Antibiotic Resistance in Enteric Bacterial Isolates from Marketed Fish in Ibadan Metropolis, Southwest Nigeria

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Abstract: Improper antibiotic usage in terms of overuse and misuse is considered the most important factor promoting the emergence, selection and dissemination of antibiotic resistant microorganisms in veterinary and human medicine. Enteric bacterial isolates from marketed fish in fifteen locations in Ibadan metropolis were randomly sampled to examine for their antibiotic resistance to some commonly used antibiotics. Bacteriological examination of collected fish intestine and gills was done using standard methods, followed by in-vitro antibiotic sensitivity test by agar gel diffusion technique. Three enteric bacteria; *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae* that were multi-drug resistant to different combinations of aminoglycosides, cephalosporins and fluoroquinolones group of drugs were isolated. The occurrence of multiple antibiotic resistances among the enteric bacterial isolates from sampled marketed fish in the study area indicate a potential public health risk as it has been shown that animals including fish can act as vectors of bacteria. It is suggested that the prophylactic use of antibiotics may be a predisposing factor in the occurrence of antibiotic resistance among enteric bacteria in fish. Therefore, it is proposed that the application of antimicrobial drugs should be strictly controlled in tropical aquaculture to prevent the dissemination of antibiotic-resistant bacteria.
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1. Introduction

Antibiotics are used in human and veterinary medicine to treat and prevent diseases and for other purposes including growth promotion in food animals (Phillips et al., 2004). Its use plays a major role in the emerging public health crisis of antibiotic resistance (Torrence, 2001). Although the majority of antibiotic use occurs in agricultural settings, relatively little attention has been paid to how antibiotic use in farm animals contributes to the overall problem of antibiotic resistance (Landers et al., 2012).

Antibiotic resistance is a looming public health crisis. International, national and local antibiotic stewardship campaigns have been developed to encourage prudent use of and limit unnecessary exposure to antibiotics, with the ultimate goal of preserving their effectiveness for serious and life-threatening infections (Avorn et al., 2001, Belongia et al., 2005). There is also considerable debate in veterinary medicine regarding use of antibiotics in animals raised for human consumption (food animals). The potential threat to human health resulting from inappropriate antibiotic use in food animals is significant, as pathogenic-resistant organisms propagated in these livestock are poised to enter the food supply and could be widely disseminated in food products (Ramchandani et al., 2005; Garofalo et al., 2007). Commensal bacteria found in livestock are frequently present in fresh meat products and may serve as reservoirs for resistant genes that could potentially be transferred to pathogenic organisms in humans (Diarrassouba et al., 2007; Mena et al., 2008). While antibiotic use in food animals may represent a risk to human and animal health, the degree and relative impact have not been well characterized (Landers et al., 2012).

Extensive use of antibiotics in fish production as growth promoters, prophylaxis and treatment of infections have led to misuse of antibiotics and consequent generation of sufficient genomic selective pressure that enable microorganisms to adapt and acquire resistance (Witte, 2001).

There is strict regulation on the use of antibiotics in food animals in the developed countries of Europe and America. However, in developing countries such as Nigeria, access to antibiotics by livestock farmers is unrestricted and as such, imprudent administration of antibiotics without veterinary prescription is common practice (Kabir et al., 2004). The potential for disease problems associated with intensive fish culture would increase the probability of the use of a number of antimicrobial drugs in their management. It has been demonstrated that indiscriminate use of antibacterial drugs and other synthetic chemotherapeutic agents to treat fish diseases and/or as feed additives has

resulted in an increase in populations of antibiotic-resistant bacteria as well as resistance plasmids in food-producing animals, fish and water microflora (Walter and Vennes, 1985). The prominently affected bacteria are members of the Enterobacteriaceae and related Gram-negative rods (Ogbondeminu and Olayemi, 1993). The undiscerning use of antibiotics in Nigerian aquaculture coupled with the proliferation of several brands of antibiotics learnt the need to evaluate the prevalence of antibiotic resistant strains of enteric bacteria in fish sold in Ibadan metropolis, Nigeria

2. Materials and Methods

The study was carried out in fifteen markets in the five local government areas that make up Ibadan metropolis. Ibadan is located in southwest Nigeria at 8°00′N 4°00′ E with estimated population of 5.6 million people (NPC, 2006). Fish intestines and gills were sampled by systematic random method whereby samples from every third fish seller in each market in the study area were collected. A total of 1,500 fish intestine and gills were sampled between February and April 2014.

The samples were processed bacteriologically by streaking the sample suspension onto nutrient agar and MacConkey agar and incubated aerobically at 37°C for 24-48 hours. The isolates were identified morphologically and biochemically based on standard bacteriological methods as described by Barrow and Feltham (1993) and Garcia and Isenberg (2007). Successively, the in-vitro antibiotic sensitivity test using agar-disc diffusion methods (Matsen and Barry, 1974) were carried out for the isolated bacteria with the antibiotic disc (Abtek biologicals, UK) of the commonly used antibiotics in Nigeria specifically; Erythromycin; ERY (10µg), Ciprofloxacin; CPR (10µg), Gentamicin; GEN (10 µg), Augmentin; AUG (30 µg), Nitrofurantoin; NIT (300 µg), Ofloxacin; OFL (10 µg), Levofloxacin; LEV (10 µg), Cloxacillin; CXC (5 µg), Ceftriaxone; CTR (30 µg), Ceftazidime; CAZ (30 µg), Ampicillin; AMP (10 µg) and Cefuroxime; CRX (30 µg) for Gram positive and negative organisms (Matsen and Barry, 1974). The zones of inhibitions produced by the bacteria isolates to the respective antibiotics were compared with the recommended standards published by CLSI (2008) for the determination of the sensitive and resistant bacteria.

3. Results

Three enteric bacteria; *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* were isolated from the fifteen markets sampled as shown in **Figure 1**. Resistances to multiple antibiotics were common; with the enteric bacteria isolates resistant to at least three antibiotics (**Table 1**). It was also observed that the fish intestines and gills were fed to feral carnivores and birds, and also collected by some small hold farmers to feed their pigs and fish.

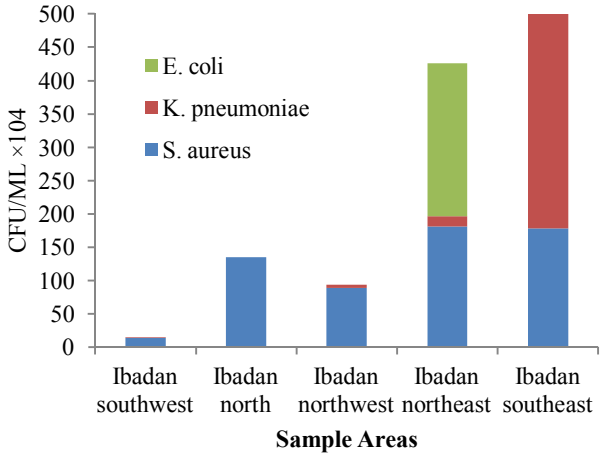

Figure 1: Distribution of enteric bacterial isolates from selected fish markets in Ibadan metropolis

Table 1: Antibiotic resistance and sensitivity profiles from selected fish markets in Ibadan

LGA	Market	Resistance profile	Sensitivity profile
Ibadan southwest	Challenge	CAZ,AMP,AUG	CRX, OFL,GEN,CPR
	Mobil	CRX,CAZ,AMP, OFL	AUG,CPR,GEN, NIT
	Apata	GEN,CAZ,AMP, CRX,AUG	OFL,CPR
Ibadan north	Bodija	CXC,ERY,GEN,AUG	CAZ,CRX,CTR,OFL
	Sango	CXC, ERY	CTR,GEN,CRX,OFL,AUG,CAZ
	Mokola	CXC,GEN,CAZ,OFL	AUG,CTR,ERY,CRX
Ibadan northwest	Onireke	CXC,ERY,AUG,CAZ,CRX,OFL	CTR,LEV,GEN
	Ogunpa	-	OFL,CTR,GEN,CXC,CRX,AUG,LEV,CAZ,ERY
	Dugbe	AUG,CXC,OFL,ERY,GEN,LEV,ERY,NIT,CRX	CAZ,CTR,CPR
Ibadan northeast	Agugu	CRX,CAZ,CXC,ERY,GEN	CPR,NIT,OFL,AUG,
	Idi-ape	CRX,AMP,NIT,AUG	CPR,CAZ,OFL,GEN
	Iwo road	GEN,ERY,CXC,NIT,CRX	OFL,AUG,CAZ,CTR,CPR
Ibadan southeast	Oja oba	ERY,OFL,CXC,CAZ,GEN	CTR,LEV,AUG,CRX
	Oranyan	ERY,CXC,GEN,AUG,LEV,CAZ,CRX,OFL	CPR,NIT,CTR
	Mapo	AUG,OFL,NIT,CRX,GEN,CPR,CAZ	LEV

CRX: Cefuroxime; NIT: Nitrofurantoin; AUG: Augmentin; OFL: Ofloxacin; AMP: Ampicillin; CAZ: Ceftazidime; GEN: Gentamicin; CPR: Ciprofloxacin; ERY: Erythromycin; CXC: Cloxacillin; CTR: Ceftriaxone; LEV: Levofloxacin

4. Discussions

The use of antibiotics in food animal production has been a great public health and food safety concern. Although these drugs are affordable and enhance the productivity of food animals, their use result in the spread of resistant bacteria and the attendant public health hazards (Amosun et al., 2012). The results of this study revealed the prevalence of antibiotic resistance in enteric bacteria isolated from fish intestines and gills in Ibadan metropolis. The majority of the bacterial strains possessed resistance to multiple antibiotics. These findings highlight the serious implications of misuse of antibiotics as frequently practiced in some livestock production in Nigeria. The results are similar to earlier reports (Hawser et al., 2010; Endimiani et al., 2012) that documented increasing enteric bacterial infections due to resistance to different antimicrobials such as flouroquinolones, cephalosporins and aminoglycosides. This calls for concern because enteric bacteria such as found in this study can cause several types of diseases and the development of antibiotic resistance by these agents poses an even greater threat to public health. Moreover, they are commensal flora of humans and various animal species colonizing multiple body sites (Vanderhaeghen et al., 2010).

The use of antibiotics in veterinary medicine, similar to its use in agriculture and aquaculture may select for resistant bacteria .The analysis of antibiotic resistance has demonstrated that identical elements are found in animals and humans and the occurrence applies to pathogenic (e.g. foodborne), opportunistic and commensal bacteria (Teuber, 2001). The potential threat to human health resulting from inappropriate antibiotic use in food animals is significant, as pathogenic-resistant organisms propagated in these livestock are poised to enter the food supply and could be widely disseminated in food products (Ramchandani et al., 2005; Garofalo et al., 2007). Commensal bacteria found in livestock are frequently present in fresh meat products and may serve as reservoirs for resistant genes that could potentially be transferred to pathogenic organisms in humans (Diarrassouba et al., 2007; Mena et al., 2008). Thus, the occurrence of antibiotic resistance in enteric bacteria isolated from marketed fish in Ibadan metropolis calls for public health concern, and may suggest a potential contribution to occurrence of resistance food-borne pathogens in the study area.

It was observed that the fish intestines and gills were scavenged on by neighbourhood dogs and cats, as well as feral birds and also used to feed small hold pigs and fish. Hence, the occurrence of multiple antibiotic resistances among the enteric bacterial isolates from fish in the study area may make their offal of public health significance. If the resistance is plasmid-mediated, as had been found commonly in fish pathogens (Torenzo et al., 1983), then there could be a problem associated with the transfer of resistance to other animals and humans. And as such, specific food items, water and direct contact may spread these bacteria from animal micro flora to human micro flora (Voss et al., 2005).

The spread of multidrug resistant pathogenic organisms constitutes a main impediment to the control of infectious diseases globally (So et al., 2012). Inappropriate antibiotic usage in terms of overuse and misuse is considered the most important factor promoting the emergence, selection and

dissemination of antibiotic resistant microorganisms in veterinary and human medicine (Goldstein and Trivedi, 2012).

In conclusion, these findings indicate a potential public health risk as it has been shown that animals including fish can act as vectors of bacteria carrying R-plasmids (Linton and Hinton, 1984). Therefore, it may be suggested from our findings that the application of antimicrobial drugs should be strictly controlled in tropical aquaculture to prevent the dissemination of antibiotic-resistant bacteria that may carry R-plasmids to fish and other animal diseases which would not respond to the usual antibiotic therapy, and which may consequently confer antibiotic resistance to otherwise antibiotic-sensitive bacterial species in animals and humans.

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