academicJournals

Vol. 11(28), pp. 314-320, 29 July, 2017
DOI: 10.5897/AJPP2017.4789
Article Number: 235215D65351
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Antibiotic sensitivity patterns of microbial isolates from fish ponds: A study in the Greater Accra Region of Ghana

John Antwi Apenteng*, Christina Osei-Asare, Esther Eshun Oppong, Ignatius Amihere and Mohammed Yahaya Hafiz

Department of Pharmaceutics, School of Pharmacy, Central University, Accra, Ghana

Received 27 April, 2017: Accepted 14 June, 2017

Intensive use of antimicrobial agents in aquaculture provides a selective pressure creating reservoirs of drug-resistant bacteria and transferable resistance genes in fish pathogens and other bacteria in the aquatic environment. From these reservoirs, resistance genes may disseminate by horizontal gene transfer and reach human pathogens, or drug-resistant pathogens from the aquatic environment may reach humans directly. This study aims at identifying the antibiotic sensitivity patterns of *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli* isolates from fish ponds in two localities (Ashiaman and Dawhenya) in the Greater Accra region of Ghana. A total of 43 isolates were tested using the Kirby-Bauer agar disc diffusion method against Ciprofloxacin (5 µg), Erythromycin (15 µg), Cefuroxime (30 µg), Gentamicin (10 µg), and Tetracycline (30 µg).*P. aeruginosa* was the most isolated organism with 90% prevalence, followed by *E. coli* (75%) and *S. typhi* (50%). All the *P. aeruginosa* isolates were resistant to Erythromycin and Cefuroxime while 90% of the *S. typhi* isolates were resistant to Tetracycline and Erythromycin. *E. coli* isolates showed 100, 93.33 and 66.66% resistance to Erythromycin, Cefuroxime and Tetracycline respectively. Gentamicin, Tetracycline and Ciprofloxacin were sensitive to 66.66, 61.10 and 50% of *P. aeruginosa* isolates respectively. Ciprofloxacin was sensitive to 90% of the *S. typhi* isolates whiles Gentamicin was sensitive to 70% of the *S. typhi* isolates. Ciprofloxacin and Gentamicin were more sensitive to *E. coli* isolates (80 and 66.66% sensitivity respectively). Multi-drug resistant strains were obtained in 77.78% of *P. aeruginosa* and 70% of *S. typhi* isolates whiles 66.67% of *E. coli* isolates were also resistant to more than two classes of the antibiotics tested. High levels of microbial resistance were observed in the isolates with 72.09% of isolates being multidrug resistant strains.

Key words: Resistance patterns, multi-drug resistance, fish ponds, antibiotics.

INTRODUCTION

With the rise in public awareness about the loss of effectiveness of antibiotics due to over use; consumer groups, public health experts and environmentalists have begun to challenge antibiotic usage in livestock, poultry

*Corresponding author. Email: j.a.apenteng@gmail.com or japenteng@central.edu.gh Tel: +233 249 449 249.

and fish farming. Antibiotics in fish farming and other animal food production is widely believed to contribute to the dramatic increase in numbers of antibiotic-resistant bacterial strains now threatening human health (Benbrook, 2002). As a result of the non-hygienic and stressful conditions present in aquaculture facilities, the risk of bacterial infections among aquaculture fish is high, therefore, heavy amount of antimicrobials are used in fish feed for preventive and curative purposes in aquaculture facilities worldwide (Sapkota et al., 2008).

The heavy use of antimicrobial agents in aquaculture has resulted in increase of strains resistance to these agents. Potentially, these resistant strains can have impact on the therapy of fish and human diseases or the environment of the fish farms (Smith et al*.,* 2003). It is therefore necessary to study the presence of clinically important bacteria in the environment and their antibiotic resistance pattern. This study shall focus on resistance strains found in fish farms and the specific isolates will *be Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*.

P. aeruginosa is a clinically important gram negative bacterium, which is responsible for a variety of systemic infections like urinary tract infections, respiratory system infections, gastrointestinal infections, dermatitis, soft tissue infections, bone and joint infections (Favero et al., 1971). The overall prevalence of antibiotic resistant *P. aeruginosa* is increasing, with up to 10% of global isolates found to be multidrug resistance strains (Gales et al., 2001). It is recognized as the second leading cause of gram negative nosocomial infection and a major treatment challenge for *P. aeruginosa* infections (Carmeli et al., 1999).

Salmonella is a Gram-negative bacterium belonging to the family Enterobacteriaceae, and known as "enteric" bacteria. *Salmonella* are found in the intestinal tract of animals and humans. Some serotypes of *Salmonella*, such as *S. typhi* and S*. paratyphi* are only found in humans (Miller and Pegues, 2005). In humans*, Salmonella* are the cause of two diseases called salmonellosis: enteric fever (typhoid), resulting from bacterial invasion of the bloodstream, and acute gastroenteritis, resulting from a food borne infection or intoxication (Foley et al., 2006). *Salmonella* bacterium is one of the commonest causes of food poisoning worldwide.

E. coli is the most prevalent facultative anaerobic species in the gastrointestinal tract of human and animals, usually a harmless microbe, but it is also a medically important bacterium causing a number of significant illnesses. *E. coli* can be easily disseminated in different ecosystems through the food chain and water. One of the possible ways of entry of various microbes could be by the adoption of improper hygienic measures during handling and processing of harvested fishes. Antimicrobial resistance in *E. coli* has been reported worldwide.

The aim of this study is to isolate and assess the prevalence of *S. typhi*, *E. coli*, and *P. aeruginosa* in fish ponds and to determine their respective antibiotic resistance patterns against some clinically used antibiotics.

MATERIALS AND METHODS

Collection of samples

A total of 20 fresh water samples were collected from Ashaiman and Dawhenya Fish Ponds. The samples were collected into a sterile sample container at four different sites of the fish ponds. They were then transferred to the Microbiology Laboratory of Central University Pharmaceutics department immediately and kept refrigerated for 24 h. A volume of 1.0 mL of the collected samples were then transferred into 10 mL peptone water and incubated at 37ºC for 48 h.

Isolation of bacteria

The initial isolation of bacteria (*P. aeruginosa*, *S. typhi* and *E. coli*) was done using various selective, differential isolation media such as Cetrimide, MacConkey and Bismuth sulphite agar. Further isolation were performed on the initial isolates using biochemical methods using fermentation, gram staining and other microbe specific media such as brilliant coliform *E. coli* media, Methyl Red Vogues Proskauer (MRVP) tests and Citrate utilization tests. A total of 43 isolates comprising the organisms of interest were obtained. All microbial media used were purchased from Oxoid, UK.

Antibiogram study

A total of 43 isolates were tested for antimicrobial drug susceptibility against five commonly used antibiotics belonging to the following antibiotic classes;(Aminoglycosides, Floroquinolones, Macrolides, Cephalosporins and Tetracyclines) by Kirby-Bauer agar disc diffusion method (Bauer et al., 1966). The isolated bacteria (*P. aeruginosa*, *S. typhi* and *E. coli*) in the nutrient broth were spread on the surface of sterile nutrient agar. Antimicrobial discs (Oxoid, UK) were placed on the surfaces of inoculated nutrient agar plates using a multidisc dispenser (Oxoid, UK). The plates were kept on the bench for 30 min prior to incubation at 37ºC for 24 h. After 24 h incubation, the plates were examined and the diameters of the inhibition zones were measured from the edge of the disc to the edge of the zone using a zone reader. Susceptibility and resistance were determined according to the interpretation criteria described in the Clinical and Laboratory Standard Institute (CLSI) Guidelines (2011) (Table 1).

Data analysis

All graphs were plotted and analysed using Microsoft Excel 2013.

RESULTS

Overall prevalence of isolates according to location

A total of 43 isolates were obtained out of which 18 were *P. aeruginosa,* 10 were *S. typhi* and 15 were *E. coli*. The

Table 1. CLSI inhibition zone diameter for *P. aeruginosa*, *S. typhi* and *E. coli*.

Antibiotic	Content	When testing	Susceptible (S)	Intermediately susceptible (I)	Resistant (R)
Cefuroxime	30 µg	Enterobacteriaceae *P. aeruginosa*	≥ 23	15-22	≤ 21
Ciprofloxacin	5 µg	Enterobacteriaceae *P. aeruginosa*	≥ 21 ≥ 21	16-20 16-20	≤ 15 ≤ 15
Erythromycin	15 µg	Enterobacteriaceae *P. aeruginosa*	≥ 23	14-22	≤ 13
Gentamicin	10 µg	Enterobacteriaceae *P. aeruginosa*	≥ 15 ≥ 15	13-14 13-14	≤ 12 ≤ 12
Tetracycline	30 µg	Enterobacteriaceae *P. aeruginosa*	≥ 15 ≥19	12-14 15-18	≤ 11 ≤ 14

Source: Clinical and Laboratory Standard Institute (CLSI) Guidelines (2011).

Table 2. Summary of prevalence of *P. aeruginosa*, *S. typhi* and *E. coli* according to location.

Location	Isolated bacteria (%)		
	P. aeruginosa	***S. typhi***	***E. coli***
Dawhenya fish pond	100	75	100
Ashaiman fish pond	87.5	43.75	68.75
Overall	90	50	75

overall prevalence (expressed as a percentage) of *P. aeruginosa*, *S. typhi* and *E. coli* in Dawhenya and Ashaiman Fish Ponds were recorded as shown in Table 2.

Antibiogram profile of isolated bacteria

Pseudomonas aeruginosa

Antibiotic sensitivity test of 18 isolates of *P. aeruginosa* showed different sensitivity and resistance patterns according different sources. Out of the five antibiotics tested, Gentamicin (CN) was sensitive to 66.66% of isolates. Tetracycline (TE) and Ciprofloxacin (CIP) were sensitive to 61.1 and 50% of isolates respectively. Ciprofloxacin (CIP) and Tetracycline (TE) were however intermediate resistant to 5.56% of isolates. Ciprofloxacin (CIP) was resistant to 44.44% of isolates. Erythromycin (E) and Cefuroxime (CXM) showed no antimicrobial activity against the isolates Figure 1.

Salmonella typhi

Antibiotic sensitivity test of 10 isolates of *S. typhi* showed different sensitivity and resistance patterns. Results from the five antibiotics tested showed that, Ciprofloxacin (CIP), Gentamicin (CN) and Cefuroxime (CXM) were sensitive to 90, 70 and 30% of isolated *S. typhi* respectively. Tetracycline (TE) and Erythromycin (E) were sensitive to 10% of isolates. Gentamicin (CN) on the other hand was intermediate resistant to 10% of isolates. The highest resistance was found with Tetracycline (TE) and Erythromycin (E), showing resistance to 90% of isolates, followed by Cefuroxime (CXM), which showed resistance to 70% isolates. Gentamicin (CN) was resistant to 20% of isolates, whereas, Ciprofloxacin (CIP) to 10% of the isolated *S. typhi* shown in Figure 2.

Antibiogram profile of *Escherichia coli*

Out of the five antibiotics tested on the 15 isolates, Erythromycin (E) was resistant to 100% of isolates, followed by Cefuroxime (CXM), which was resistant to 93.33% of isolates. Ciprofloxacin (CIP), Gentamicin (CN), and Tetracycline (TE) showed resistant to 13.33%, 26.66%, and 66.66% of isolated *E. coli* respectively. Ciprofloxacin (CIP), Gentamicin (CN), and Cefuroxime (CXM) were intermediate resistant to 6.66% of isolates. Ciprofloxacin (CIP) on the other hand showed the highest sensitivity against 80% of isolates. Gentamicin (CN) and Tetracycline (TE), showed sensitivity to 66.66 and 33.33% of isolates respectively which is shown in Figure 3.

Multidrug resistant organisms

The presence of multidrug resistance strains were also studied among isolates. Of the isolates, 31 (72.09%) were resistance to more than two different classes of antibiotics out of which 14 (77.78%) isolates of

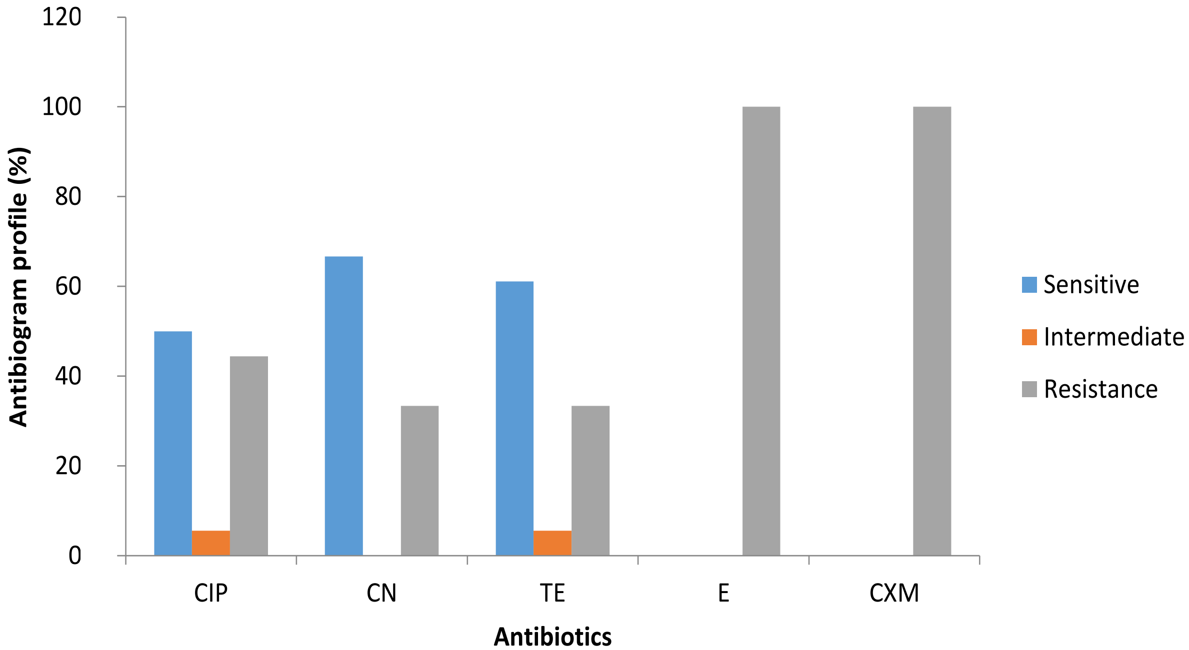

Figure 1. Antibiogram profile of *P. aeruginosa* isolates from Dawhenya and Ashaiman Fish Ponds. Ciprofloxacin (CIP), Gentamicin (CN), Tetracycline (TE), Erythromycin (E), and Cefuroxime (CXM).

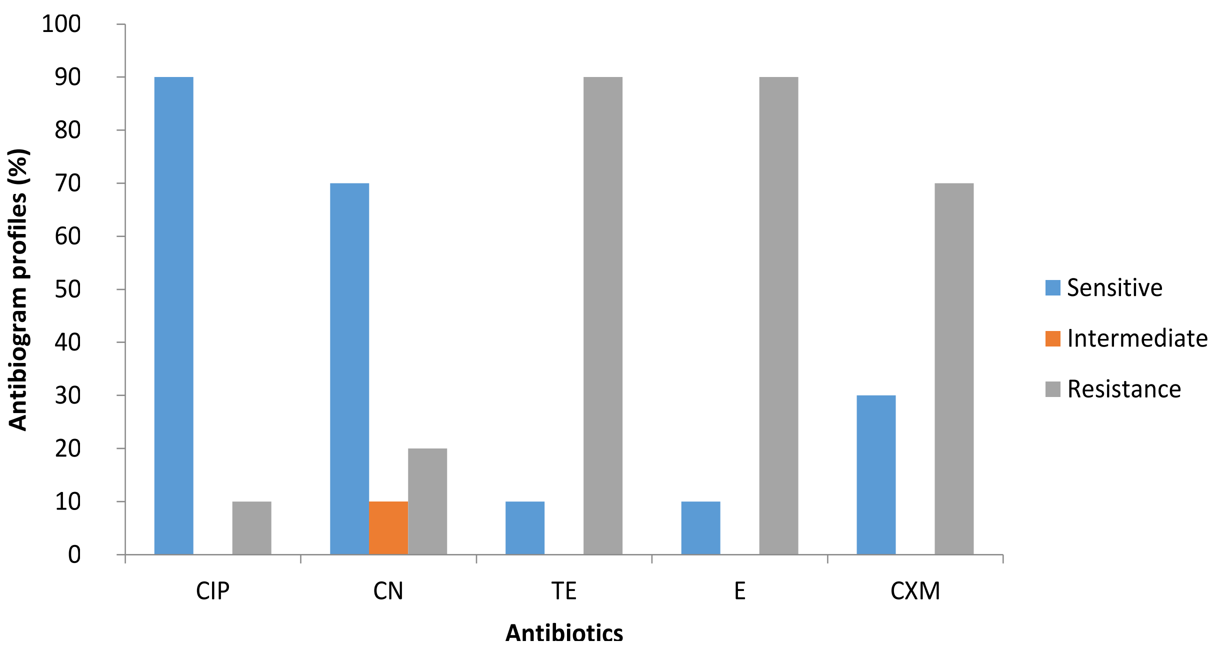

Figure 2. Antibiogram profile of *S. typhi* isolates from Dawhenya and Ashaiman Fish Ponds. Ciprofloxacin (CIP), Gentamicin (CN), Tetracycline (TE), Erythromycin (E), and Cefuroxime (CXM).

P. aeruginosa, 7 (70%) isolates of *S. typhi* and 10 (66.67) isolates of *E. coli* were all found to be multidrug resistant strains. *P. aeruginosa* had the highest number (14 out of 18 isolates) of multidrug resistant strains, followed by *S. typhi*.

DISCUSSION

Aquaculture is growing rapidly in many regions of the world, and aquaculture products constitute an important food supply with increasing economic importance. Use of

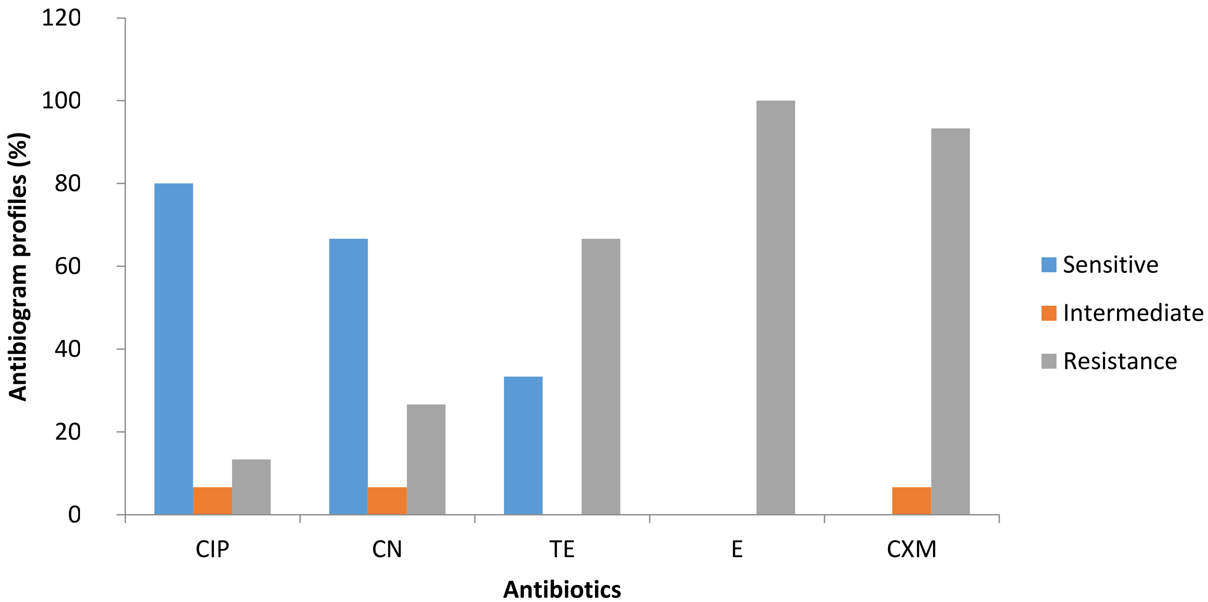

Figure 3. Antibiogram profile of *E. coli* isolates from Dawhenya and Ashaiman Fish Ponds. Ciprofloxacin (CIP), Gentamicin (CN), Tetracycline (TE), Erythromycin (E), and Cefuroxime (CXM).

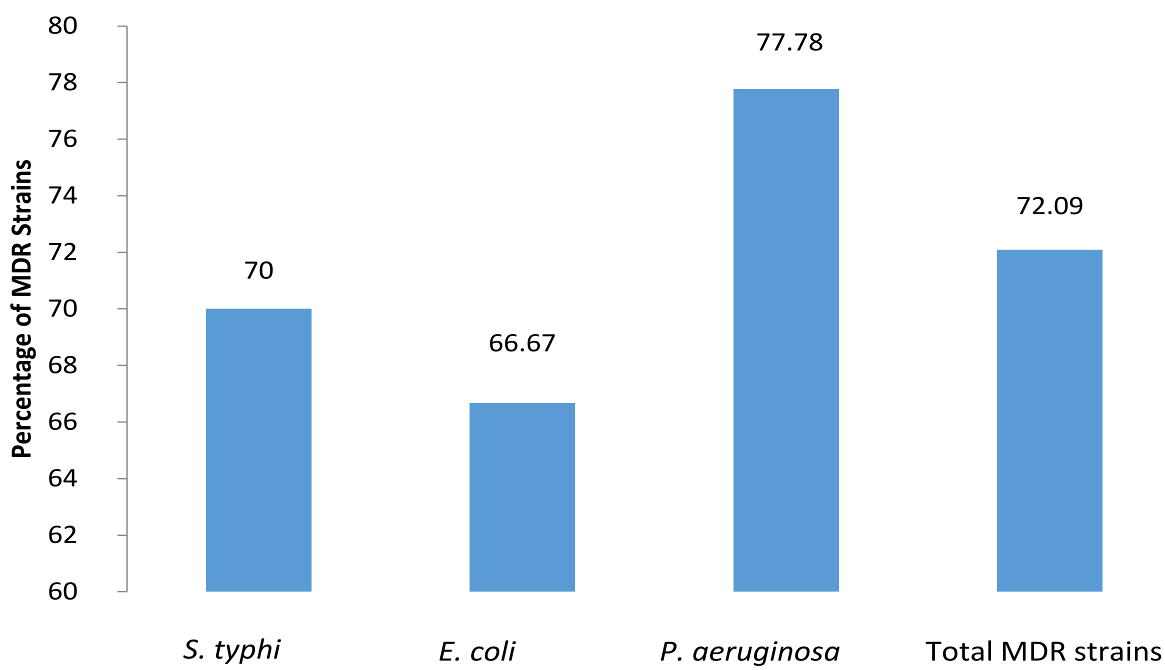

Figure 4. Multi-drug resistance (MDR) strains of isolates.

antimicrobial agents in aquaculture has resulted in the emergence of reservoirs of antimicrobial-resistant bacteria in fish and other aquatic animals, as well as in the aquatic environment (Akinbowale, 2006; Schmidt et al., 2000). The three bacteria studied in this project are of public health importance (Bangtrakulnonth et al., 2004; Nadeem et al., 2009; Ministry of Health, 2002) and presence of resistance strains in fish pond water should be of much concern because, the people around and the environment are exposed to these microorganisms. A total of 43 isolates were obtained in this study of which 18(41.36%) were *P. aeruginosa*, 15(34.88%) were *E. coli* and 10(23.26%) were *S. typhi*. Among the organisms isolated from Ashaiman and Dawhenya Fish Ponds, *P. aeruginosa* was the mostly found organism with 90% prevalence followed by *E. coli* (75%) and *S. typhi* (50%). Uğur et al. (2012) reported in their study in Turkey that, *Pseudomonas* species was the most frequently detected microorganism in fish ponds.

All the *P. aeruginosa* isolates obtained in this study were resistance to Erythromycin and Cefuroxime. This is similar to a study conducted by Sivanmaliappan and Sevanan (2011) in Coimbatore, India where 100% of *P. aeruginosa* isolates were resistant to Erythromycin. A study conducted in Egypt by Gad et al. (2008) also showed that 100% of *P. aeruginosa* isolates were resistant to Cefuroxime. Resistance to Cefuroxime is largely due to the production of extended spectrum β-lactamase (ESBL) enzymes by the bacteria. Resistance to Cefuroxime by *P. aeruginosa* could also be due to a combination of mechanisms such as the expression of chromosomal AmpCcephalosporinases and over expression of active efflux systems (McGowan, 2006). Resistance to Erythromycin may be due to the use of this agent in fish farming. Gentamicin, Tetracycline and Ciprofloxacin were potent against *P. aeruginosa* isolates with 66.66, 61.1% and 50% susceptibility, respectively. This is comparable to a study carried out in Dhaka, Bangladesh where both Gentamicin and Tetracycline were found to be sensitive to 93.70% of *P. aeruginosa* isolates (Nasreen et al., 2015). A similar observation has also been reported in Bangladesh where Ciprofloxacin was highly sensitive to *P. aeruginosa* isolates (Hossain et al., 2013). Gentamicin and Ciprofloxacin resistance to *P. aeruginosa* isolates is due to the ability of *P. aeruginosa* to acquire further resistance mechanisms to these agents (Streteva and Yordanov, 2009). This could be the reason why *P. aeruginosa* isolates were resistant (33.33%) to Gentamicin and (44.44%) to Ciprofloxacin, even though Gentamicin and Ciprofloxacin are hardly used in fish farming. *P. aeruginosa* isolates resistance to Tetracycline (33.33%) could be due to its frequent use in aquaculture facility.

Infection by *Salmonella* is a common cause of food poisoning in humans (Hobbs and Robert, 1993). In this study *S. typhi* was the least isolated organism compared to *P. aeruginosa and E. coli*. Erythromycin and Tetracycline exhibited the highest resistance to *S. typhi* (90%) isolates whiles Cefuroxime showed resistance against 70% *S.typhi* isolates. According to Chanda et al. (2011), most farms use tetracycline and erythromycin to eliminate pathogenic problems. Antibiotic resistance develops when microorganisms are exposed to effective doses of an antibiotic within a shorter period or when the microorganisms are exposed to smaller concentrations or residues of the antibiotic over a longer period of time (Todar, 2008). These theories may support the high resistance observed for Tetracycline and Erythromycin as a result of prolong exposure to microorganisms. It was observed that the most sensitive antibiotics to *S. typhi* isolates were Ciprofloxacin and Gentamicin. A similar result was obtained in a study in Bangladesh where *S. typhi* was mostly sensitive against Ciprofloxacin and Gentamicin (Mannan et al., 2014). A total of 100, 93.33, and 66.66% of the *E. coli* isolates exhibited resistance to Erythromycin, Cefuroxime and Tetracycline respectively. A similar result was obtained by Kibret and Abera (2011), where Erythromycin and Tetracycline were resistance to 89.4 and 72.6% to *E. coli* isolates respectively. A study conducted by Ahmed et al. (2015) in Islamabad showed complete resistance of *E. coli* isolates to Cefuroxime. Ciprofloxacin and Gentamicin revealed the highest sensitivity against the *E. coli* isolates with 80 and 66.66% sensitivity, respectively. This in accordance with a study carried out in Nigeria where 78.9% of *E. coli* isolates showed sensitivity to Ciprofloxacin and same percentage was obtained for Gentamicin (Reuben and Owuna, 2013).

In general, the relatively higher proportion of susceptible responses of *E. coli*, *S. typhi*, *and P. aeruginosa* isolates to Ciprofloxacin and Gentamicin suggest the effectiveness of these agents in the treatment of infections caused by *E. coli*, *S. typhi* and *P. aeruginosa*. *P. aeruginosa* was the most isolated organism from the fish ponds. This is because *P. aeruginosa* thrives very well at habitats with adequate amount of moisture (Hardalo et al., 1997). Out of the 43 isolates, 31 (72.09%) were resistant to more than two different classes of antibiotics. *P. aeruginosa* had the highest percentage of multidrug resistance strains accounting for 77.78%. According to Gales et al. (2001), up to 10% of global *P. aeruginosa* isolates are found to be multidrug resistance. The high number of resistance of *E. coli*, *S. typhi*, and *P. aeruginosa* found in the two fish ponds serves as a reservoir which can be easily transferred to other pathogens and even humans.

Conclusion

The study has shown the presence of resistant strains in the fish ponds at Dawhenya and Ashiaman. All the *P. aeruginosa* isolates were resistant to Erythromycin and Cefuroxime whiles 90% of the *S. typhi* isolates were resistant to Tetracycline and Erythromycin. Cefuroxime, Erythromycin and Tetracycline respectively showed 100, 93.33 and 66.66% resistance to *E. coli* isolates. Ciprofloxacin and Gentamicin showed the highest sensitivity when tested on all the isolates. *P. aeruginosa* had the highest number of multi-drug resistant strains. This study has therefore proved the need for the monitoring of antibiotics usage in fish farming and the adoption of proper hygienic measures in aquaculture facilities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

Authors acknowledge the immense contributions of Mr Harry Oblie Laryea, Mr Kwame Koomson and Mr Christopher Kyei Mensah of Central University School of Pharmacy for their technical support.

REFERENCES

Ahmed I, Sajed M, Sultan A, Murtaza I, Yousaf S, Maqsood B, Vanhara P, Anees, M (2015).The Erratic Antibiotic Susceptibility Patterns of Bacterial Pathogens Causing Urinary Tract Infections**.** EXCLI J. 14:916-925

Akinbowale OL, Peng H, Barton MD. (2006). Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. J. Appl. Microbiol. 100(5):1103-13.

B.C. Ministry of health (2002) Hamburger Disease (Haemorrhagic Colitis) Fact sheet. Available at: https://www.healthlinkbc.ca/hlbc/files/documents/healthfiles/hfile02.pdf

Bangtrakulnonth A, Pornreongwong S, Pulsrikam C, Sawanpanyalert P, Hendriksent RS, Wong DMA, Aarestrupt FM (2004). Salmonella serovars from humans and other sources in Thailand, 1993-2002. Emerg. Infect. Dis. 10(1):131-6.

Bauer AW, Kirby WMM, Sherris JC, Truck M(1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45(4):493-496.

Benbrook CM (2002). "Antibiotic Drug use in U.S. Aquaculture." Institute for Agriculture and Trade Policy. Available at: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.188.5770&rep=rep1&type=pdf.

Carmeli Y, Troillet N, Karchmer AW, Samore MH (1999). Health and Economic Outcomes of Antibiotic Resistance in *Pseudomonas aeruginosa*. Arch. Intern. Med.159(10):1127-1132.

Chanda S, Kaneria M, VaghasiyaY (2011). Evaluation of antimicrobial potential of some Indian medicinal plants against some pathogenic microbes. Indian J. Nat. Prod. Resour. 2: 225-228.

Clinical and Laboratory Standard Institute (CLSI) guidelines (2011). http://vchmedical.ajums.ac.ir/_vchmedical/documents/CLSI%202011.pdf

Favero MS, Carson LA, Bond WW, Peterson NJ (1971). *Pseudomonas aeruginosa*: Growth in Distilled Water. Science 173(3999):836-838.

Foley SL, White DG, McDermott PF, Walker RD, Rhodes B, Fedorka-Cray PJ, Simjee S, Zhao S (2006) Comparison of subtyping methods for differentiating *Salmonella enterica* serovar *Typhimurium* isolates obtained from food animal sources. J. Clin. Microbiol. 44:3569-3577.

Gad GF, el-Domany RA, Ashour HM (2008). Antimicrobial susceptibility profile of *Pseudomonas aeruginosa* isolates in Egypt. J. Urol. 180(1):176-81.

Gales AC, Jones RN, Turnidge J, Rennie R, Ramphal R (2001) Characterization of *Pseudomonas aeruginosa* isolates: Occurrence Rates, Antimicrobial Susceptibility Patterns, and Molecular Typing in the Global SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin.Infect. Dis. 32: S146-S155.

Hardalo C, Edberg SC (1997). *Pseudomonas aeruginosa*: Assessment of risk from drinking water. Crit. Rev. Microbiol. 23: 47–75.

Hobbs BS, Roberts D (1993).Bacterial and other microbial agents of food poisoning and food- borne infection. Food poisoning and food hygiene. Edward Arnold, London.pp: 26-50.

Hossain MG, Saha S, Rahman MM, JK, Mamum AA (2013). Isolation, Identification and Antibiogram Study of *Pseudomonas aeruginosa* from cattle in Bangladesh. J. Vet. Adv. 3(7):180-185.

Kibret M, Abera B (2011). Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. Afr. Health Sci. 11(3):40-45.

Mannan A, Shohel M, Rajia S, Mahmud NU, Kabir S, Hasan I. (2014). A cross sectional study on antibiotic resistance pattern of *Salmonella typhi* clinical isolates from Bangladesh. Asian Pac. J. Trop. Biomed. 4(4):306-311.

Miller S, Pegues D (2005). *Salmonella* Species, including *Salmonella typhi* in Mandell, Douglas and Bennetts Principle and Practice of Infectious Diseases, 6th Edition Chapter 220: 2636-2650 https://www.doody.com/rev400images/pdf/2010/9780443068393.pdf

McGowan JE (2006). Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. Am. J. Med. 119(6):S29-S36.

Nadeem SG, Quasmi SA, Afaque F, Saleem M, Hakim ST (2009). Comparison of the in vitro susceptibility of clinical isolates of Pseudomonas aeruginosa in a local hospital setting in Karachi, Pakistan. Br. J. Med. Pract. 2(4):35-39.

Nasreen M, Sarker A, Malek MA, Ansaruzzaman MD (2015). Prevalence and Resistance Pattern of *Pseudomonas aeruginosa* isolated from surface water. Adv. Microbiol. 5(01):74-81.

Reuben RC, Owuna G (2013).Antimicrobial Resistance Patterns of Escherichia Coli O157:H7 from Nigerian Fermented Milk Samples in Nasarawa State, Nigeria. Int. J. Pharm. Sci. Invent. 2(3):38-44.

Sapkota A, Sapkota AR, Kucharski M, Burke J, Mckenzie S, Walker P, Lawrence R (2008). Aquaculture practices and potential human health risks: Current Knowledge and future priorities. Environ. Int. 34(8):1215-1226.

Schmidt AS, Bruun MS, Dalsgaard I, Pedersen K, Larsen JL (2000). Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four Danish rainbow trout farms. Appl. Environ. Microbiol. 66 (11):4908-15.

Sivanmaliappan TS and Sevanan M. (2011). Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* from Diabetes Patients with Foot Ulcers. Int. J. Microbiol. Available at: http://dx.doi.org/10.1155/2011/605195

Smith P, Hiney MP, Samuelsen OB (2003). Bacteria resistance to antimicrobial agents used in fish farming: a critical evaluation of method and meaning. Ann. Rev. Fish Dis. 4:273-313.

Streteva T and Yordanov D (2009). *Pseudomonas aeruginosa* - a phenomenon of bacterial resistance. J. Med. Microbiol. 58(9):1133-1148.

Todar K (2008). *Pseudomonas aeruginosa*. Todar's Online Textbook of Bacteriology pp 1. Available at: http://textbookofbacteriology.net/pseudomonas.html

Ugur A, Ceylan O, Ashm, B (2012). Characterization of *Pseudomonas Spp*. from seawater of the Southwest coast of Turkey. J. Biol. Environ.Sci. 6(16):15-23.