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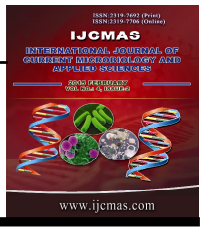

Original Research Article

Evaluation of Prevalence and Antimicrobial Resistance using Enterococci Isolates from Pigs and Poultry Birds in Abia State, Nigeria

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Keywords

Resistance patterns, Resistance surveillance, *Enterococci* species, Poultry, Pigs, Prevalence

A B S T R A C T

Changes in resistance patterns of microorganism from the usage of antimicrobials in animal productions are phenomena of increasing importance. The aim of this research was to investigate the effect on the resistance patterns of *Enterococcus* from antimicrobial use in pigs and poultry birds in Nigeria. This was carried out through antimicrobial resistance evaluation in *Enterococcus* isolated from pigs and poultry in Nigeria. Faecal samples were collected for a period of ten months. Isolates were identified based on colonial morphology on Bile aesculin agar, gram reaction, and lack of catalase production, aesculin hydrolysis and by conventional methods. These *Enterococcus* strains were tested against ten antimicrobial agents to determine the prevalence of multiple antimicrobial resistances among them. The results revealed that more than 50% of the isolates were multiple antimicrobial resistant. Poultry strains showed higher resistance levels (30-50%) than strains obtained from pigs (20-30%) when they were tested against quinolones. Seventy percent of *Enterococcus* species isolated from both animals' species showed resistance, most commonly to erythromycin and floxapen. There was low level of resistance of *Enterococcus* spp. to gentamycin (3%). Percentage of resistance to floxapen (80%), erythromycin (72%) and norfloxacin (60%) were high. Eight resistance patterns of *Enterococcus* isolates from pigs and poultry were found. Resistance in poultry birds of *Enterococcus* species to norfloxacin/erythromycin was most frequent, while resistance to norfloxacin/erythromycin/streptomycin was most frequent in pig isolates. These results indicate a high level of multi-resistance in *Enterococcus* bacteria in Nigeria, suggesting that an antimicrobial resistance surveillance program is needed in Nigeria in order to detect bacterial resistance.

Introduction

Over the past fifty years, antimicrobials have been used in a variety of settings including human medicine, veterinary medicine and plant agriculture. Emergence of multiple-drug-resistant bacteria and the role of potential reservoirs of pathogens is

poorly understood (Bengis *et al*., 2004). The development of antimicrobial resistance among bacteria is a point of concern in both human and animal medicine. Frequent empirical-based antimicrobial therapy of humans, the addition of antibiotics as growth promotions in livestock feeds and antibiotic misuse have been implicated in the selective proliferation of resistant strains (Hershberger *et al.*, 2005).

Enterococcui species are known commensal organisms of the gastrointestinal tract and are also opportunistic pathogens associated with significant morbidity and mortality of humans and animals (Eller-broek *et al*., 2004). *Enterococcus* species as an example of indicator bacteria are part of human intestinal flora and in other mammals, birds and insects. Studying antimicrobial resistance in indicator bacteria from healthy animals would be extremely valuable because of their ability to acquire and could serve as a reservoir for genes that encode antimicrobial resistance, and given the right conditions, could transfer or disseminate these resistance genes to pathogenic or zoonotic bacteria (Moubareeck *et al*., 2003, Lester *et al*., 2006).

Factors other than the use of antimicrobial drugs could influence the maintenance and development of antimicrobial resistance of enteric bacteria in the gastrointestinal tract (Dewulf *et al*., 2007). The use of antimicrobials in animals is of concern (Mora *et al*., 2005) since resistant organisms might be excreted in the faeces of animals, following administration of antimicrobials, and contribute to the reservoir of resistant bacteria in the environment (Ahmed *et al*., 2010).

In Nigeria, as in the rest of the world, antimicrobial agents are the main therapeutic tool for treatment of bacterial diseases in human and veterinary medicine. However, even though it is known that pathogenic bacteria is being isolated from dairy cattle that is resistant and multidrug resistant (MDR) against different antimicrobial agents (San Marten *et al*., 2003), there is no information about such risk in indicator bacteria isolated from pigs and poultry. Therefore, the present study considered the use of *Enterococcus* species to evaluate the prevalence and antimicrobial resistance in pigs and poultry birds.

Materials and Methods

Farms: Culture collection

Faecal samples from 72 piggery farms and 90 poultry farms in Abia State were collected between May 2012 and February 2013. A random sample of various sizes and location of poultry and swine farms was selected. Animals of different species were geographically separated. Faecal samples were collected by rectal swabs from pigs and by cloacae swabs from poultry birds. All samples (360 from pigs and 360 from poultry birds) were immediately placed on ice and kept at refrigerator temperature during transport. A questionnaire was used to collect information from the farmers regarding use of antimicrobials in the animals.

Identification of *enterococci*

Upon arrival to the laboratory one swab stick was directly cultured onto Bile-esculin agar (BBL). Presumptive enterococci were identified using the tests proposed by Fracklam *et al* (2007), on the basis of aesculin hydrolysis in the presence of bile, cellular morphology following gram stain and results of biochemical testing, including catalase production, growth in 6.5% Nacl broth, pigment production, haemolytic

activity, arginin hydrolysis, tolerance to tellurite, utilization of pyruvate, motility and acid production from the following carbohydrates: arabinose, mannitol, raffinose, sucrose and sorbitol (Teixeria and Fracklam, 2003).

Antimicrobial susceptibility testing

Antibiograms was evaluated for 330 isolates by the disk diffusion method, according to the Clinical Laboratory Standard Institute (CLSI, 2006) guidelines. Inoculums were prepared from the overnight cultures incubated on plate count agar at 37^{o}C and the suspension was adjusted to equal the 0.5 McFarland standards. The following 10 antimicrobial agents were tested: ciprofloxacin (10mcg), norfloxacin (10mcg), gentamycin (10mcg), lincocin (20mcg), streptomycin (30mcg), rifampicin (20mcg) erythromycin (30mcg), ampiclox (20mcg), floxapen (10mcg) and chloramphenicol (30mcg). *E.faecalis* ATCC 29212 and ATCC 51299 were used as quality control organisms. Interpretation of susceptibility tests were classified as sensitive, slightly sensitive and resistance as recommended by Weinstein *et al*, (2004).

Statistical analysis

The frequency of antimicrobial resistance profiles of Enterococcus isolates was compared between pigs and poultry birds from Abia State, Nigeria, using the chi-squared test with a significant level of $P < 0.05$.

Result and Discussion

Table 1 showed sample sites, number of samples and number of isolates. This show that samples were collected from two species of animals i.e. pigs and poultry. These species of animals are reared on a large-scale intensive system in the southeast geopolitical zone. Faecal samples were collected from the three different zones of Abia States with one hundred and twenty samples collected from each zone. The number of *Enterococcus* isolates slightly varied among the locations and was constant among the species of animal. Result below showed that a total number of 165 isolates of *Enterococcus* were isolated from pigs and the same number of isolate from poultry birds. The highest number of *Enterococcus* (60 isolates) was isolated from Abia South in both pigs and poultry birds.

Table 2 show the percentage of antimicrobial resistance of *Enterococcus* isolates from pigs. These strains showed high degree of resistance to chloramphenicol (85.8%) and erythromycin (71.8%), and low level of resistance to ampiclox (2.3%), Lincocin (2.4%), gentamycin (2.3%) and streptomycin (4.3%). Intermediate resistance to ciprofloxacin (48.5%) and Floxapen(48%) were more prevalent.

Table 3 show the percentage of antimicrobial resistance to *Enterococcus* isolates from poultry birds. These strains showed high resistance to chloramphenicol (76.7%) and erythromycin (71.7%). This is lower when compared to strains (isolates) from pigs i.e. 85.6% and 71.8%. There was low level of resistance to lincocin (1.2%) and gentamycin (6.7%) only. Resistance to streptomycin in poultry isolates was higher (23.7%) than in pig isolates (4.3%), while isolates resistance to lincocin was lower in poultry isolates (1.2%) than in pig isolates (2.4%). Intermediate resistance to norfloxacin (40.3%), streptomycin (40.3%), ampiclox (51.5%) and floxapen (50.5%) were more prevalent.

Eight resistance patterns were found as shown in Table 4. The predominant pattern

was resistance to norfloxacin/ erythromycin/streptomycin (36 in Abia North, 38 in Abia South and 40 in Abia central). Moderate resistance patterns were seen in Nor/Ery (22 in Abia North, 29 in Abia South and 21 in Abia central). The patterns varied from one zone to another.

Complete patterns of *Enterococcus* species multiple resistant isolates are shown in Table 5. Resistance in poultry birds of *Enterococcus* species to norfloxacin/ erythromycin was most frequent (32in Abia North, 33 in Abia South and 35 in Abia central), while resistance to norfloxacin/erythromycin/streptomycin was most frequent in pigs isolates (36 in Abia north, 38 in Abia South and 40 in Abia central).

Resistance to antimicrobials of isolated enterococci from pigs, performed by agar disc diffusion method is shown in Figure 1. The resistance profiles of the isolates varied among the three zones. Of the 165 isolates 30%, 28% and 20% were resistant to amplicox in Abia North, Abia South and Abia central respectively. The isolates were highly sensitive to gentamicin, lincocin and chloramphenicol, and resistant to floxapen and erythromycin.

The resistance profiles of isolated enterococci from poultry birds are shown in Figure 2. Resistance of isolates from pigs to streptomycin (44%) and norfloxacin (67%) were significantly higher ($P<0.05$) than poultry birds.

The isolates were highly sensitive to chloramphenicol, lincocin and gentamicin. Isolates resistant to floxapen (80% for Abia North, 70% for Abia South, 80% for Abia central), erythromycin (75% for Abia North, 70% for Abia central) was detected.

The enterococci have emerged as important opportunistic pathogens with a remarkable capacity of expressing resistance to several groups of antimicrobial agents, limiting the number of therapeutic options. Species of the genus *Enterococcus* comprise a large proportion of the normal microbial associated with the gastrointestinal tract of humans and animals (Hayes *et al.*, 2003). Their presence in the environment is an indicator of faecal contamination (Mannu *et al*., 2003). The ubiquitous nature of the enterococci and resistance to adverse environmental conditions take account for the ability to colonize different habitats, and for their potential for easy spreading through the food chain. Therefore, the presence of antimicrobial resistance enterococci in food animals has been a matter of growing concern.

This study provides clear evidence that resistance in enterococci to chloramphenicol (85.8% for pigs, 76.7% for poultry birds) and erythromycin (71.8% for pigs; 71.7% for poultry birds) is common in various food animals and that the increase in consumption of antibiotics by animals has been accompanied by an increase in antimicrobial resistance. The potential role that antimicrobial use in veterinary medicine and animal husbandry plays in the transfer of antimicrobial-resistant bacteria to humans remains unresolved. Considering the effect antimicrobial resistance has on human health and also its economic impact, measures to preserve these agents and delay the development of resistance are urgently needed (Turnnidge, 2004). This include judicious use of antimicrobials for human infections, control measures to prevent spread of resistant pathogens in health care facilities and decrease in resistance in reservoirs on farms and in the environment.

In this study we demonstrated the widespread occurrence of antimicrobial resistance to erythromycin (71.7%) in Enterococcal isolates of pigs and poultry birds is likely related to the wide use of this class of antimicrobials in animal husbandry activities (Chopra and Roberts, 2001). Aarestrup *et al* (2002), pointed out that it is common to find resistance to erythromycin in pathogenic, zoonotic and indicator bacteria such as *E.coli*, *E.faecium and E.faecalis*, probably a consequence of selection pressure by the massive use of this drug. In Nigeria, there is no formal guideline about the total use of erythromycin in animal production. This antimicrobial agent has been used for decades in our country as a therapeutic agent and growth promoter. This could explain the high resistance levels seen in *Enterococus* species to erythromycin.

Eight resistant patterns were observed. The most common pattern of resistance was Norfloxacin/Erythromycin/Streptomycin in pigs and Norfloxacin/Erythromycin in poultry birds. Enterococci are intrinsically resistant to several antimicrobials and also readily accumulate mutations and exogenous genes that confer additional resistance (Arias and Murray, 2012). Resistance to erythromycin was highly prevalent, which is consistent with previous studies on erythromycin-resistant *Enterococcus* from other regions (Gomes *et al.*, 2008; Koluman, 2009). In the current study, we found no statistically significant differences in the majority of antimicrobial resistance profiles of isolates from pigs vs poultry birds ($P>0.05$).

Resistance to high-level gentamycin was not observed, whereas only (4.3%) isolated from pig samples showed high level streptomycin resistance. In previous reports, high level streptomycin resistance. (HLSR) was also more frequent that high level gentamycin resistant (HIGS) (Johnston and Jaykus, 2004; Fracalanzza *et al*., 2007). In opposition, Teuber *et al* (2009), found out 80% of enterococci isolated from cheese with high-level gentamycin resistance, and in Brazil, Gomes *et al* (2008), reported 22% of *E.Faecalis* with high level gentamycin resistance.

The enterococci are intrinsically resistant to a large range of antimicrobials of therapeutic use, and they are also known by the ability to acquire and transfer resistance markers by a process mediated by genes present in plasmid and transposons that facilitate their dissemination (Murray, 1990). Data on antimicrobial resistance of enterococci associated with food animals indicate that there is a strong epidemiological evidence of a link between the use of antimicrobials in human medicine and animal husbandry and the emergency, spreading and persistence of resistant strains in animal products (Van den Bogaard and Stobbering, 2000). The major risk related to the kresistance traits is that they are for the most part transferable to enterococci and more virulent pathogens as well.

While interpretation of our results is limited by availability of comparable of our results is limited by both pigs and poultry birds. We are confidence that further efforts in large-scale antimicrobial resistance surveillance and molecular characterization of resistance genes will eventually allow the resistance potentials introduced in this study to be measured at an increased resolution.

Table.1 Prevalence of Enterococcus from pigs and poultry birds in Abia State, Nigeria

Animals	**Location/sites**	**Number of samples**	**Number of *E.coli* isolates**
Pigs	Abia North	120	55
	Abia South	120	60
	Abia central	120	50
Poultry birds	Abia North	120	55
	Abia South	120	60
	Abia central	120	50
	Sub total	360	165
Total		720	330

Table.2 Antimicrobial resistance of Enterococcus isolates from pigs in Abia State
Number of isolates =165

	Sensitivity		**Intermediate**	**Resistance**	**Resistance**	
Antimicrobial Agents	N	%	N	%	N	%
Ciprofloxacin	49	29.8	80	48.5	36	21.7
Norfloxacin	10	6.0	47	28.5	108	65.5
Gentamicin	162	97.7	0	0.0	3	2.3
Lincocin	161	97.6	0	0.0	4	2.4
Streptomycin	28	16.7	66	40.0	71	4.3
Rifampicin	112	68.0	36	21.3	17	10.7
Erythromycin	30	18.0	17	10.2	118	71.8
Ampiclox	66	40.1	95	57.6	4	2.3

Table.3 Antimicrobial resistance of Enterococcus isolates from poultry birds in Abia State.
Number of isolates =165

	Sensitivity		Intermediate	Resistance	Resistance	
Antimicrobial Agents	N	%	N	%	N	%
Ciprofloxacin	113	68.3	38	9.8	14	21.8
Norfloxacin	38	23.0	66	40.3	61	36.7
Gentamicin	116	70.5	48	22.8	11	6.7
Lincocin	163	98.8	0	0	2	1.2
Streptomycin	60	36.0	66	40.3	39	23.7
Rifampicin	147	89.3	0	0.0	18	10.7
Erythromycin	30	18.0	17	10.3	118	71.7
Ampiclox	68	41.2	85	51.5	12	7.3
Floxapen	49	29.8	80	50.5	36	21.7
Chloramphenicol	34	20.0	5	3.3	126	76.7

Table.4 Resistance pattern of Enterococcus isolates from pigs in the 3 zones of Abia State

Antimicrobial agents	**Number of resistant isolates**		
Multi-resistance patterns	Abia North N=55	Abia South N=60	Abia central N=50
Nor-Ery	22	29	21
Nor-Ery-Str	36	38	40
Amp-Ery	9	7	8
Lin-Flo-Ery-Str	2	4	5
Amp-Nor-Ery Chl-Ery-Gen-Str	- -	- -	- -
Lin-Flo-Str	1	7	4
Cip-Str	6	7	9

Nor = Norfloxacin, Ery = Erythromycin, Str = Streptomycin, Amp = Ampiclox, Lin = Lincocin, Flo = Floxapen, Gen = Gentamycin

Table.5 Resistance patterns of Enterococcus isolates from poultry birds in the 3 zones of Abia State

Antimicrobial agents	Number of resistant isolates		
Multi-resistance patterns	Abia North N=55	Abia South N=60	Abia central N=50
Nor-Ery	32	33	35
Nor-Ery-Str	18	22	25
Amp-Ery	7	10	5
Lin-Flo-Ery-Str	5	7	4
Amp-Nor-Ery	5	4	4
Chl-Ery-Gen-Str	-	-	-
Lin-Flo-Str	2	2	3
Cip-Str	13	19	18

Figure.1 Antimicrobial percentage resistance profiles of Enteroccocus isolates from pigs in the three ones of Abia State

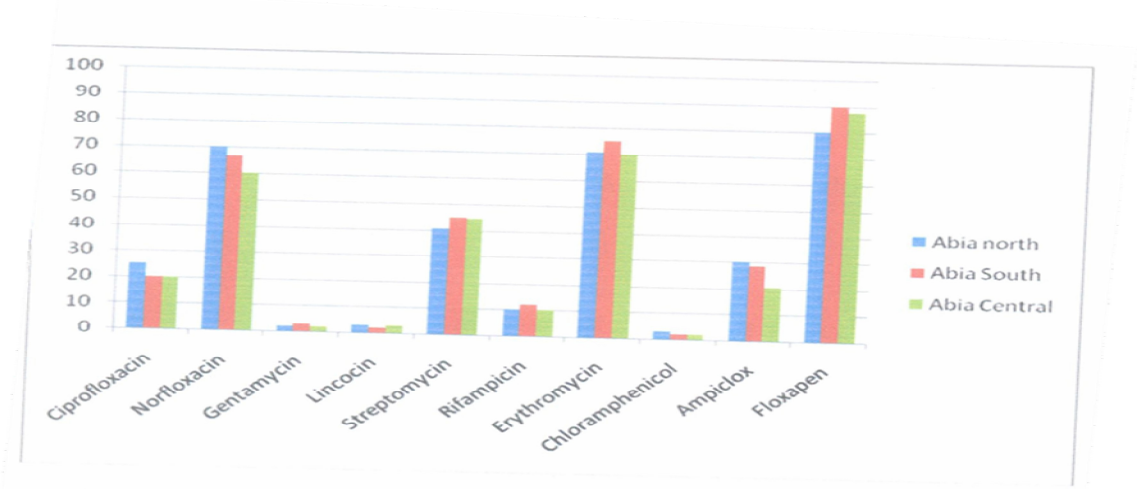
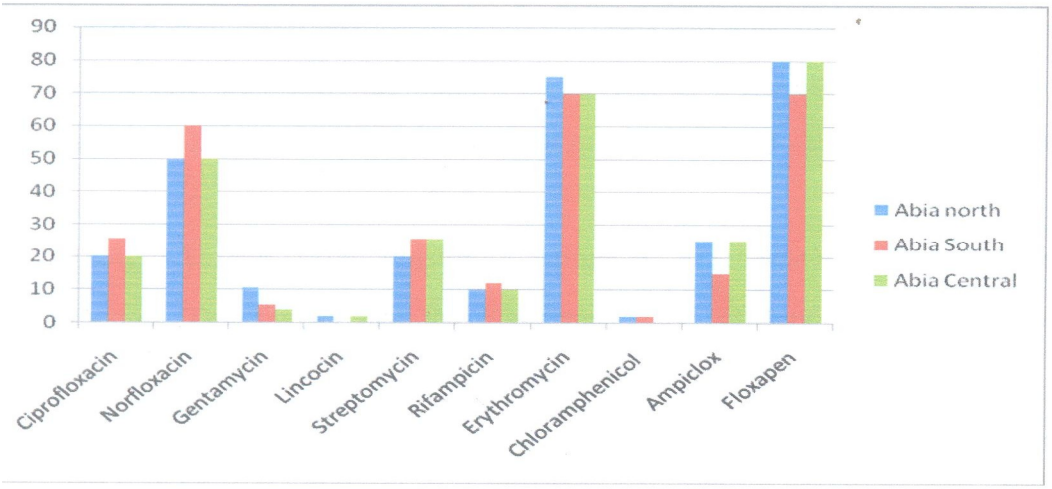


Figure.2 Antimicrobial percentage resistance profiles of Enteroccocus isolates from poultry birds in the three zones of Abia State



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