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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Antimicrobial resistance of *Salmonella* isolated from swine in Abia State, Nigeira

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Abstract

The aim of the study was to evaluate the antimicrobial resistance against *Salmonella* isolated from swine farms in Abia State, Nigeria. *Salmonella* isolates (n=400) were obtained from 260 faecal samples and 90 environmental samples. Of the faecal samples comprises 80 piglets, 80 growers and 80 finishers, isolates were classified into susceptible, intermediate or resistant based on NCCLS guideline. Two-third of the isolates (6.4%) were susceptible to all of the 10 antimicrobials in the antibiotic disc. No noticeable resistance was recorded for amoxicillin, ceftriaxone, cephalothin, ciprofloxacin and nalidixic acid. About 0.8% isolates were resistant to gentamycin. The frequencies of resistance were higher for tetracycline (36.49%), streptomycin (23.5%), ampicillin (9.8%) and kanamycin (14.8%). The absence of resistance to the fluoroqniolones and low resistance to amoxicillin, gentamycin are findings that would be of great importance to public and animal health. The level of common resistance observed for kanamycin, streptomycin, ampicillin and tetracycline indicates the need for a more effective use of these antimicrobials.

Keywords: *Salmonella*; resistance; multi-drug resistance; swine

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Introduction

Salmonella species are the second leading cause of bacterial food borne disease worldwide. In Canada, approximately 6000 to 9000 cases of human Salmonellosis are reported annually, and for each reported illness, 13-17 cases are unreported (Thomas et al., 2006). Antimicrobial resistant *Salmonella* can cause even greater morbidity than their susceptible counterparts due to treatment failure and increased infection severity (Verma et al., 2005).

Although pork is not a major cause of Salmonellosis in America, it has been responsible for disease outbreaks of multi-resistant *Salmonella* in humans elsewhere (Molbak et al., 1999; Hedberg, 2002). Most *Salmonella* infections are acquired from contaminated food; therefore, studying antimicrobial resistance in live pigs indirectly estimates the potential for cross-contamination at slaughter and the risk to consumers from resistant organisms in pork (Van den Bogaard et al., 2000; Angulo et al., 2000).

Due to growing concern over antimicrobial resistance and its adverse effect in human and animal health, international public health has recommended antimicrobial that nations should develop surveillance programs for humans safety (World Health Organization, 1997; World Health Organization for Animal Health, 2001).

More recently, antimicrobial resistance of *Salmonella* collected from healthy swine at slaughter (Poppe et al., 2001; Sorensen et al., 2001; Larkin et al., 2004) and healthy swine at farm (Poppe et al., 2001) has been examined.

The aim of this study was to evaluate the antimicrobial resistance profiles of *Salmonella* isolated from piglets grower and finisher swines in Abia State, Nigeria.

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Materials and Methods

Collection of samples

Faecal samples were collected from different pig farms in Abia State. The farms were located at Ogbor Hill, Osisioma, Ekaegbara, Ugunagbo, Umudike in Aba South, Aba North, Ikwuano and Ugunagbo local government areas of Abia State. Faecal samples were collected from piglets, growers and finisher using sterile spatula. An average of 20 samples was collected at a time. Samples were transported in Ice Park to Veterinary Microbiology Laboratory of Michael Okpara University for analysis. The samples were collected between March and August 2010.

Salmonella isolates

Approximately 1g of faeces was added to 9 ml of selective enrichment broths (selenit-F-broth) and mixed very well by steady shaking and later incubated at 37^{o}C overnight. The broths were later sub-cultured onto brilliant green agar (BGA) (Oxoid Ltd, Detroit, Mich, USA) and incubated aerobically at 37^{o}C for 24 hours. On examination, suspected isolates of *Salmonella spp.* showed pink colonies. They were thereafter subjected to various biochemical test using standard methods (McFadden, 2000). Identified *Salmonella* isolates were confirmed using commercially available *Salmonella* polyvalent antiserum (Difio Ltd, Detroit, Mich., USA) in a slide agglutination test. Isolates that were positive were recorded.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out in Veterinary Microbiology Laboratory of Michael Okpara University of Agriculture Umudike, Abia State. All isolates (n=400) were tested for susceptibility to 10 antimicrobials using the microdilution technique and the MIC (minimum inhibitory concentration) panel (sensitive diagnostic systems, Westlake, OH) and the manufacturers instruction was used. The break points for resistance of antimicrobials were $\geq$32µ/ml: for amoxicillin, 32µ/ml: for ampicillin, 64µ/ml: for kanamycin, 32µ/ml: nalidixic acid, 64µ/ml: streptomycin, 16µ/ml: Tetracycline, 4µ/ml: ciprofloxacin, 16µ/ml: Gentamycin, 64µ/ml: ceftriaxone, 32µ/ml: cephalothin. Panels were used 24 hours after inoculation. Results were recorded as sensitive, resistant and intermediate according to National Committee for Clinical Laboratory standards Guidelines (2002). The control organism used for this study is typed *Echerichia coli* ATCC 25922.

Data manipulation and analysis

The susceptible data were programmed into a Microsoft excel spreadsheet and evaluated for accuracy by checking each entry against the original raw data copy. For the purpose of proper analysis, isolates possessing intermediate susceptibility to the tested antimicrobials were classified susceptible. The tabulation frequency of outcome was variable and was determined for each of the 10 antimicrobials used for antibiotic susceptibility testing. The correlation among antimicrobial resistance traits was examined at isolate level using Pearson's correlation coefficients (Dahoo et al., 2003).

Results

Of the 400 *Salmonella* isolates obtained, 262 (16.5%) were susceptible to all the 10 antimicrobials. Hence, 138 of the 400 (34.5%) *Salmonella* isolates were resistance to one antimicrobial or the other. For amoxicillin, ceftriaxone, cephalothin, ceproflaxin and nalidixic acid, there was no recorded resistance. Thus, amoxicillin (3.2%) cephalothrin (2.7%) and ceptriaxone (2.5%) was observed for intermediate susceptibility Gentamycin recorded (0.8%). The antimicrobials that registered higher frequencies of resistance were observed for ampicillin (9.6%) kanamycin (14.8%) streptomycin (23.5%) and tetracycline (36.4%). The pattern of distribution of antimicrobial resistance at various concentrations (µg/ml) is shown in Table 1.

Among 138 isolates resistant to at least one antimicrobial, 46 isolates were resistant to one antimicrobial or other. Resistance to only one antimicrobial drug was frequently observed due to tetracycline (36 isolates). From Table 2, tetracycline and streptomycin recorded the highest resistant level which belonged to Eleke's farm. Statistically significant correlations for resistance among various antimicrobials at the isolate level are presented in Table 3.

Discussion

In this study, 66.4% of *Salmonella* isolates were highly susceptible compared to that reported in other studies (Farrington et al., 2001; Gebreyes et al., 2004). The difference may be due to difference in sampling method. The findings of the present study are in agreement with a previous report (Deckert et al., 2003). The study also showed that of all the *Salmonella* isolates obtained, tetracycline (47.8%) was the highest. In nalidixic acid, ciprofloxacin, cephalothin and amoxicillin, no noticeable resistance was recorded. In previous study, it was observed that clinical *Salmonella* isolates from United States of America, 2001 cases showed high level resistance to tetracycline (56%) streptomycine (48%) and ampicillin (38%) while the resistance frequency was low to amoxicillin, gentamycin and cephalothin (Deckert et al., 2003). Resistance was detected more in tetracycline and streptomycin (Table 2). Carlson et al. (2003) reported

Table 1: The distribution of MICs (μg/ml) among 400 *Salmonella* isolates collected from 10 swine farms in Abia State

	Number of isolates at each MIC (μg/ml)								N = 400 isolates			
Antimicrobial	0.015	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
Ceftriaxone		N		310	2							
Cephalothin							145	118	32	4^x	0^y	
Ciprofloxacin	249	11					0^y	0^y				
Amoxicillin					4	260	32		16	9^x	0^y	
Ampicillin								290	3	0^y	0^y	
Gentamyxin				120	152	28	4		0^y	1^x		
Kanamycin										280	0^y	32^x
Nalidixic acid								282	30	2	0^y	
Streptomycin										240	84^x	
Tetracycline									192	120		

Y = the number of resistant isolates; X = the number of isolates with intermediate susceptibility

Table 2: The resistant distribution among 138 *Salmonella* resistant isolates by farm location

	Number of resistant *Salmonella* isolates by antimicrobial				
Farm location	A	G	Kv	Sv	Tv
Eke farm (Ogbor Hill)	13	-	12	14	28
Illoh farm (Ogbor Hill)	9	-	12	11	14
Eleke farms (Ogbor Hill)	28	12	8	43	54
Obinna farm (Osisioma)	5	-	-	15	28
Friday farm (Osisioma)	-	-	1	3	-
Tony farm (Osisioma)	-	4	2	6	21
Love farm (Ugunagbo)	11	-	7	5	17
Goodluck farm (Ekeagbara)	3	-	-	1	3
Happy farm (Ugunagbo)	-	1	-	-	19
Umudike farm (Ikwuano)	-	-	-	-	3
Total 388	69	7	42	98	172

A = Ampicillin, G = Gentamycin; K = Kanamycin; S = Streptomycin; T = Tetracycline

Table 3: Pearson correlation coefficients for resistance to various antimicrobials among *Salmonella* species isolated from pigs

Antimicrobial	Statistically significant	($P \leq 0.05$)	Pearson's correlation coefficients
Amoxicillin (Am)	G^x (0.80)		
Kanamycin (K)	A^Y (0.15)	G (0.25)	St (0.86)
Streptomycin (S)	A (0.50)	K (0.30)	G (0.12)
Tetracycline (T)	A (0.28)	K (0.32)	K (0.20)

X = Gentamycin; Y = Ampicillin

that such type of resistance might be linked genetically with resistance to other antimicrobials.

The study suggests that there was high level correlation between occurrences of resistance to various antimicrobials as reflected in (Table 3). The result of this study shows that antimicrobial resistance and multi-drug resistance occurs in *Salmonella* isolates among piglets (growers and finisher swine).

Antimicrobials are basically used to minimize the severity of clinical cases of *Salmonella* in swine, prevent spread of infection and disease (Schwartz, 1999). The need for further study is necessary especially in the area of monitoring of antimicrobial resistance to evaluate the overall resistance trends and to detect emerging resistance phenotypes in swine.

Conclusion

Salmonella prevalence is high in swine under study. More isolates were obtained from finishers. Gentamycin recorded the least resistance to salmonella isolate.

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