al.,* 2008). Also, previous studies have reported the isolation of *Staphylococcus aureus* from chickens (Capita *et al.,* 2002; Persoons *et*

al., 2009) corroborating the findings in the present study.

The increasing incidence of antimicrobial resistance among clinical bacterial isolates is a global phenomenon that has generated a lot of concerns in human and veterinary clinical practices. The quinolones have been described as an exceptionally important and rapidly developing group of antimicrobial drugs introduced into human and veterinary medicine for a wide variety of antimicrobial purposes (Orden *et al.,* 2000). The efficacy of these antimicrobial agents in the treatment of infectious diseases is being threatened by the emergence of resistant bacterial strains. In the present study, there was a high rate of quinolone resistance among the bacterial isolates. Generally, the highest rate of resistance was to norfloxacin followed by ciprofloxacin. Resistance to nalidixic acid was the lowest. Salehi and Bonab (2006) reported high rate of quinolone resistance (ciprofloxacin, 67 %; norfloxacin, 68%; enrofloxacin, 78%; and nalidixic acid, 100%) in avian pathogenic *E. coli* isolates similar to the range observed in the present study. However, the rate of nalidixic resistance in the present study did not at any point exceed 50% in all the bacterial species identified. Other workers also reported rates of quinolone resistance among *E. coli* isolates of chicken origin similar to those observed in the present study (Miles *et al.,* 2006). In contrast to the findings in the present study, a lower rate of quinolone resistance that range from 0.5% to 5.9% for nalidixic acid and 0.0% to 4.9% for enrofloxacin and ciprofloxacin were reported in *Escherichia coli* isolates of ruminant origin (Orden *et al.,* 2001). Similar to the findings in the present study, Agbaje *et al.,* (2010) reported high rates of quinolone resistance in *Salmonella*. However, Forrest *et al.* (2009) did not detect fluroquinolone resistance in non-typhoidal *Salmonella* isolated from humans. Oyekunle *et al.* (2003) also reported a zero quinolone resistance in *Salmonella*. In the present study, the highest levels of quinolone resistance were in *Staphylococcus aureus* followed by *Klebsiella, E. coli* and least in *Salmonella*. This probably suggests a higher rate of emergence of quinolones resistance in other bacteria other than the enterobacteriaceae. Although the rate of resistance was particularly high in the first three years (April 2005 – March 2008), it appeared that there was a decline in the incidence of resistance to the quinolones over the last two years (April 2009- March 2011). The reason for this decline is not clear and needs to be investigated.

The high rate of quinolone resistance among avian pathogenic bacterial isolates in the present study may be related to high antibiotic usage in poultry production (Alo and Ojo, 2007; Ogunleye *et al.,* 2008). In Nigeria, quinolones are increasingly being preferred over other antimicrobial agents both as prophylaxis in raising chicks and as therapeutic agents in treatment of bacterial infections (Alo and Ojo, 2007). In the study area, norfloxacin and enrofloxacin are commonly used in poultry production while nalidixic acid ciprofloxacin are rarely used (Ogunleye *et al.,* 2008). Since the mode of action of the quinolones is similar, resistance to a member may induce resistance to others members. An association has been described between the emergence of fluoroquinolone resistant zoonotic pathogens and the use of these drugs in animals (Blanco *et al.,* 1997). In addition, antimicrobial resistance traits in bacteria are often resident within transmissible mobile genetic elements which can be shared among bacteria (Lee *et al*., 2006). Resistant bacteria may transfer their resistant traits to other bacteria

within the same environment through mobile genetic elements such as plasmids and transposons (Lee *et al.*, 2006). Exchange of resistant genetic materials is particularly common among enteric bacteria. This contributes significantly to the persistence, spread and overall prevalence of antimicrobial resistance among bacteria within a community.

Resistance of avian pathogenic bacteria to quinolones may lead to increase in economic loss from bacterial infections that are refractory to antibiotic therapy. Resistance to quinolones among avian pathogens is also of public health implication as the resistant bacterial strains could be transmitted to humans. *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* are among major zoonotic bacteria implicated in human food-borne infections (Mead *et al.,* 1999).

TABLE I: Rate of occurrence of four bacteria pathogens in chicken carcasses in Abeokuta, Nigeria over a six-year period.

Year (Number of isolates)	Number (%) of identified bacterial species				
	E. coli	*Salmonella spp*	*Klebsiella spp*	*Staphylococcus aureus*	Total (%)
I	23 (45.1)	13 (25.5)	8 (15.7)	7 (13.7)	51 (100.0)
II	15 (50.0)	14 (46.7)	1 (3.3)	0	30 (100.0)
III	17 (41.5)	15 (36.6)	4 (9.8)	5 (12.2)	41 (100.0)
IV	16 (50.0)	12 (37.5)	4 (12.5)	0	32 (100.0)
V	17 (51.5)	16 (48.5)	0	0	33 (100.0)
VI	7 (53.8)	6 (46.2)	0	0	13 (100.0)
Total (%)	95 (47.5)	76 (38.0)	17 (8.5)	12 (6.0)	200 (100.0)

Year I: April 2005- March 2006
Year II: April 2006- March 2007
Year III: April 2007- March 2008
Year IV: April 2008- March 2009
Year V: April 2009- March 2010
Year VI: April 2010- March 2011

TABLE II: Rates of quinolone resistance in bacterial isolates from dead chickens from commercial poultry farms in Abeokuta, Nigeria over a six-year period (Mach, 2005 to April, 2011).

Year	Bacterial Isolates	Number of isolates	Norfloxacin n (%)	Ciprofloxacin n (%)	Enrofloxacin n (%)	Nalidixic acid n (%)
I	*E. coli*	23	14 (60.9)	10 (43.5)	0	8 (34.8)
	Salmonella	13	11 (84.6)	7 (53.8)	0	3 (23.1)
	Klebsiella spp	8	6 (75.0)	2 (25.0)	0	4 (50.0)
	Staphylococcus aureus	7	7 (100.0)	4 (57.1)	0	2 (28.6)
	Sub-total	**51**	**38 (74.5)**	**23 (45.1)**	**0**	**17 (33.3)**
II	*E. coli*	15	9 (60.0)	9 (60.0)	4 (26.7)	0
	Salmonella	14	9 (64.3)	7 (50.0)	0	0
	Klebsiella spp	1	0	0	0	0
	Staphylococcus aureus	0	0	0	0	0
	Sub-total	**30**	**18 (60.0)**	**16 (53.3)**	**4 (13.3)**	**0**
III	*E. coli*	17	13 (76.5)	12 (70.6)	14 (82.4	0
	Salmonella	15	6 (40.0)	6 (40.0)	8 (53.3)	0
	Klebsiella spp	4	4 (100)	4 (100)	4 (100.0)	0
	Staphylococcus aureus	5	3 (60.0)	3 (60.0)	4 (80.0)	0
	Sub-total	**41**	**26 (63.4)**	**25 (60.9)**	**30 (73.2)**	**0**
IV	*E. coli*	16	3 (18.8)	3 (18.8)	3 (18.8)	1 (6.3)
	Salmonella	12	2 (16.7)	3 (25.0)	2 (16.7)	1 (8.3)
	Klebsiella spp	4	1 (25.0)	1 (25.0)	1 (25.0)	0
	Staphylococcus aureus	0	0	0	0	0
	Sub-total	**32**	**6 (18.8)**	**7 (21.9)**	**6 (18.8)**	**2 (6.3)**
V	*E. coli*	17	0	4 (23.5)	0	0
	Salmonella	16	0	6 (37.5)	0	0
	Klebsiella spp	0	0	0	0	0
	Staphylococcus aureus	0	0	0	0	0
	Sub-total	**33**	0	**10 (30.3)**	**0**	**0**
VI	*E. coli*	7	0	0	1 (14.3)	0
	Salmonella	6	0	0	1 (16.7)	0
	Klebsiella spp	0	0	0	0	0
	Staphylococcus aureus	0	0	0	0	0
	Sub-total	**13**	**0**	**0**	**2 (15.4)**	**0**
Overall total		200	88 (44.0)	81 (40.5)	42 (21.0)	19 (9.5)

Year I: April 2005- March 2006
Year II: April 2006- March 2007
Year III: April 2007- March 2008
Year IV: April 2008- March 2009
Year V: April 2009- March 2010
Year VI: April 2010- March 2011

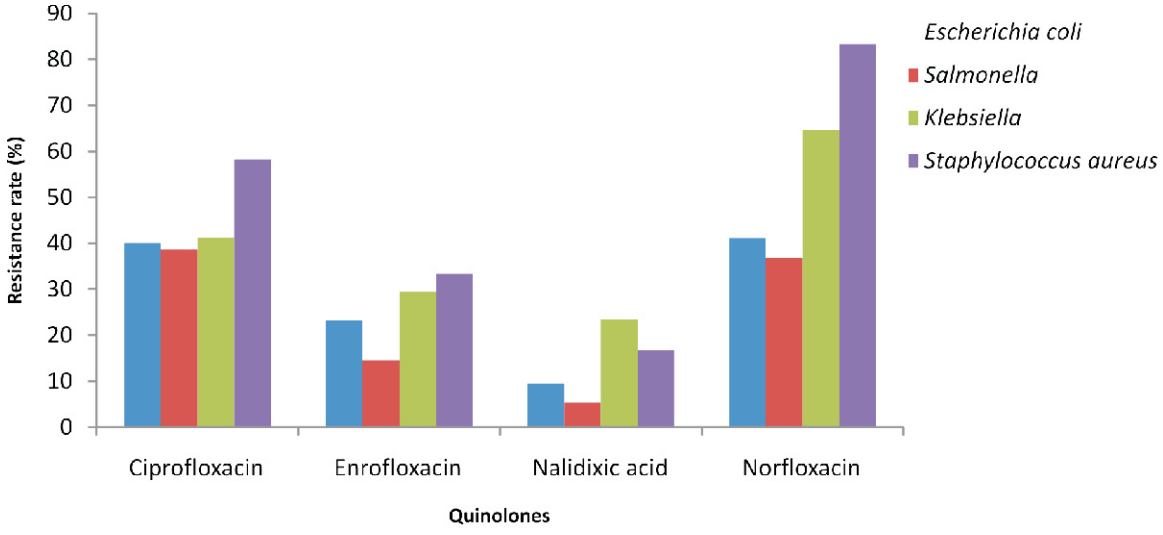

Figure 1:Overallquinolone resistance in bacterial pathogens isolated from chicken carcasses over a six-year period

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