

Occurrence of Antibiotic Residues and Antibiotic-Resistant Bacteria in Nile Tilapia Sold in Accra, Ghana: Public Health Implications

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Abstract

In Ghana there are concerns that antibiotics may be used inappropriately to boost fish production, though no study has investigated this problem. To provide preliminary insights into public health aspects of the problem, we investigated the occurrence of antibiotic residues and antibiotic-resistant bacteria in Nile tilapia (*Oreochromis niloticus*), a fish commonly cultivated and consumed in Ghana. Two hundred Nile Tilapia fish were randomly sampled from four major markets in Accra, the capital city of Ghana. One hundred samples were screened for antibiotic residues using a microbial inhibition plate test that detects sixteen different antibiotics commonly used in animal husbandry and aquaculture. The other 100 samples were cultured for bacteria using direct culture methods, and the isolates were tested against seven antibiotics by the Kirby Bauer method. The overall prevalence of antibiotic residues in the fish samples was 7%. Bacteria that were isolated from the fish samples were *Shigella sonnei* (10%), *Enterobacter cloacae* (7%), *Escherichia coli* (6%), *Salmonella* Typhi (3%), *Klebsiella pneumoniae* (2%), and *Proteus mirabilis* (2%). All bacteria isolated were susceptible to gentamicin and ciprofloxacin but resistant to ampicillin. Multi-drug resistance (ie resistance to three or more different classes of antibiotics) occurred in 86.7% of the isolates. Nile Tilapia sold in Accra is a source of multi-drug resistant bacteria. Consumption of the fish can also lead to significant exposure to antibiotic residues.

Keywords: antibiotic residues, multi-drug resistance, fish, *Shigella sonnei*

1. Introduction

Antibiotics are used in aquaculture for several reasons including treatment and prevention of fish diseases, increment in fish reproduction and tranquilization such as in weighing (Cabello, 2006; Kim *et al.*, 2011). Common antibiotics used in aquaculture include tetracyclines, penicillins, chloramphenicol, fluoroquinolones, sulfonamides and macrolides (Cabello, 2006; Kim *et al.*, 2011; Samanidou & Evaggelopoulos, 2007). The dependency on antibiotics in aquaculture can have negative effects on the health of humans, as well as damage to the environment (Kim *et al.*, 2011; Buschmann *et al.*, 2006; Sorum, 2006). Certain antibiotics in fish products, particularly penicillin can evoke allergic reactions even if small amounts are ingested; it is known that about 10% and 3.4% of humans have allergic sensitivity to penicillin and sulphonamide respectively (Wassenaar, 2005; Sundlof, 2004). Chloramphenicol can cause aplastic anaemia, while broad-spectrum antibiotics such as metronidazole may adversely affect a wide range of intestinal flora and consequently cause gastrointestinal disturbance (Wassenaar, 2005; Sundlof, 2004). To protect public health against toxicological risk of antibiotics used in animal husbandry and aquaculture, maximum residue limits have been established for various antibiotics as well as acceptable daily intakes (FAO, 2005; JECFA, 2005). Additionally, withdrawal periods have been defined for these antibiotics to prevent animal source food contamination.

The widespread use of antibiotics in aquaculture has resulted in the emergence of antibiotic-resistant bacteria in aquaculture environments. In Australia, a study on fish and fish ponds showed widespread resistance among *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* (Ndi & Barton, 2012). L'Abée-Lund & Sorum (2001) have reported the presence of tetracycline resistance associated with *tet* (A) and *tet* (E) resistance genes in transposons and integrons in multi-resistant *Aeromonas salmonicida* isolated from fishes from Finland, France,

Japan, Norway, Scotland, Switzerland, and the United States. In Turkey, a study showed varying levels of multi-resistance in some gram-negative bacteria from wastewater river containing fishes (Toroglu & Korkmaz, 2005). A study in Nigeria with *Salmonella Enteritidis* recovered from fishes revealed varying levels of resistance (Raufu *et al.*, 2014).

Commercial aquaculture now exists in a huge scale in many developed and developing countries. In Ghana, the aquaculture industry is relatively young and faced with significant challenges involving production intensification and disease control (ATFALCO, 2012). In order to address some of these issues, antibiotics are sometimes used in fish cultivation. Currently, there are concerns that antibiotics may be used inappropriately in aquaculture in Ghana, though there has been no study to investigate the problem. To provide preliminary insights into the public health aspects of the problem, we investigated the occurrence of antibiotic residues and antibiotic resistant bacteria in Nile Tilapia (*Oreochromis niloticus*), a fish commonly cultivated and consumed in Ghana.

2. Methods

2.1 Study Area, Design and Sampling

The study was carried out in four markets in Accra, including Agbobgloshie, Kaneshie, Makola and Weija markets from May 2014 to July 2015. Accra is the capital city of Ghana and has a population of 2.5 million people (Population and Housing Census, 2010). The four sites selected for the study are markets where residents of Accra commonly purchase fish. A wide range of fishes are sold on these markets, however only a few types including tilapia and catfish are from aquaculture sources. In Ghana, Tilapia is the major fish species cultivated and constitutes over 80 percent of aquaculture production (ATFALCO, 2012).

Two hundred fresh fish samples of Nile Tilapia (*Oreochromis niloticus*) were randomly sampled from the four markets. The fish samples were transported to the bacteriology laboratory of School of Biomedical and Allied Health Sciences, University of Ghana in Accra for analysis. The first 100 fish samples collected were screened for antibiotic residues while the other 100 samples were cultured for bacteria followed by antimicrobial susceptibility testing of the isolates.

2.2 Determination of Antibiotic Residues in Fish Samples

Fish samples were screened for antibiotic residues using the microbial inhibition method described by Koenen-Dierick *et al.* (1995). Briefly, 0.5 McFarland's standard suspension of *Bacillus subtilis* ATCC 6633 was prepared and used to inoculate the surface of Muller-Hinton agar (MHA) plates. A sterile cork borer of 3 mm in diameter was used to create four wells on the agar plates. Using a sterile forceps and scalpel blade, 3mm of the gill of each tilapia sample was cut and transferred aseptically into the cut wells. For each MHA plate, positive and negative controls were set up with penicillin (1mg/mL) and sterile distilled water respectively. The agar plates were incubated at 37 °C for 18–24 hours. After incubation, the plates were examined for zones of inhibition. Any well containing fish gills with ≥ 1 mm zone of inhibition was considered positive for antibiotic residue (Koenen-Dierick *et al.*, 1995)

2.3 Isolation and Identification of Bacteria from Fish Samples

Sterile swab sticks were used to swab the gut regions of the fish samples and inoculated into Brain-heart infusion broth and *Rappaport-Vassiliadis* broth. The inoculated broths were incubated at 37°C for 18-24 hours and subcultured onto MacConkey, Blood and Xylose Lysine Deoxycholate agar plates. After incubation at 37°C for 18-24 hours, bacterial isolates were identified based on colonial morphology, Gram stain and a battery of biochemical tests such as the triple sugar iron test, catalase test, urease test, indole test and citrate utilization test (Baron *et al.*, 1994).

2.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of bacterial isolates were determined by the Kirby Bauer method (Bauer *et al.*, 1966; CLSI, 2010). On the agar plate of the test organism, a sterile loop was used to touch four to five morphologically similar colonies and inoculate 5 mL sterile peptone water until the turbidity was comparable to 0.5% McFarland's standard. A loopful of the suspension was transferred to a Mueller-Hinton agar plate, and a sterile cotton swab was used to streak the entire surface of the plate. The lid of the agar plate was left ajar for 3 to 5 minutes to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks. The antibiotics tested included ceftriaxone (30 µg), gentamicin (10 µg), cefuroxime (30 µg), meropenem (10 µg), amikacin (30 µg), cotrimaxole (25 µg) and ciprofloxacin (5 µg), ampicillin (10 µg), cefotaxime (30 µg), chloramphenicol (30 µg). The agar plates were incubated at 37 °C for 18–24 hours, after which zone diameters around the antibiotic discs were measured and classified as sensitive or resistant based on the CLSI break point system (CLSI, 2010). Quality control testing was done by using *Escherichia coli* ATCC25922 strain for

validation of the susceptibility testing process.

2.5 Data Analysis

The data were analysed using STATA 11 (Strata Corp., College Station, TX, USA). Descriptive analysis including frequencies and percentages were computed for antibiotic residues and various bacterial organisms in the fish samples. The Chi-square test was used to compare these parameters among the various markets and a p value <0.05 was considered to be significant. Analysis of frequencies and percentages were also done for resistant and multi-drug resistance isolates. Multidrug resistance was defined as resistance to three or more classes of antibiotics.

3. Results

3.1 Prevalence of Antibiotic Residues in Fish Samples

The prevalence of antibiotic residues in fish sampled from the markets is shown in Table 1. While none of the fish samples from Agbobloshie market tested positive, prevalence of antibiotic residues in samples from Kaneshie and Makola markets were 11.8% and 9.1% respectively. The overall prevalence of antibiotic residues in the fish samples was 7%; generally, there was no significant difference in the prevalence of fish contamination among the different markets at $p < 0.05$.

Table 1. Prevalence of antibiotic residues in fish sampled from markets in Accra (Ghana)

Name of Market	Number of samples tested	Number of positive samples	% of positive samples
Agbobloshie	33	0	0
Kaneshie	34	4	11.8
Makola	33	3	9.1
Weija	0	0	0
Overall	100	7	7

No fish samples from Weija market were tested for antibiotic residues; No significant difference in positive samples from the different markets.

3.2 Bacteria Isolated from Fish Samples

For the 100 fish samples screened by culture, 6 different types of bacterial pathogens were isolated from 46 samples. As shown in Table 2, the most prevalent organism was *Shigella sonnei* (10%), followed by *Enterobacter cloacae* (7%), *Escherichia coli* (6%), *Salmonella Typhi* (3%) and *Klebsiella pneumoniae/Proteus mirabilis* (2%).

Unlike the other study markets, fish samples from Makola market harboured all the 6 bacterial pathogens while samples from Agbobloshie market were least contaminated. Generally, there was no significant difference in the types of bacteria isolated from the different markets ($p > 0.05$).

Table 2. Bacteria isolated from fish sampled in markets in Accra (Ghana)

Bacteria	Market				
	Agbobloshie	Kaneshie	Makola	Weija	Overall
	n (%)	n (%)	n (%)	n (%)	n (%)
<i>Shigella sonnei</i>	0 (0)	5 (20)	4 (16)	1 (4)	10 (10)
<i>Enterobacter cloacae</i>	0 (0)	4 (16)	2 (8)	1 (4)	7 (7)
<i>Escherichia coli</i>	1 (4)	0 (0)	2 (8)	3 (12)	6 (6)
<i>Salmonella Typhi</i>	0 (0)	0 (0)	1 (4)	2 (8)	3 (3)
<i>Klebsiella pneumoniae</i>	0 (0)	1 (4)	1 (4)	0 (0)	2 (2)
<i>Proteus mirabilis</i>	0 (0)	0 (0)	2 (8)	0 (0)	2 (2)

n indicates number of samples that tested positive for bacteria

% indicates corresponding percentage of positive samples

3.3 Antibiotic Resistance of Bacteria Isolated from Fish Samples

All the 30 isolates from fish samples were susceptible to gentamicin and ciprofloxacin but resistant to ampicillin. Prevalence of multi-drug resistance among the 30 isolates was 86.7%. Multi-drug resistance occurred among all the different bacterial pathogens (Table 3). In many cases, resistance profiles of the multi-drug resistant isolates

were highly varied within the same species (Table 4). Further analysis on the prevalence of resistance to various antibiotics was done for only *Shigella sonnei* as isolates of the other bacterial pathogens were insufficient for analysis. For *S. sonnei*, antibiotic resistance was observed for eight of the ten drugs tested, increasing across chloramphenicol/ceftriaxone/cefuroxime (25%), amikacin (33%), cefotaxime (42%), meropenem (67%), cotrimoxazole (83%) and ampicillin (100%) (Figure 1).

Table 3. Prevalence of multi-drug resistance among bacteria isolated from fish samples

Bacteria	Number of isolates	Number of Multi-drug resistant isolates (%)
<i>Enterobacter cloacae</i>	7	4 (57.1)
<i>Escherichia coli</i>	6	6 (100)
<i>Klebsiella pneumoniae</i>	2	2 (100)
<i>Proteus mirabilis</i>	2	1 (50)
<i>Salmonella Typhi</i>	3	3 (100)
<i>Shigella sonnei</i>	10	10 (100)

4. Discussion

This study is probably the first to report on contamination of antibiotic residues in fish in Ghana. By comparison, studies on fish in Nigeria and Vietnam reported antibiotic residue contamination of 61.3% (Olatoye & Basiru, 2013) and 29.4% (Pham *et al.*, 2015) respectively. In Ghana, prevalence of antibiotic residues has been reported in several animal foods including beef (30.8%), chevon (29.3%), pork (28.6%), mutton (24%), and egg (6.8%) (Donkor *et al.*, 2011). Generally, in the developed world, antibiotic residue contamination of animal source food is low, and in Europe prevalence rates of <1% are generally reported (Commission of the European Communities, 2005). The relatively lower prevalence of antibiotic residues in food in the developed world can be attributed to quality assurance programmes such as educational programmes, widespread testing of food for antimicrobial drugs, and financial penalties (Commission of the European Communities, 2005; McKenzie & Hathway 2006). The aquaculture industry in Ghana is still young and the concept of using antibiotics in cultivation may not be widespread among farmers yet, which may explain the relatively lower prevalence of antibiotic residues in fish in this study.

Table 4. Resistance profile of multi-drug resistant bacteria isolated from fish

Isolates	Number of isolates	Resistance pattern
<i>Enterobacter cloacae</i>	2	TET-COT-MEM-AMP
	2	CTR-MEM-AMP
<i>Escherichia coli</i>	4	TET-CRX-CTX-MEM-AM
	1	CRX-MEM-AMP
	1	CRX-CTX-MEM-AMP
<i>Klebsiella pneumoniae</i>	1	TET-COT-CTX-CTR-AMP
	1	TET-COT-CTX-CTR-MEM-AMP
<i>Proteus mirabilis</i>	1	TET-COT-MEM-AMP
<i>Salmonella Typhi</i>	1	TET-COT-CRX-CTX-MEM-AMP
	1	CRXT-CTX-MEM-AMP
	1	COT-CRX-MEM-AMP
<i>Shigella sonnei</i>	2	TET-COT-CRX-CTX-MEM-AMP
	3	COT-TET-AMK-AMP
	1	TET-AMK-AMP
	1	TET-COT-CRX-CHL-MEM-AMP
	1	TET-CRX-CTX-MEM-AM
	1	TET-COT-CHL-CTX-MEM-AMP
	1	COT-CRX-MEM-AMK-AMP

TET= tetracycline COT= cotrimoxazole GEN= gentamicin CRX= cefuroxime CHL= chloramphenicol CTX= cefotaxime CTR= ceftriaxone MEM= meropenem AMK= amikacin CIP= ciprofloxacin AMP= ampicillin

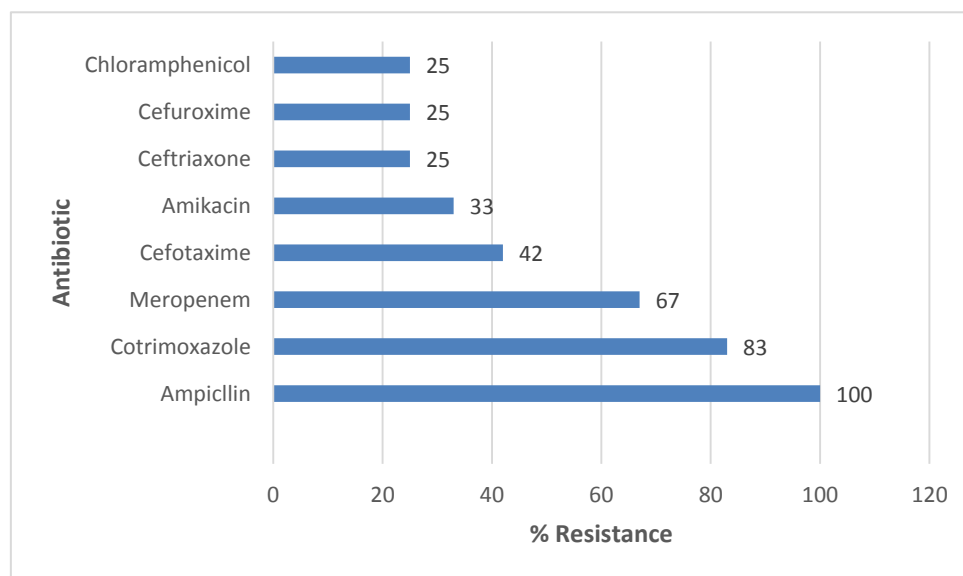


Figure 1. Percentage resistance of *Shigella sonnei* to various antibiotics

The microbial inhibition plate test used to screen fish for antibiotic residues in this study has a low sensitivity for macrolides but has a high sensitivity for several other antibiotics that are known to be used in animal husbandry in Ghana, such as β -lactams, tetracyclines, aminoglycosides, sulphonamides, and quinolones (Koenen-Dierick, *et al.*, 1995; Sekyere, 2014). Overall, the test used can detect sixteen different antibiotics commonly used in animal husbandry and aquaculture. With such a wide coverage of antibiotics, we expect that our screening captured to a large extent any antibiotics used in aquaculture in Ghana. Though the test used detects a wide range of antibiotic residues in food above maximum residue limits, it is unable to differentiate the residues. Assays that detect specific antibiotics in animal food are rather expensive and include chemical methods such as chromatography and mass spectrometry (Samanidou & Evaggelopoulos, 2007).

An overall tilapia contamination of 7% with antibiotic residues in this study translates into an average risk of exposure every fifteenth time a consumer takes in the commodity. The risk of exposure of a human population to food contaminated with antibiotic residues depends mainly on the extent of food contamination and the consumption rate of the food (Lu & Sam, 2002; MacNeil, 2005). In this study, because we did not quantify specific drugs in the fish samples and evaluate consumption trends of the commodity, it is difficult to measure the actual risk of specific drugs to consumers. Consumption rate of tilapia in Ghana is known to be very high and some of the samples, which tested positive for antibiotic residues in this study, had large zones sizes (>31mm) reflecting high concentration of antibiotic residues. These observations along with a fish contamination rate of 7% reflect a potentially high risk of exposure to antibiotic residues in Tilapia, though further studies are needed to throw more light on this. In Ghana, there are no national limits for antibiotic residues in fish currently. With the increasing public health concerns of antibiotic residues in fish sold in the country, it is about time for legislative establishment of such limits.

The various bacteria reported in this study have been previously isolated from fish (Onyango *et al.*, 2009; Marcel *et al.*, 2013; Plumb, 1999). All the organisms isolated are enteric, suggesting that faecal pollution may have played a role in the microbial contamination of the fish samples (Ashbolt *et al.*, 2015). Consumption of fish contaminated by some of these organisms particularly, *Shigella sonnei* and *Salmonella* Typhi could pose serious threat to public health. *Shigella* causes shigellosis or bacillary dysentery and is specific host-adapted to humans (Shears, 1996). It has a low infectious dose and some of its outbreaks have been linked to fish consumption (Shears, 1996). Recent epidemiological evidence indicates that *S. sonnei*, which has historically been more commonly isolated in developed countries, is undergoing an unprecedented expansion across industrializing regions (Thompson *et al.*, 2015). Consequently, it is interesting to note that *S. sonnei* was the predominant *Shigella* spp. isolated in this study, though it was previously reported to be relatively uncommon in Ghana compared to several other *Shigella* spp. (Opintan & Newman, 2007). Further studies are needed to evaluate the public health significance of *S. sonnei* in Ghana in light of its changing epidemiology. *S. Typhi* causes typhoid fever, an infection, which is endemic in some parts of Ghana (Marks *et al.*, 2010). Though *S. Typhi* has been isolated from several types of food in Ghana, the current study is probably the first report on fish. Generally, *Salmonella* is responsible

for a huge public health problem associated with fish and fishery products and its presence in food reflects poor standard of hygiene (Heinitz *et al.*, 2000). In light of this study, the recent efforts to expand aquaculture in Ghana could be associated with an increased risk of typhoid fever unless precautions are taken to protect public health.

Though fish samples from Agbogbloshie market were least contaminated by bacteria and were also free of antibiotic residues, it is difficult to attribute these observations to better food safety and hygienic practices at this market. This is because fish samples on the different markets originated from farm sources and may have moved through various channels before reaching the markets. For example, cross-contamination of food with microbes commonly occurs during food transportation (Stier, 2004), while drug residue contamination of food is mainly a farm level issue (Aning *et al.*, 2007).

The extent of antibiotic resistance observed among bacteria isolated from fish is high and similar to what has been reported for clinical isolates in Ghana especially for tetracycline, chloramphenicol, ampicillin and cotrimoxazole (Opintan & Newman, 2007; Newman *et al.*, 2011; Labi *et al.*, 2014). These antibiotics have been on the market in Ghana for a long time and have been subjected to a high rate of usage leading to emergence and spread of resistant strains at a high level (Donkor *et al.*, 2012; Donkor *et al.*, 2011; Tetteh-Quarcoop *et al.*, 2013). Of concern is the extremely high prevalence of multi-drug resistance in pathogens of public health significance such as *Shigella sonnei* and *Salmonella* Typhi. This could have serious public health implications, especially in developing countries like Ghana where antibiotic treatment options are limited. Multi-drug resistance in enteric bacteria is mediated by mobile genetic elements such as plasmids and transposons that carry resistant genes (von Wintersdorff, *et al.*, 2016; AbdelRahim *et al.*, 2015; Codjoe & Donkor, 2017). Several lines of evidence indicate that such genetic elements can be transferred from indigenous microbial flora of fish to human pathogens (Marshall & Levy, 2011; Sorum, 2006; Akinbowale *et al.*, 2006). It is important to note the high susceptibility of enteric bacteria to ciprofloxacin and gentamicin in this study, despite the high levels of resistance to several antibiotics in this study. This could be because these antibiotics are hardly used in aquaculture in Ghana. There is the need to limit the use of these drugs in Ghana through their prudent usage. The design of this study did not permit us to collect data on antibiotic use in aquaculture and thus it is difficult to relate this to the pattern of resistance observed. There is the need for studies to understand the epidemiology of antibiotic use in aquaculture in Ghana, as this will be an important step in solving the huge problem of antibiotic resistance in the aquaculture environment that has been exposed by this study.

There are a few limitations of the study. Firstly, the method used to screen fish samples for antibiotic residues could not identify and quantify specific antibiotics. Secondly, since the study was done at the market level, we do not have information on the origin of the fish samples. Additionally, we did not collect data on certain biological parameters of the fish samples such as sex, weight and length.

In conclusion, Nile Tilapia sold in Accra is a source of multi-drug resistant bacteria and consumption of the fish could also lead to significant exposure to antibiotic residues. Though fish is normally consumed in the cooked state thus decreasing the potential of infection, they may play a role in spreading multi-drug resistant bacteria to other food sources in the environment. The study has revealed a potentially serious public health problem. There is the need to create awareness about this among policy makers and also test for drug residues at fish distribution centres. There is also the need to include the aquaculture environment in antibiotic-resistance management and surveillance programmes in Ghana.

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