Correlation between the use of Antimicrobials and the Occurrence of Antimicrobial Resistant Bacteria in Poultry and Pig Farms

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Abstract- Antimicrobials are valuable therapeutics whose efficacy is seriously compromised by the emergence and spread of antimicrobial resistance. A survey was carried out to evaluate the relationship between the use of antimicrobials in animal production and the occurrence of antimicrobial resistant organisms. The survey was conducted between November, 2012 to May 2013 using structured questionnaires. Responses to the questionnaires were analyzed using linear regression and correlation variables. Results showed that correlation between the use of antimicrobials and the occurrence of antimicrobial resistant bacteria were both positive and negative on one hand and significant and non-significant on the other hand at 0.01 and 0.05 in both poultry and pig farms. Escherichia coli isolates had a negative (-0.20) non significant ($P > 0.050$) correlation with increase in dosage of antimicrobial given. Negative, non-significant ($P > 0.05$) correlations were found between dosage of antimicrobials given and number of Enterococcus isolates (-0.19). In Table 2, the correlations between the variables were almost positive except between dosage of antimicrobials given and number of Enterococcus isolates where there was no correlation.

Keywords: *antimicrobial usage, occurrence, resistant bacteria, poultry and pig farms.*

GJMR-C Classification : *NLMC Code: QW 4*

CORRELATIONBETWEENTHEUSEOFANTIMICROBIALSANDTHEOCCURRENCEOFANTIMICROBIALRESISTANTBACTERIAINPOULTRYANDPIGFARMS

Strictly as per the compliance and regulations of:

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Abstract- Antimicrobials are valuable therapeutics whose efficacy is seriously compromised by the emergence and spread of antimicrobial resistance. A survey was carried out to evaluate the relationship between the use of antimicrobials in animal production and the occurrence of antimicrobial resistant organisms. The survey was conducted between November, 2012 to May 2013 using structured questionnaires. Responses to the questionnaires were analyzed using linear regression and correlation variables. Results showed that correlation between the use of antimicrobials and the occurrence of antimicrobial resistant bacteria were both positive and negative on one hand and significant and non-significant on the other hand at 0.01 and 0.05 in both poultry and pig farms. *Escherichia coli* isolates had a negative (-0.20) non significant ($P>0.050$) correlation with increase in dosage of antimicrobial given. Negative, non-significant ($P>0.05$) correlations were found between dosage of antimicrobials given and number of Enterococcus isolates (-0.19). In Table 2, the correlations between the variables were almost positive except between dosage of antimicrobials given and number of Enterococcus isolates where there was no correlation. Results from linear showed that farm size and level of education were significant at 5% and 10% in poultry and pig farms respectively. The results of this study suggest that the amounts and patterns of antimicrobials used in food animals is the major determinant for the propagation of resistant bacteria in the animal reservoir. However, further studies are needed for other determinants that may play a part in the propagation of resistant bacteria in animal reservoir.

Keywords: antimicrobial usage, occurrence, resistant bacteria, poultry and pig farms.

I. Introduction

There has been massive use of antimicrobials in animal husbandry. The most abundant use of antimicrobials worldwide is in livestock; they are typically distributed in animal feed and water for purposes such as disease prevention and growth (Silbergeld *et al.*, 2008). Debates have arisen surrounding the extent of the impact of these antimicrobials, particularly antimicrobial growth promoters, on human antimicrobial resistance. Although some sources believe that there remains a lack of knowledge on which antimicrobial use generates the most risk to humans (Landers *et al.*, 2012).

The use of antibiotics has been linked to the rise of resistance in every drug and species where it has been studied, including humans and livestock. The use of antimicrobials in various forms in widespread throughout animal industry. The practice of using antimicrobials for growth stimulation is problematic as it is the longest use of antimicrobials worldwide (Silbergeld *et al.*, 2008). Its sub therapeutic use results in bacteria resistance (Silbergeld *et al*, 2008) and every important class of antimicrobial are being used in this way, making every class less effective (Sillbergeld *et al.*, 2008).

There has been a study on whether there was a connection between resistance and the practice of feeding a drug related to vancomycin to animals as a growth stimulant (Landers *et al.*, 2012). Vancomycin-resistant enterococci can spread from animals to humans (Wegner, 2003) CC 398 is a methicillin-resistant *Staphylococcus aureus* which was produced by the use of antibiotics in livestock production (Peter *et al.*, 2008). The appearance of carbepenem resistant entero-bacteriaceae has been attributed in part to antibiotic in livestock (Carlet *et al.*, 2012). The overuse of fluoroquinolone and other antibiotics fuels antimicrobial resistance in bacteria, which can inhibit the treatment of antimicrobial-resistant infections (Nauhauser, *et al.*, 2003). Widespread use of fluoroquinolones as a first-line antibiotic has led to decreased antimicrobial sensitivity, with negative implications for serious bacterial infections such as those associated with cystic fibrosis, where quinolones are among the few viable antibiotics (Ziganshina and Squire, 2008).

Although microbial resistance results primarily as a consequence of selection pressure placed on a susceptible microbes by the use of therapeutic agents, a variety of social and administrative factors also contribute to the emergence and spread of resistance. The aforementioned factors necessitated the need to carry out this study.

II. Materials and Methods

a) Poultry and Pig Farms

A total of 70 poultry and 50 pig farms were randomly selected from the 17 local government areas of Abia State, Nigeria were selected. The poultry and pig

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farms that participated in this study were managed intensively and were classified as large and commercial poultry and pig farms.

b) Survey Questionnaire

A survey instrument (questionnaire) on antimicrobial usage was developed for collecting information on antimicrobial usage. The questionnaires were administered by the author to the manager or the owners of each farm. The questionnaire sought information like dosage of antimicrobials given, frequency of antimicrobial use, duration of administration, who makes prescription etc as well as personnel data.

c) Statistical Analysis

Answers to the questionnaires were analyzed using linear regression where X is the independent variables and Y is the dependent variables. Correlation analysis was done to determine the relationship between antimicrobial usage and the occurrence of antimicrobial resistant bacteria in poultry and pig farms at 0.01 and 0.05 levels.

III. RESULTS

A significant reason for high selection pressure in the face of modest antimicrobial expenditure is inappropriate antimicrobial use. Table 1 shows the correlation between the use of antimicrobials and the occurrence of antimicrobial resistant bacteria in pig farms. The correlation among some variables was both positive and negative on one hand, and significant and non-significant on the other hand. For instance, the correlation between dosage of antimicrobial given (X_1) and frequency of antimicrobial use (X_2) was positive (0.46) and significant ($P<0.05$). This implies that the dosage and frequency of antimicrobial have positive association such that increase in the frequency of use will lead to increase in the dosage of antimicrobials. Dosage of antimicrobial given and number of *Escherichia coli* isolates had a negative (-0.20) non-significant ($P>0.05$) correlation, implies that *E.coli* isolates will decrease with increase in dosage of antimicrobials.

Table 1 : Correlation between the use of Antimicrobials and the Occurrence of Antimicrobial Resistant Bacteria in Pig Farms

	X1	X2	X3	X4	X5	X6
X1	1					
X2	0.46**	1				
X3	0.28*	0.36**	1			
X4	0.30*	0.23	0.16	1		
X5	-0.20	-0.31*	-0.27*	0.10	1	
X6	-0.01	-0.19	-0.15	0.15	0.93	1

** = correlation is significant at 0.01 levels
*= correlation is significant at 0.05 levels
X1= Dosage of antibiotics given
X2 = Frequency of antimicrobial use
X3 = number of animals in the flock that received antimicrobials
X4 = Completion of antimicrobial treatment
X5= Number of E. coli isolates
X6 = Number of Enterococcus isolates

Generally, positive and significant ($P<0.05$) correlations existed between each of dosage of antimicrobial given and frequency of antimicrobial use, dosage of antimicrobial given and number of animals in the flock that received antimicrobial; dosage of antimicrobial given and completion of antimicrobial treatment and frequency of antimicrobial use and number of animals in the flock that received antimicrobial with correlation coefficient of 0.46, 0.28, 0.30 and 0.36 respectively.

Correlation between each of frequency of use of antimicrobials and completion of antimicrobial treatment; number of animals in the flock that received antimicrobials and completion of antimicrobial treatment; completion of antimicrobial treatment and number of *E. coli* isolates; completion of antimicrobial treatment and number of Enterococcus; and number of

E.coli isolates and number of Enterococcus isolates were positive and non significant ($P>0.05$) with respective correlation coefficients of 0.23, 0.16, 0.10, 0.15 and 0.93 respectively.

Negative significant ($P<0.05$) correlations existed between frequency of use of antimicrobials and number of *E.coli* isolates (-0.31) and between number of animals in the flock that received antimicrobials and number of *E.coli* isolates (-0.27), while negative non-significant ($P>0.05$) correlations were found between dosage of antimicrobials given and number of *E.coli* isolates (-0.20) dosage of antimicrobial given apnd number of Enterococcus isolates (-0.01); frequency of antimicrobial use and number of Enterococcus isolates (-0.19); and number of Enterococcus isolates and number of animals in the flock that received antimicrobials (-0.15).

Table 2 : Correlation between the use of Antimicrobials and The Occurrence of Antimicrobial Resistant Bacteria in Poultry Farms

	X1	X2	X3	X4	X5	X6
X1	1					
X2	0.12	1				
X3	0.30*	0.58**	1			
X4	0.27*	0.32*	0.36**	1		
X5	0.39**	0.24*	0.29*	0.41**	1	
X6	0.00	0.26*	0.26*	0.17	0.50*	1

*** = correlation is significant at 0.01 levels*
** = correlation is significant at 0.05 levels*
X1 = Dosage of antibiotics given
X2 = Frequency of antimicrobial use
X3 = number of animals in the flock that received antimicrobials
X4 = Completion of antimicrobial treatment
X5 = Number of E. coli isolates
X6 = Number of Enterococcus isolates

Table 2 above showed the correlation between the use of antimicrobials and the occurrence of antimicrobial resistance bacteria in poultry farms. The correlations between the variables were almost positive except between dosage of antimicrobial given and number of Enterococcus isolates where there was no correlation. Dosage of antimicrobial given and frequency of antimicrobial use; completion of antimicrobial treatment and number of Enterococcus isolates each had positive non-significant ($P>0.05$) correlation with coefficients of 0.12 and 0.17 respectively. Other positive correlations were all significant ($P<0.05$).

Table 3 shows the regression of dependent variables, the most common antimicrobial use (Y1) and frequency of use (Y2) on the dependent variables using four functional forms- linear, semi-log, double log and exponential in poultry farms. The values outside the parenthesis between Y1 and Y2 and each of the X's are the regression coefficients, while those in the parenthesis are the t-statistics. For instance, the linear regression coefficient between Y1 and each of X1, X2 and X3 are 0.46, 0.11 and 0.43 respectively and that of Y2 are -0.02, 0..17 and -0.12 respectively.

Table 3 : Regression Analysis in Poultry Farms Regression Functions

Explanatory variable	Linear		Semi-log		Double log		Exponential	
	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
Constant	-2.10	1.36	-0.27	1.50	-0.50	0.37	-1.41	0.37
	(-1.19)*	(1.45)*	(-0.31)*	(3.18)**	(-1.18)	(1.33)*	(-1.66)*	(0.67)
X1	0.46	-0.02	1.14	-0.12	0.57	-0.10	0.23	-0.23
	(2.75)**	(-0.22)	(2.65)**	(-0.53)	(2.76)	(-0.76)	(2.88)**	(-0.44)
X2	0.11	0.17	-0.29	0.41	-0.20	0.25	-0.9	0.10
	(-0.59)	(1.71)	(-0.81)*	(2.16)**	(-1.15)*	(2.21)**	(-0.98)	(1.70)*
X3	0.43	-0.12	0.78	-0.17	0.37	-0.11	0.20	-0.08
	(1.87)*	(-0.15)	(1.89)*	(-0.78)*	(1.88)*	(-0.86)	(1.79)*	(-1.13)*
X4	0.34	-0.52	0.51	-0.31	0.25	-0.15	0.17	-0.08
	(1.37)*	(-1.15)	(1.14)*	(-1.33)*	(1.19)*	(-1.05)*	(1.39)*	(-1.00)*
X5	-0.06	0.35	-0.18	0.56	-0.12	0.37	0.05	0.21
	(-0.27)	(2.90)**	(-0.40)	(2.41)**	(-0.59)	(2.62)**	(-0.44)	(2.90)**
X6	-0.01	-0.05	-0.10	-0.10	-0.05	-0.06	0.00	-0.04
	(-0.05)	(-0.50)	(-0.31)	(-0.58)	(-0.31)	(-0.67)	(0.01)	(-0.66)
X7	-0.001	0.09	0.14	0.29	0.13	0.16	0.02	0.08
	(-0.004)	(0.79)*	(0.20)	(0.77)*	(0.38)	(0.71)	(0.18)	(1.24)*
X8	0.64	1.36	0.92	0.16	0.46	0.07	0.33	0.06
	(1.55)	(0.63)	(1.51)	(0.49)	(1.59)*	(0.37)	(1.64)*	(0.49)
R^2	0.24	0.36	0.24	0.36	0.11	0.38	0.27	0.40
Error term	1.81	1.89	1.81	1.92	1.80	1.87	1.83	1.81
F statistics	1.35*	2.46**	1.35*	2.41**	1.68*	2.62**	1.60*	2.86**

*** = significant at 5%*
** = significant at 10%*
R^2 = coefficient of determination

Values in parenthesis are + statistics of individual X variables

X = Independent variable(X1 =level of education; X2 = farm size, X3 = Reason for antimicrobial use, X4 = Duration of administration, X5 = who makes the prescription, X6= Reason for treatment using antimicrobial, X7= Frequency of consulting a veterinarian, X8= Availability of veterinarian when needed.

Y = dependent variable (Y1 = the most common antimicrobial use, Y2 = frequency of use)

The linear regression coefficient between Y1 and X1 indicated that a unit increases in level of education led to 0.46 increases in the most common antimicrobial use, and this was significant at 5%. Thus level of education is a determinant factor in the use of antimicrobials. Increase in the farm size in poultry farming will lead to increase use of a particular antimicrobial due to increased assessment of market information. Thus social factor may play an important role in the success or otherwise of poultry farming.

Table 3 also showed that the coefficient of multiple determinant (R^2) for Y1 and Y2 in linear, semi-log,, double log and exponential regression functions were 0.24,0.36, 0.24, 0.36, 0.11, 0.38, 0.27 and 0.40 respectively. The R^2 indicates the total variation in Y (dependent variable) that is caused by X's (the independent variables). The values of R^2 were greatly low, below 50%, the highest being 0.40, between frequency use and the independent variables. This indicates that about 40% of the total variation in the most common antimicrobial use was caused by the combined effect of the X1-X2.

Table 4 showed the regression of dependent variables, the most common antimicrobial use (Y1) and frequency of use (Y2) on the dependent variables using four functional forms- linear, semi-log, double log and exponential in pig farms. The values outside the parenthesis between Y1 and Y2 and each of the X's are the regression coefficients (bs), while those in the

parenthesis are the t-statistics. Taking X1, X2 and X3 as example, the linear regression coefficients between Y1 and each of X1, X2 and X3 are 0.08, 0.22 and -0.19 respectively and that of Y2 are 0.17, 0.69 and -0.03 respectively.

The semi-log regression coefficients of these variables are- 0.04, 0.48 and 0.22 for Y1and 0.27, 1.38 and 0.17 for Y2 respectively. The double log regression coefficients of these variables are -0.03, 0.24 and -0.14 forY1 and 0.17, 0.61 and -0.06 for Y2 respectively. Similar results for the exponential regression are 0.04, 0.11 and-0.11 for Y1 and 0.09, 0.31 and -0.04 for Y2 respectively. The linear regression coefficient between Y1 and X1 indicated that a unit increases in level of education led to 0.08 increases in the most common antimicrobial used, and this was not significant. Similarly, as farm size increased, frequency of use of antimicrobial increased in pig farms and this was significant at 10%.

In Table4, the coefficient of multiple determinants (R^2) for Y1 and Y2 in linear, semi-log, double log and exponential regression functions were 0.15, 0.34, 0.20, 0.31, 0.20, 0.34, 0.14 and 0.37 respectively. The values of R^2 were generally smaller than 50%, the highest being 0.37, between frequency of use and the independent variables. This indicates that about 37% of the total variation in the most common antimicrobial used was caused by the combined effect of the VII-VIII.

Table 4 : Regression Analysis in Pig Farms Regression Functions

Explanatory variable	Linear		Semi-log		Double log		Exponential	
	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
Constant	2.27 (1.86)*	0.20 (0.16)	2.63 (3.67)***	0.82 (1.12)*	0.95 (2.45)**	0.17 (0.58)	0.75 (0.13)*	-0.03 (-0.06)
X1	0.08 (0.60)	0.17 (1.19)*	-0.04 (-0.13)	0.27 (0.78)*	-0.03 (-0.18)	0.17 (1.22)*	0.04 (0.52)	0.09 (1.58)*
X2	0.22 (0.90)*	0.69 (2.77)**	0.48 (0.98)*	1.38 (2.66)**	0.24 (0.93)*	0.61 (2.93)**	0.11 (0.82)*	0.31 (3.08)***
X3	-0.19 (-0.80)*	0.03 (0.13)	-0.22 (-0.45)	0.17 (0.33)	-0.14 (-0.55)	-0.06 (-0.28)	-0.11 (-0.86)*	-0.04 (-0.377)
X4	-0.25 (-1.10)*	0.24 (1.01)*	-0.42 (-0.88)*	0.43 (0.85)*	-0.27 (-1.03)*	-0.14 (0.67)	-0.15 (0.23)	0.08 (0.83)*
X5	0.37 (1.96)*	0.38 (1.90)*	0.80 (2.44)**	0.54 (1.54)*	0.43 (2.39)**	0.22 (1.56)*	0.19 (1.87)*	0.15 (1.93)*
X6	0.03 (0.17)	0.15 (0.96)*	0.06 (0.21)	0.24 (0.85)*	0.60 (0.42)	0.14 (1.24)*	0.03 (0.73)	0.09 (1.41)*
X7	-0.18 (-1.0)*	-0.02 (-0.10)	-0.43 (-1.28)*	0.08 (0.22)	-0.23 (-1.24)*	0.07 (0.46)	-0.09 (-0.97)*	0.00 (0.05)
X8	-0.19 (-0.72)	0.16\ (0.59)	-0.32 (-0.86)*	0.20 (0.50)	-0.13 (-0.65)	0.06 (0.38)	-0.07 (-0.50)	0.06 (0.50)
R^2	0.15	0.34	0.20	0.31	0.20	0.34	0.14	0.37

Error term	1.47	1.63	1.49	1.65	1.51	1.77	1.49	1.76
F statistics	0.77	2.20**	0.99	1.96*	0.99	2.26**	0.72	2.52**

***, **, * = significant at 1%, 5% and 10% respectively

R^2 *= coefficient of determinant*

Values in parenthesis are t-statistics of individuals X variables

X = Independent variable (X1 = level of education, X2 =farm size, X3 = regression for antimicrobial use, X4 = duration of administration, X5 = who makes the prescription, X6 = reasons for treatment using antimicrobials, X7 = frequency of consulting a veterinarian, X8 = availability of veterinarian when needed)

Y = Dependent variable (Y1 = the most common antimicrobial use, Y2 = frequency of use)

IV. DISCUSSIONS

Information on the occurrence of antimicrobial resistance is needed at the local, national and international levels to guide policy and detect changes that require intervention strategies. Such monitoring programs should be continuous and standardized, enabling comparison between countries as well as overtime. Comparing different antimicrobials, we have shown that resistance gene abundance and penetration on average are higher for drugs used in animals, even when compensating for differences in many resistance genes are known. This is consistent with expectations from previous research into a "farm-to-flock" connection (Marshall and Levy, 2011).

We first analyzed some general trends such as the connection between the use of antimicrobials in animal husbandry and the spread of resistance, previously suggested from studies of one or a few antimicrobials at a time (Bager *et al.*, 1997). We observed a clear and significant increase in resistance gene abundance both for antimicrobials approved for animal use and for older antimicrobials that have been longer in the market. These effects are independent and hold even when controlling for differences in number of genes active against each antimicrobial class or subclass. The Danish antimicrobial resistances, on the other hand, has a relative bias toward bacitracin and vancomycin and to a lesser extended toward streptomycin, spectromycin and chloramphenicol. Notably, a vancomycin analog (avoparcin) has been previously administered to animals in Europe (Barton, 2000), and was subsequently banned as its use was linked to a rapid European increase in vancomycin-resistant enterococci (VRE) (Aarestrup, 2012).

In Tables 1 and 2, there was a positive correlation between the use of antimicrobials and the occurrence of antimicrobial resistant bacteria in poultry and pig farms. These correlations were significant at both 0.01 and 0.05 levels. For instance, in Table 1, increase in the frequency of antimicrobial use leads to the development of antimicrobial resistance to *E. coli*. In the poultry farms, increase in the frequency of use and dosage of antimicrobial leads to antimicrobial resistance to *E. coli* and Enterococcus. This is in agreement with comparative study done by de Jong *et al*; (2012) and Borg (2012) showing that resistance potential correlates significantly with out-patient antimicrobial use.

To further investigate the effect of agricultural use of antimicrobial on the antimicrobial resistance (Table 3); we collected data on level of education, farm size, reasons for antimicrobial use, duration of administration who makes the prescription, reason for treatment using antimicrobials, frequency of consultancy a veterinarian,, availability of veterinarian when needed. The linear regression coefficient between Y1 and X1 indicated that a unit increases in the most common antimicrobial use, and this was significant at 5%. Thus level of education is a determinant factor in the use of antimicrobials. In Table4, the linear regression coefficient between Y1 and X1 indicated that a unit increases in level of education leads to 0.08 increases in the most common antimicrobial use, and this was not significant. Similarly, as farm size increased, frequency of antimicrobial use increased in pig farms and this was significant at 1%. Samples from some animal species are, on average, more similar in their antimicrobial resistance potential to samples from different animals species, and this similarly does not decrease noticeably with time. This is consistent with earlier research on individual antimicrobials (Johnson *et al*; 2011) showing that resistance determinants, once introduced into the microbial flora, can persist for a long time at low abundance, which might also explain the high vancomycin resistance potential in the Danish population despite its animal-use analog being banned since 1995 (Aarestrup, 2012).

Thus, we conclude that the use of antimicrobials in animals contribute to resistance development in commensal bacteria. Thus, the outcome of our investigation covering a vast range of antimicrobials should provide a profound molecular basis for the ongoing debate on the appropriate use of antimicrobials in agriculture and medicine.

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