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Vol. 9(4), pp. 35-46, August 2017
DOI: 10.5897/JMA2017.0387
Article Number: D983F7F65898
ISSN 2141-2308

http://www.academicjournals.org/JMA

Journal of Microbiology and Antimicrobials

Full Length Research Paper

Antibiotic resistance patterns of bacterial isolates from hatcheries and selected fish farms in the Ashanti region of Ghana

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Received 7 June, 2017; Accepted 3 August, 2017

Antibiotic resistance has become an increasingly global health problem. This has resulted in a limited number of antibiotics being effective in the treatment of various infections. Antibiotic resistant organisms have been isolated from fish ponds in various studies in different parts of the world. This study was carried out to assess some fish farming practices among catfish and tilapia farmers which may contribute to antibiotic resistance and also to determine the susceptibilities of *Staphylococcus aureus*, *Escherichia coli*, *Shigella* species, *Salmonella typhi* and *Pseudomonas aeruginosa* isolated from fish pond water, catfish gut and tilapia gut from 11 farms and two hatcheries to selected reference antibiotics including penicillin, ampicillin, flucloxacillin, erythromycin, tetracycline, sulphamethoxazole/trimethoprim, cefuroxime, gentamicin, ciprofloxacin and chloramphenicol using the disc diffusion method. With the exception of gentamicin and ciprofloxacin, there was varying resistance of more than 60% to the other antibiotics. Most of the bacterial isolates exhibited high resistance (≥70%) to penicillin, ampicillin, flucloxacillin and tetracycline whilst low resistance was observed in all isolates to gentamicin (1.7 to 5.6%) except in *P. aeruginosa*. 44 to 92.9% of isolates of organisms showed resistance to more than three antibiotics. The bacterial isolates from the sampled fishes exhibited multidrug resistance although there was no recent history of use of antibiotics in most of the farms studied.

Key words: Bacterial isolates, antibiotics, antibiotic resistance, fish farms.

INTRODUCTION

Antibiotic resistance is one of the major health challenges, which is largely attributed to varying factors such as indiscriminate use of antibiotics both in humans and in food producing animals (Huttner et al., 2013;

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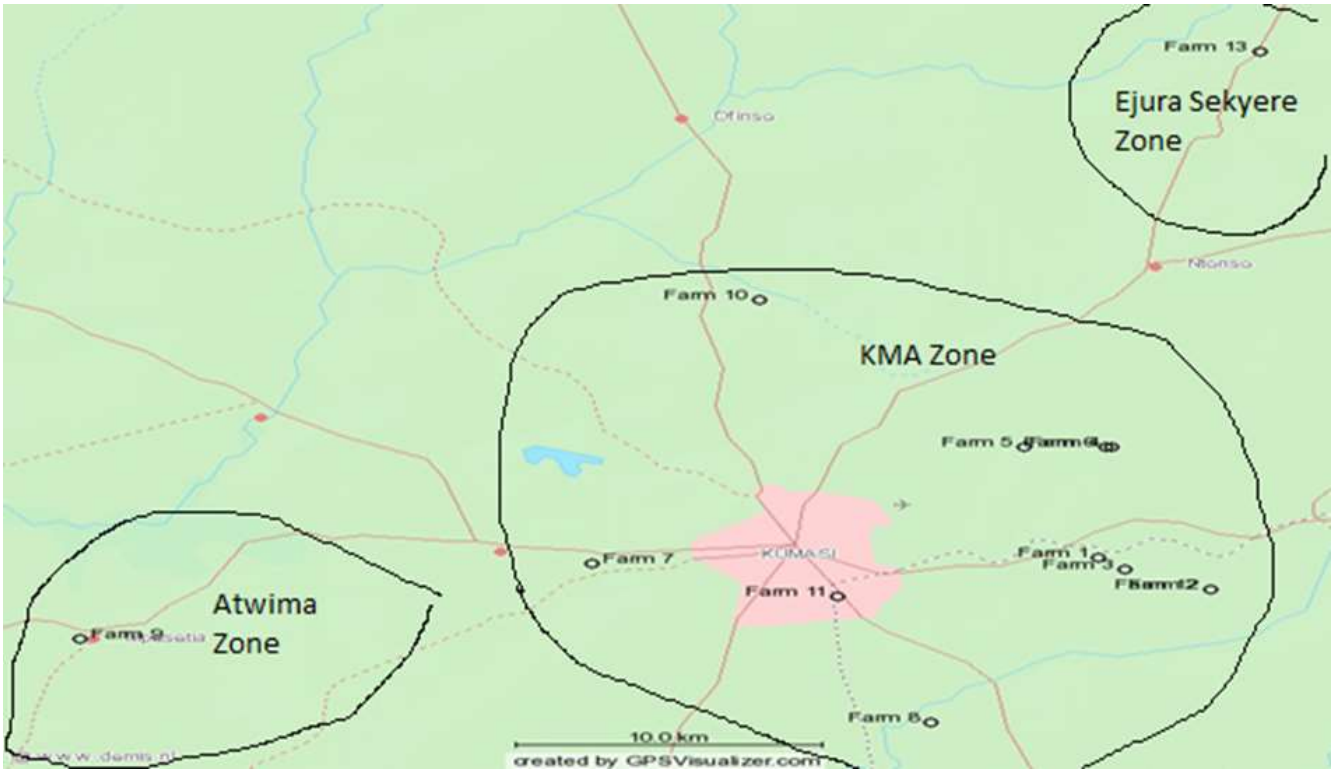

Figure 1. Geographical representation of studied farms.

Laxminarayan et al., 2013). Antibiotics have been used in aquatic environments mainly to prevent diseases or to treat diseases in fish production and may be administered through feed or direct application in pond water (Romero et al., 2012). Antibiotics and pesticides used in fish farms may accumulate in the water and sediments of fish farms and receiving water bodies. These residues in fish tissues may consequently affect consumers (Abu et al., 2010; Pouliquen et al., 2009).

The use of antibiotics in aquaculture affects a wide range of bacteria and has potential impact on other components of the aquatic system such as receiving water bodies as well as in fish pathogens (Gordon et al., 2007). Bacteria may be transferred from the aquatic environment to humans through direct contact with water and in the handling processes of the fish and direct consumption (Chenia and Vietze, 2012; Lowry and Smith, 2007). Human pathogens such as *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp., *Escherichia coli* and *Pseudomonas aeruginosa* have been isolated from fish farms and some of these isolates showed high resistance to commonly used antibiotics in humans. These antibiotics include penicillin, tetracycline, cephalosporin, sulphonamide, quinolone and macrolide (Chenia and Vietze, 2012; Karki et al., 2013; Newaj-Fyzul et al., 2008).

Ghana has a thriving freshwater aquaculture industry with tilapia and catfish being the most farmed species in freshwater farms. Tilapia farming alone contributes 88% of total fish farming in Ghana (Onumah et al., 2010). Few studies have been done on the bacteria flora of the fish farms in Ghana, including the bacteria flora of fish feed and on farms using agricultural waste as well as flora of sewage treatment plant used as fish pond (Ampofo and Clerk, 2003, 2010). This study sought to determine the antibiotic susceptibility profiles of bacterial isolates from selected tilapia and catfish farms and hatcheries in Ashanti region of Ghana.

MATERIALS AND METHODS

Farms studied for sampling

Ten farms from the Kumasi zone and one farm from the Atwima zone were selected for sampling of water and fish in the Ashanti region of Ghana. Farms which have not been in active production for at least 6 months were not selected for the study. The farms were all integrated fish farms (organic manure is used in the ponds). Two fish hatcheries, located in the Kumasi and Ejura-Sekyere zones which serve as the official source of fingerlings (mainly tilapia) for majority of farmers in the Ashanti Region were also studied (Figure 1).

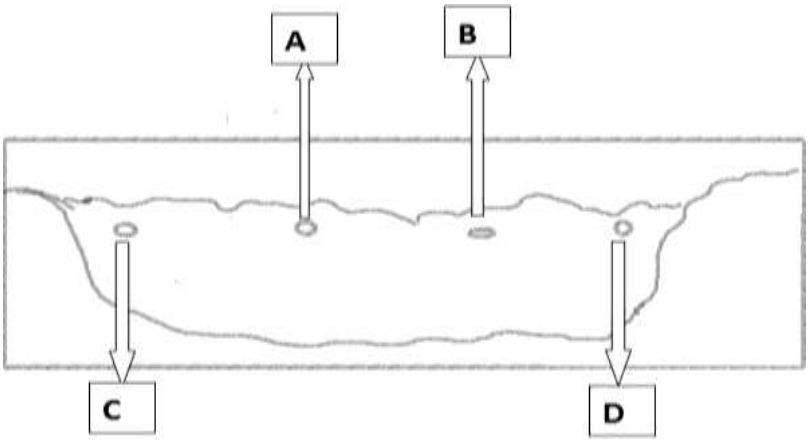

Figure 2. Schematic representation of a fish pond showing relative positions of sampling sites (A-site 1, B-site 2, C-site 3, and D-site 4) from which water samples were collected.

Collection of samples from water

One hundred milliliters (100 mL) of water samples was collected approximately 15 cm below water surface using sterile glass bottles with stoppers from a minimum of four different sites of all fish farms (ponds) between 8:00 and 11:00 GMT (Figure 2). The water samples were transported to the laboratory in boxes with ice and isolation of bacterial agent was done within 24 h of picking the water samples (Gordon et al., 2007).

Preparation of culture media and isolation of bacteria

One milliliter (1 mL) each of composite samples from each farm was aseptically transferred into 10 mL sterile tryptone soya broth, an enrichment medium, and then incubated at 37°C for 18 h (Elhadi, 2014). Samples from various farms were aseptically streaked unto mannitol salt agar (MSA), eosin methylene blue (EMB) agar, Salmonella-Shigella agar (SSA) and Pseudomonas cetrimide agar (to isolate *S. aureus, E. coli, Shigella* spp. and *S. typhi* and *P. aeruginosa,* respectively.

Collection of bacterial samples from fish

Apparently healthy fish samples (tilapia and catfish) were obtained from the 11 selected farms and two hatcheries with the aid of cast net for fish from fish farms and scoop nets for hatcheries. The fishes were cleaned with ethanol and dissected aseptically using a sterile scalpel and approximately 2.5 cm of the gut excised. Contents of the excised guts were transferred into 10 mL sterile tryptone soya broth and incubated for 24 h at 37°C. The samples were aseptically streaked unto respective media for the isolation of bacteria including *S. aureus, E. coli, Shigella* spp*., S. typhi* and *P. aeruginosa* using a sterile inoculating loop.

Isolation of bacteria from samples

Different selective media (purchased from ThermoFisher Scientific, Waltham, MA, USA) were streaked with the samples to isolate the organisms of interest. Mannitol salt (MS) agar for *S. aureus*, eosin methylene blue (EMB) was used to isolate *E. coli*, Salmonella-Shigella (SS) agar was used to isolate *Salmonella* spp. and *Shigella* spp. and Pseudomonas cetrimide agar was used to isolate *P. aeruginosa*. Blood agar was used to demonstrate haemolysis characteristics of *S. aureus* isolates (Benson, 2002). *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. typhi* ATCC 14028, *Shigella flexneri* ATCC 12022 and *P. aeruginosa* ATCC 29213 were used as positive control organisms for isolation. Colonies on selective media were examined microscopically to determine the morphology of cells by Gram-staining.

Biochemical tests

Biochemical tests conducted included coagulase, catalase, oxidase, citrate, indole, methyl red (MR) Voges Proskaeur (VP), Baird-Parker, triple sugar iron (TSI) agar tests. *S. aureus* was confirmed using coagulase test, catalase test, isolation on Baird-parker agar and B-hemolysis on blood agar. Enterobacteria, *E. coli*, *S.typhi* and *Shigella* spp. were confirmed by biochemical tests including indole test, citrate test, methyl red, Voges-Proskaeur tests, oxidase test, and reactions on triple sugar iron (TSI). *E. coli* ATCC 25922, *S. typhi* ATCC 14028 and *S. flexneri* ATCC 12022 were used as positive controls.

Antimicrobial susceptibility testing

Susceptibility of identified bacterial isolates from each sample to selected antibiotics was performed by the disk diffusion method according to CLSI (2014) guidelines using antibiotic discs on Mueller-Hinton agar. Antibiotic discs (purchased from Abitek Biologicals Limited, Liverpool, United Kingdom) used were: penicillin (10 units), ampicillin (10 µg), flucloxacillin (5 µg), tetracycline (30 µg), cefuroxime (30 µg), trimethoprim-sulphamethoxazole (1.25/23.75 µg), gentamicin (10 µg), ciprofloxacin (5 µg) and chloramphenicol (30 µg). In accordance with the CLSI (2014) guidelines for efficiency of the disk, *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 29213 were used as controls.

Table 1. Isolation of organisms from selected farms.

Farm	NCWS	NCTS	NCCS	NI	% No. of isolates
Farm 1	1	1	0	14	4.9
Farm 2	4	3	2	56	19.4
Farm 3	1	1	0	13	4.5
Farm 4	1	1	1	11	3.8
Farm 5	1	1	0	6	2.1
Farm 6	1	1	1	21	7.3
Farm 7	1	1	0	10	3.5
Farm 8	1	1	1	13	4.5
Farm 9	1	1	1	8	2.8
Farm 10	1	1	0	24	8.3
Farm 11	1	1	1	17	5.9
Farm 12	4	1	1	55	19.1
Farm 13	2	2	0	40	13.9
Total	**20**	**16**	**8**	**288**	**100.0**

NCWS, Number of composite water samples; NCTS, number of composite tilapia gut samples; NCCS, number of composite catfish samples; NI: Number of isolates from farm.

Table 2. Frequency of isolation of organisms from fish ponds.

	Organisms					
	S. aureus	***E. coli***	***Shigella* spp.**	***S. typhi***	***P. aeruginosa***	**Total**
Frequency	72	58	47	49	62	**288**
Percent (%)	25.0	20.1	16.3	17.0	21.5	**100.0**

Statistical analysis

Microsoft Excel and Statistical Package for Social Science (SPSS, Chicago, Illinois, USA) version 22 was used to analyze the data on the survey of antimicrobial use on fish farms as well as the frequency of detection of resistant bacteria isolates from different farms and sources. The level of resistance to antibiotics from the various sources was compared using the chi-squared and students t-test at a 0.05 level of significance with 95% confidence interval. A one-way ANOVA test and a further post-hoc tukey's test were carried out to analyze the differences in antibiotic resistant bacteria from the various sources.

RESULTS

One composite sample each of water and fish gut contents was collected from each farm depending on the types of fish farmed on respective farms. A total of 44 composite samples were collected from the 13 farms. These include 20 water samples, 8 composite catfish gut samples and 16 composite tilapia gut samples (Table 1). Out of 645 bacterial isolates, 288 isolates were confirmed by biochemical tests as *S. aureus, E. coli, Shigella* spp., *S. typhi* and *P. aeruginosa* (Table 2).

Identification of isolated organisms

The microorganisms of interest were identified using biochemical tests and Gram staining technique. S. aureus were the most isolated whilst Shigella spp. were the least isolated from the farms (Table 2). *S. aureus* (23.2%), *E. coli* (19%), *P. aeruginosa* (22.5%), S. typhi (18.3%) and *Shigella* spp. (16.9%) were isolated from water samples from fish ponds. Tilapia samples for the study were also observed to habour *S. aureus* (26.3%), *E. coli* (22.1%), *P. aeruginosa* (23.2%), S. typhi (12.6%) and *Shigella* spp. (15.8%). In the intestine of catfishes, *S. aureus* (27.5%), E. coli (19.6%), *P. aeruginosa* (15.7%), S. typhi (17.6%) and *Shigella* spp. (19.6%) were isolated. Overall, *S. aureus* was the most isolated and *Shigella* spp. was the least isolated in water sample from fishponds as well as tilapia and catfish samples (Figure 3).

Antibiotic susceptibility tests

Susceptibilities of the identified and confirmed bacterial isolates to penicillin, ampicillin, flucloxacillin, erythromycin,

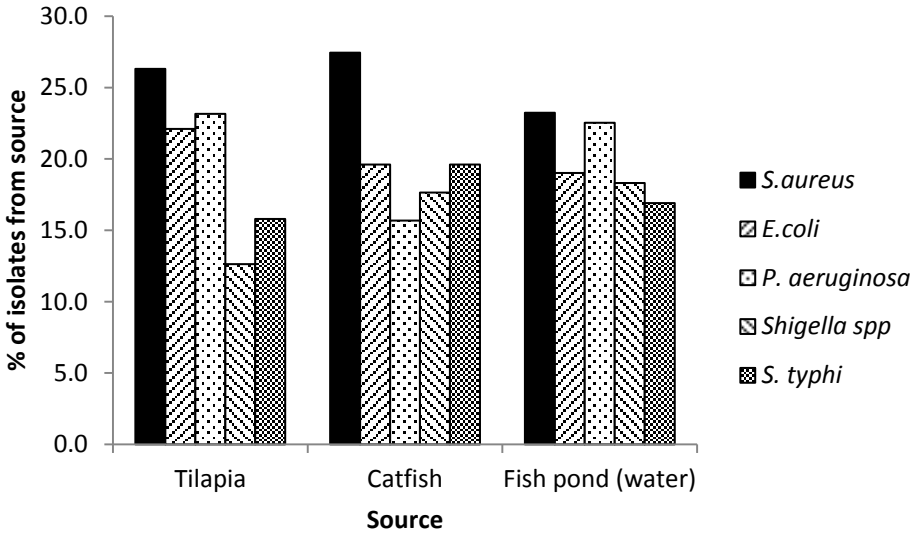

Figure 3. Microbial isolates from water (fish pond), tilapia and catfish.

Table 3. Resistance of isolates to antibiotics from fish farms and hatcheries.

Antibiotic	Farm				(Chi-sq p)[a]
	Fish farm		Hatchery		
	N	%	N	%	
PEN	54	100.00	18	100.00	-[b]
AMP	150	100.00	76	100.00	-[b]
FLX	54	100.00	18	100.00	-[b]
ERY	40	74.10	17	94.40	0.167
TET	127	84.70	69	90.80	0.440
COT	102	68.00	51	67.10	0.780
CRX	119	79.30	56	73.70	0.161
GEN	20	10.40	5	5.20	0.332
CPR	65	33.90	25	26.00	0.367
CHL	90	93.80	50	86.20	0.057

PEN, Penicillin (10 units); AMP, ampicillin (10 µg); FLX, flucloxacillin (5 µg); ERY, erythromycin (15 µg); TET, tetracycline (30 µg); COT, trimethoprim/sulphamethoxazole (1.25/23.75 µg); CRX, cefuroxime (30 µg); GEN, gentamicin (10 µg); CPR, ciprofloxacin(5 µg); N, number of isolates a, p-value comparing antibiotic resistance of isolates from main farm to isolates from hatchery; b = p-value not computed.

tetracycline,sulphamethoxazole/trimethoprim, cefuroxime, gentamicin, ciprofloxacin and chloramphenicol were determined using disc diffusion method (Hudzicki, 2009). The susceptibility of the individual bacterial isolates to the reference antibiotics was done in triplicate and the standard deviations (SD) calculated. The SD of the susceptibilities of the individual bacterial isolate was found to be negligible and the isolates were grouped according to their resistance pattern (based on the zones of growth inhibition) to individual antibiotic as recommended by CLSI (2014) guidelines. The results were interpreted according to CLSI (2014) guidelines.

All isolates of *S. aureus*, *E. coli*, *S. typhi* and *Shigella* spp. were resistant to ampicillin. All *S. aureus* isolates exhibited 100% resistance to penicillin, ampicillin and flucloxacillin. With the exception of gentamicin and ciprofloxacin, there was varying resistance of more than 60% to the other antibiotics (Table 3). Generally, isolates showed high resistance to penicillin, ampicillin, flucloxacillin and tetracycline. Low resistance was observed in all isolates to gentamicin (1.7 to 5.6%) except with *P. aeruginosa* isolates which showed 29.0% resistance to gentamicin.

All *E. coli* isolates were resistant to ampicillin and

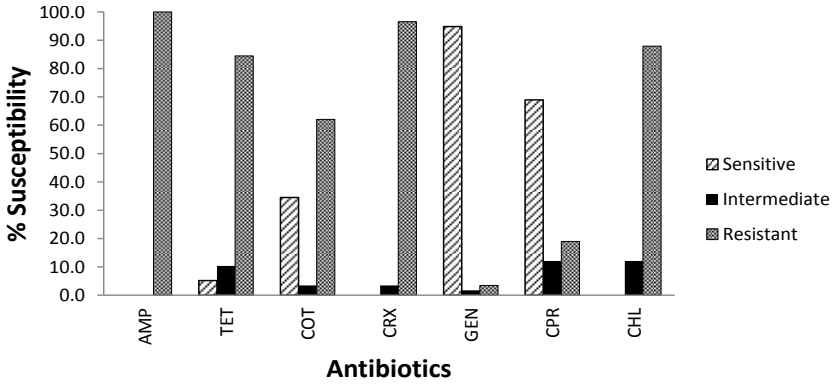

Figure 4. Susceptibility of *E. coli* isolates from fish farms to selected antibiotics. AMP, Ampicillin (10 µg); TET, tetracycline (30 µg); COT, trimethoprim/sulphamethoxazole (1.25/23.75 µg) CRX, Cefuroxime (30 µg); GEN, gentamicin (10 µg); CPR, ciprofloxacin (5 µg); CHL, chloramphenicol (30 µg).

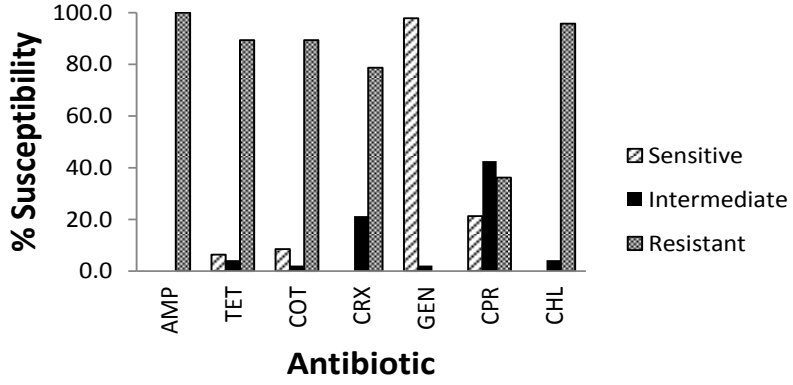

Figure 5. Antibiotic susceptibility of *Shigella* spp, isolates from fish farms. AMP, Ampicillin (10 µg); TET, tetracycline (30 µg); COT, trimethoprim/sulphamethoxazole (1.25/23.75 µg); CRX, cefuroxime (30 µg); GEN, gentamicin (10 µg); CPR, ciprofloxacin (5 µg); CHL, chloramphenicol (30 µg).

resistance to tetracycline, cotrimoxazole, cefuroxime and chloramphenicol was between 62.1 and 96.6%. Resistance of *E. coli* isolates to gentamicin and ciprofloxacin were 3.4 and 19.0%, respectively (Figure 4).

Resistance of *Shigella* spp. isolates to ampicillin was 100%. Resistance of *Shigella* spp. to ciprofloxacin, tetracycline, cotrimoxazole, cefuroxime and chloramphenicol were 36.3 to 95.7%. *Shigella* spp. isolates were susceptible to gentamicin (Figure 5).

S. typhi isolates exhibited 100, 89.8 and 87.8% resistance to ampicillin, tetracycline and chloramphenicol, respectively. Resistance of *S. typhi* isolates to cotrimoxazole, cefuroxime, ciprofloxacin and gentamicin were 61.2, 59.2, 18.4 and 4.1%, respectively (Figure 6). *P. aeruginosa* isolates showed resistance of 29.03 and 51.61% to gentamicin and ciprofloxacin, respectively (Figure 7).

S. aureus isolates showed 100% resistance to penicillin, ampicillin and flucloxacillin. Resistance to erythromycin, tetracycline, cotrimoxazole and cefuroxime was 62.5 to 86.1%. Resistance to gentamicin and ciprofloxacin was 5.6 and 29.2%, respectively (Figure 8).

Multidrug resistant isolates

Multi drug resistant (MDR) bacteria were defined as isolates with acquired resistance to three or more antibiotics indicated in their respective CLSI (2014) panels. Almost 90% of bacterial isolates exhibited

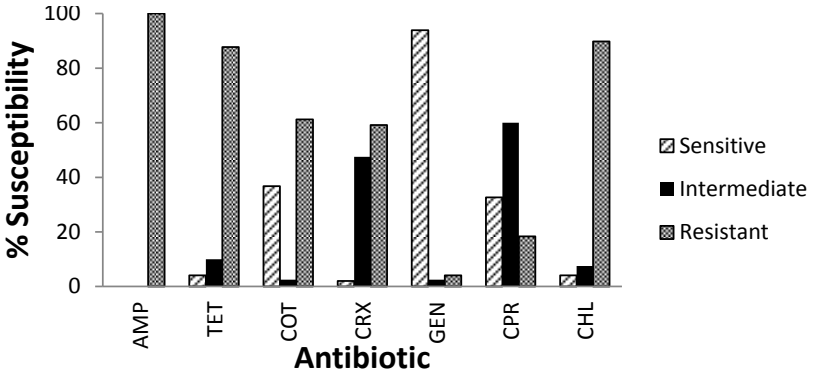

Figure 6. Antibiotic susceptibility of *S. typhi* isolates from fish farms. AMP, Ampicillin (10 µg); TET, tetracycline (30 µg); COT, trimethoprim/sulphamethoxazole (1.25/23.75 µg); CRX, cefuroxime (30 µg); GEN, gentamicin (10 µg); CPR, ciprofloxacin (5 µg); CHL, chloramphenicol (30 µg).

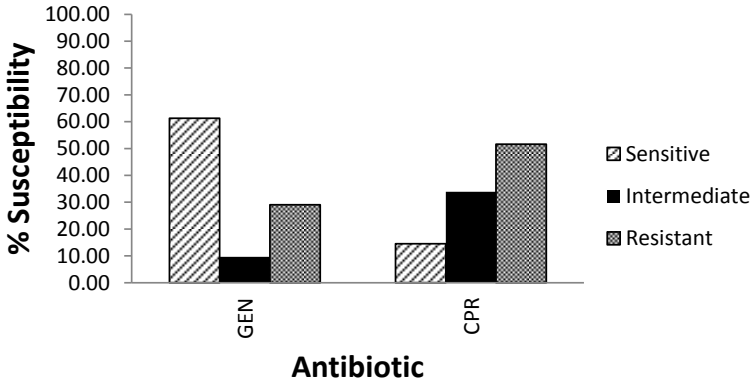

Figure 7. Antibiotic susceptibility of *P. aeruginosa* isolates from fish farms. GEN, Gentamicin (10 µg); CPR, ciprofloxacin (5 µg).

resistance towards 3 or more antibiotics. *S. aureus*, *E. coli*, *Shigella* spp. and *S. typhi* isolates were resistant to 5 antibiotics by 25.3, 29.8, 39.7 and 12.2%, respectively. *S. aureus* isolates showed multidrug resistance to up to 8 antibiotics (Figure 9).

Resistant bacterial isolates from farms and hatcheries

Isolates from fish farms were compared to isolates from hatcheries to determine if there was any difference in resistance patterns between isolates from either source. Isolates from both main farms and hatcheries exhibited >50% resistance to antibiotics with the exception of gentamicin and ciprofloxacin. Isolates from both hatcheries and fish farms were 100% resistant to penicillin, ampicillin and flucloxacillin. Resistance of isolates from main fish farms to tetracycline was 84.70% as compared to 90.80% resistance of isolates from the hatcheries. Resistance of isolates to gentamicin was the lowest at 10.40 and 5.20% in fish farms and hatcheries, respectively. Using the students' t-test, the observed differences in resistance to antibiotics in main farms compared to hatcheries were insignificant for all antibiotics (Table 3).

Resistant isolates from water, catfish and tilapia

A one-way analysis of variance (ANOVA) (to determine differences between the means of more than two independent groups) was employed to determine whether there was significant differences in source of isolates and their resistance to reference antibiotics revealed statistically significant difference (p=0.033) in resistant

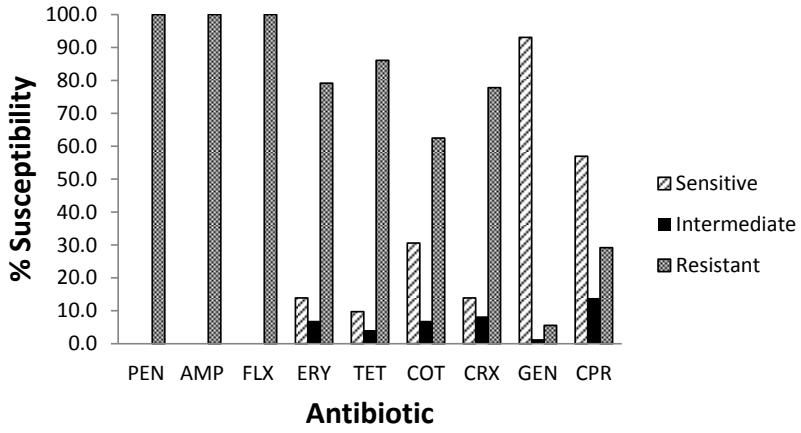

Figure 8. Antibiotic susceptibility of *S. aureus* isolates from fish farms. PEN, Penicillin (10 units); AMP, ampicillin (10 µg); FLX, flucloxacillin (5 µg); ERY, erythromycin (15 µg); TET, tetracycline (30 µg); COT, trimethoprim/sulphamethoxazole (1.25/23.75 µg); CRX, cefuroxime (30 µg); GEN, gentamicin (10 µg); CPR, ciprofloxacin (5 µg).

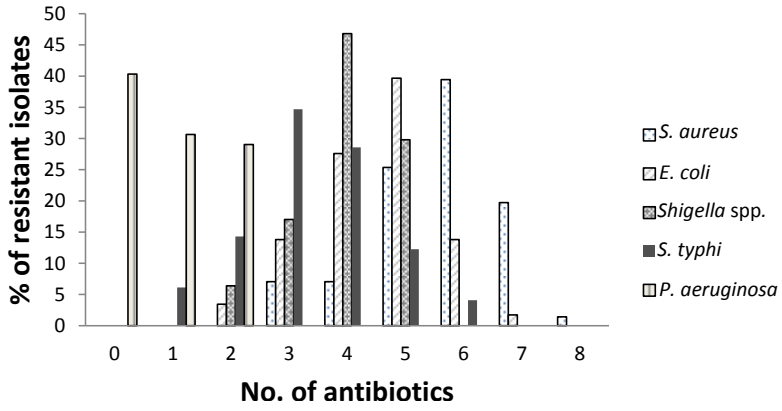

Figure 9. Number of antibiotics to which isolates are resistant to.

isolates from water, catfish and tilapia, only for tetracycline resistance (F=3.455, *p*=0.033). Post-hoc Tukey's tests on tetracycline resistant isolates from the three sources revealed statistically significant difference (*p*=0.027) between resistant isolates from water and isolates from tilapia). However, there was no significant difference between tetracycline resistant isolates from water and catfish (*p*=0.874) and isolates from tilapia compared to catfish (*p*=0.256). For the other antibiotics, there was no significant difference (p>0.05) in resistance of isolates from water compared to isolates from tilapia and catfish (Table 4).

DISCUSSION

Antibiotic resistant isolates have often been isolated from fish farms especially farms with history of antibiotic use. In this study, *S. aureus, E. coli, S. typhi, Shigella* spp. and *P. aeruginosa* were isolated from the selected fish ponds. These bacterial species have been isolated from fishes raised in fresh, brackish water and pond water (Osungbemiro et al., 2014; Uddin and Al-Harbi, 2012).

The presence of these enteric bacteria in both water and fish samples is an indication of faecal contamination as these pathogens are normally found in warm-blooded animals and they rarely form part of the normal fish flora. In a study of fertilized ponds in Ghana, Ampofo and Clerk (2010) isolated *Pseudomonas* spp*., Salmonella* spp*., E. coli, S. aureus* and *Shigella* spp. from tilapia in fertilized fish ponds which confirms the study findings. *Pseudomonas* spp. have also been identified as fish pathogens and can remain present in tilapia even when they are processed (Najiah et al., 2009; Rice, 2009). This

Table 3. Resistance of isolates to antibiotics from fish farms and hatcheries.

Antibiotic	Farm				(Chi-sq p)[a]
	Fish farm		Hatchery		
	N	%	N	%	
PEN	54	100.00	18	100.00	-[b]
AMP	150	100.00	76	100.00	-[b]
FLX	54	100.00	18	100.00	-[b]
ERY	40	74.10	17	94.40	0.167
TET	127	84.70	69	90.80	0.440
COT	102	68.00	51	67.10	0.780
CRX	119	79.30	56	73.70	0.161
GEN	20	10.40	5	5.20	0.332
CPR	65	33.90	25	26.00	0.367
CHL	90	93.80	50	86.20	0.057

PEN, Penicillin (10 units); AMP, ampicillin (10 µg); FLX, flucloxacillin (5 µg); ERY, erythromycin (15 µg); TET, tetracycline (30 µg); COT, trimethoprim/sulphamethoxazole (1.25/23.75 µg); CRX, cefuroxime (30 µg); GEN, gentamicin (10 µg); CPR, ciprofloxacin(5 µg); N, number of isolates a, p-value comparing antibiotic resistance of isolates from main farm to isolates from hatchery; b = p-value not computed.

Table 4. Resistance of isolates from water, catfish and tilapia to different antibiotics.

Antibiotic	Source						(p=value)[b]
	Water		Tilapia		Catfish		
	N	%	N	%	N	%	
PEN	33	100	25	100	14	100.0	na
AMP	111	100	72	100.0	43	100.0	na
FLX	33	100	25	100.0	14	100.0	na
ERY	27	81.8	20	80.0	10	71.4	0.612
TET	101	91.0	56	77.8	39	90.7	0.033
COT	79	71.2	48	66.7	26	60.5	0.402
CRX	83	74.8	60	83.3	32	74.4	0.390
GEN	16	11.2	8	8.5	1	2.0	0.255
CPR	38	26.6	35	37.2	17	33.3	0.423
CHL	71	91.0	42	89.4	27	93.1	0.840

PEN, Penicillin (10 units); AMP, ampicillin (10 µg); FLX, flucloxacillin (5 µg); ERY, erythromycin (15 µg); TET, tetracycline (30 µg); COT, trimethoprim/sulphamethoxazole (1.25/23.75 µg); CRX, cefuroxime (30 µg); GEN, gentamicin (10 µg); CPR, ciprofloxacin (5 µg); b = p-value comparing resistant isolates from water to catfish and tilapia; N, number of isolates; na, non-applicable.

can pose a threat to public health as they can be transferred to humans and a possible transfer of resistant bacterial strains to humans.

The use of organic manure by fish farmers may contribute to antibiotic resistance of bacterial isolates from their farms and this occurs through the transfer of antibiotic residues and resistant bacteria to fish farms if the commercial farms from which the manure is sourced use antibiotics (Elsaidy et al., 2015). From the survey, 93.6% of respondents use poultry manure in fertilizing their ponds. This could be a possible source of enteric bacteria in both the pond water and fish samples as observed in studies of integrated fish farms in Vietnam where tetracycline resistant *Enterococcus faecium*, *Enterococcus faecalis*, and other *Enterococcus* spp. in the water-sediment and manure samples isolated in the ponds were found to have originated mainly from the pig manure (Dang et al., 2011). A study in Egypt by Elsaidy et al. (2015) reported higher incidence of both *E. coli* and *Salmonella* spp. in water and fish raised in ponds which received unfermented chicken manure than those which received fermented chicken manure. The use of fermented chicken manure as a bacteriologically safe fish pond fertilizer was recommended or made in the report.

Isolates of *S. aureus, E. coli, S. typhi* and *Shigella* spp. showed 100% resistance to ampicillin. This finding is

comparable to a report by Newaj-Fyzul et al. (2008), where 92.0% of five genera of bacteria isolated from tilapia and cohosalmon hatcheries were ampicillin resistant and in which various resistant phenotypes to penicillin, vancomycin, chloramphenicol, tetracycline, sulphamethoxazole/trimethoprim and gentamicin were found in 20 to 100% of isolates. Even though antibiotics were not previously used in the hatcheries prior to their study, Karki et al. (2013) also reported ampicillin resistance in bacteria isolated from the hatcheries in the USA and recommended further study to determine the source of antibiotic resistance in the hatcheries. Su et al. (2011) observed that enterobacteria isolated from integrated fish farms in China showed high antibiotic resistance to ampicillin (80%), tetracycline (52%) and trimethoprim (50%) and indicated high multiple antibiotic resistances in isolates from animal manure on the farms. *E. coli* isolates exhibited least resistance to gentamicin (1.7%) and ciprofloxacin (19%). In a study of cultured catfish in Malaysia, *E. coli* isolates were 100% susceptible to norfloxacin, sulphamethoxazole/trimethoprim and chloramphenicol but exhibited 35.3, 23.5 and 11.8% resistance to ampicillin, tetracycline and nitrofurantoin, respectively (Samuel et al., 2011).

Generally, resistance of bacterial isolates from the hatcheries to antibiotics was slightly lower than that of isolates from the main farms. However, there was higher resistance to tetracycline and erythromycin in isolates from hatcheries than from main farms. There was 100% resistance of isolates from both hatcheries and main farms to ampicillin, penicillin and flucloxacillin (Table 3). The differences in resistance to the antibiotics from the main fish farms and hatcheries were however, not significant (p>0.05). Both hatcheries use tetracycline for prophylaxis in the fingerlings and this could account for the high resistance of 90.8% of bacaterial isolates from the hatcheries. Karki et al. (2013) reported that bacterial isolates from hatchery-raised tilapia and coho salmon were all resistant to ampicillin and penicillin but were sensitive to gentamicin. The isolates also showed varying resistance to chloramphenicol, tetracycline, vancomycin and streptomycin. However, the hatcheries in that study had history of none use of antibiotics in the rearing of the fingerlings.

Isolates from both hatcheries and main fish farms showed high resistance of 90 and 84% to tetracycline, respectively. There has been report of tetracycline resistant determinants in isolates of *Salmonella* spp. from tilapia in South African fish ponds where there had not been recent use of tetracycline (Chenia and Vietze, 2012). Seyfried et al. (2010), suggested that aquatic environments may harbour tetracycline resistant genes regardless of the use of tetracycline or not, and the source of water for the hatchery tanks (well and tap water) may also contribute to the resistance of isolates to antibiotics as bacteria flora in the fish gut may be acquired from the water or feed. The resistant bacteria though they may not be pathogenic, can transfer resistance to other bacteria.

Though there was no history of recent use of antibiotics on the main farms, high resistance of isolates to antibiotics studied was recorded. This is similar to a study by Shah et al. (2012), where bacteria isolated from water, pond sediment and fish from fish ponds in Pakistan and Tanzania with no recorded history of antibiotic use showed resistance to tetracycline, sulphamethoxazole/trimethoprim, amoxicillin and chloramphenicol and they hypothesized the contribution of integrated fish farming practices using domestic farm waste as a likely source of resistance genes in the aquaculture environment. Schmidt et al. (2001) also reported the presence of resistant genes in aeromonads isolated from a fish farm with no history of recent antibiotic use for therapeutic purposes nor in feed and suggested that the resistance may due to the persistence of previously acquired resistance genes in the pond environment.

There was no significant difference (p>0.05) in resistance to antibiotics in isolates from water compared to isolates from catfish and tilapia except in tetracycline (p=0.033). There was statistically significant (p=0.027) higher levels of tetracycline resistant isolates from pond water compared to tilapia but not catfish. The increase in tetracycline resistant isolates in pond water may be attributed to the excretion of tetracycline resistant isolates into the water which subsequently donate resistant genes to other bacteria in the pond water. The presence of tetracycline residues in water may also lead to selective pressure (Petersen et al., 2002).

There was high level of resistance to ampicillin (100%), tetracycline (84.5 to 91.5%), cefuroxime (59.2 to 96.6%), sulphamethoxazole/trimethoprim (61.2 to 89.4%) and chloramphenicol (87.9 to 95.7%) in the enteric bacteria (*E. coli, S. typhi* and *Shigella* spp.), which ordinarily are not part of fish flora but an indication of faecal contamination as shown in other studies where various antibiotic resistant pathogenic bacteria such as *E. coli*, *S. typhi*, *Shigella* spp. have been isolated from animal manure. The presence of these enteric bacteria in this study may be due to the use of organic manure from commercial farms, especially poultry as most farmers interviewed use poultry manure to fertilize the ponds. Donkor et al. (2012) and Turkson et al. (2008) reported that different classes of antibiotics including penicillin, tetracycline and sulphonamide are used in livestock and poultry farming in Ghana and may contribute to antibiotic resistance. The use of manure on the farms may have contributed to high antibiotic resistance in the bacteria isolates from the main farms as reported in various studies (Petersen et al., 2002; Shah et al., 2012).

The presence of these highly resistant pathogens which

are also pathogens of humans is a public health threat as these resistant bacteria may cause diseases in humans. These include gastroenteritis, diarrhoea, shigellosis and salmonellosis (Novotny et al., 2004). The resistant bacteria may transfer resistance directly to humans or indirectly by transferring resistant determinants to other pathogenic bacteria of humans. These multidrug resistant bacteria may be a problem as therapeutic failure may occur in the event of an outbreak of fish diseases among the fish on these farms and infections caused by these resistant bacteria in humans.

The emergence of multidrug resistance (MDR) bacteria is of major concern globally. This is due to the fact that MDR bacterial infections are difficult to treat in aquaculture, livestock and humans. MDR was observed in all but *Pseudomonas* spp. with most isolates of *S. aureus, S. typhi,* and *E. coli* and *Shigella* spp. showing MDR. Though there are reports of multidrug resistance in *Pseudomonas* spp*.*, because only two antibiotics were tested against *P. aeruginosa isolates* in this study, multidrug resistance cannot be concluded. The use of more than one antibiotic on fish farms may result in selective pressure leading to multiple antibiotic resistant isolates on fish farms as reported by Sarter et al. (2007) where bacteria of the genus *Enterobacteriacae*, *Pseudomonads* and *Vibrionaceae* showed multiple resistance to antibiotics including oxytetracycline, chloramphenicol, trimethoprim-sulphamethoxazole, nitrofurantoin, nalidixic acid, and ampicillin. Infections caused by multidrug resistant organisms may be difficult to manage or can lead to high mortality since these organisms have multiple resistance mechanisms which enables them to inactivate antibiotics (Magiorakos et al., 2012). Hence, the observed trend of multidrug resistant strains poses a major public health concern globally and there is need to come with effective policies and implementation plans to address these concerns.

Conclusion

S. aureus, E. coli, Shigella spp*., S. typhi* and *P. aeruginosa* isolated from pond water hatcheries and guts from catfish and tilapia from fish farms exhibited multidrug resistance to the reference antibiotics.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to the staff of Ministry of Fisheries, Ashanti region, Ghana for their support in accessing the fish farms and farmers who volunteered to be part of the study.

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