"Studies on occurrence of multiple antibiotic resistant bacteria in fish pond water and sediment in Offatedo, Osogbo Osun state, Nigeria"

Original Research Article

STUDIES ON OCCURRENCE OF MULTIPLE ANTIBIOTIC RESISTANT BACTERIA IN FISH POND WATER AND SEDIMENT IN OFFATEDO, OSOGBO OSUN STATE, NIGERIA

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ABSTRACT

To control infectious diseases, strategies such as vaccination and use of antimicrobial agents are employed in aquaculture as in other areas of animal production. Use of 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. This study was carried out with the aim of isolating and characterizing multiple antibiotic resistant bacteria from water and sediment of two fish ponds in Offatedo, Osogbo Nigeria. Sediment and water samples were collected with sterile sample bottle which was placed against the movement of the water current. One milliliter of pond water and 1g of pond sediment was suspended into 9ml of sterile distilled water and serially diluted up to 10^{-6}. Muller Hinton Agar (MHA) was prepared in two 250ml conical flasks and each were supplemented separately with $20\,\mu g/ml$ of Oxytetracycline and Doxycycline respectively, Aliquot of 0.5ml each of serially diluted pond water and sediment was plated out in four replicates and incubated at 35^0c for 24hours. The isolated bacteria were characterized according to the procedure described in Bergey Manual of bacteriology.

Following Identification, minimum inhibitory concentration (MICs) were determined for all Isolates using agar dilution method as described by the European Committee for Antimicrobial Susceptibility Testing. The isolates obtained from the experimental pond and control pond reveal that there is high population of tetracycline resistant bacteria (TRB) in water

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and sediment of both ponds. This may be as a result of the use of antibiotics in the pond which creates a selective pressure on the bacteria flora of the pond. The high level of resistance to the antibiotics used in this study may be as a result of adaptation of the bacteria species to stress imposed by antimicrobial agents.

In the light of this study, to control and prevent effectively the development and spread of antimicrobial resistance from fish farms in Nigeria and environmental hazards associated with residues of antibiotics used in fish production. There should be reduction in the use of antimicrobial agent in aquaculture production. Also there should be a regulatory framework at the national level to ensure registration, approval, monitoring and control the use of antimicrobial agent in aquaculture for public health safety.

INTRODUCTION

Aquaculture is developing rapidly in many regions of the world, and aquaculture products constitute an important food supply with increasing economic importance (Ole *et al*., 2009). To control infectious diseases, strategies such as vaccination and use of antimicrobial agents are employed in aquaculture as in other areas of animal production. Use of 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*et al*., 2006). Similarly, residues of antibiotic used in aquaculture have been reported to be toxic to non-target organisms present in the pond ecosystem.

It is well documented that consumption of antibiotics in food can generate problems of allergy and toxicity (Cabello, 2004),allergy to antibiotic and problems of toxicity can also be created for unprotected workers in the aquaculture industry through the use of large amounts of antibiotics that come in contact with the skin, intestine and bronchial tract as workers medicate food, (in feed mills), distribute and administer medicated food to fish (Li *et al*., 2003).The apparent increase in the occurrence of antibiotics resistance among bacteria from various areas of animal production during the past years and its possible implication for public health have in many countries led to an intensified surveillance for bacteria resistance (Anna *et al*., 2008).

In Nigeria, the common antibiotics reportedly used in aquaculture industry in Southwestern and Northern regions include Furazolidone, Streptomycin, Erythromycin, Tetracycline, Ampicillin, Oxytetracycline, Chlortetracycline, Penicillin, Sulphonamides, Colistin, Tylosin, Neomycin and Nitrofurantoin banned by National Agency for food and Drug

Administration and control (NAFDAC,1996) because of its mutagenic potentials.(Adelowo *et al.*, 2009; Adelowo and Fagade, 2009).This study was carried out with the aim of isolating and characterizing multiple antibiotic resistant bacteria from water and sediment of two fish ponds in Offatedo, Osogbo Nigeria

MATERIALS AND METHODS

LOCATION AND SELECTION OF SITES

The two ponds used for this study are located at Offatedo, Ede Road, Osun State, Nigeria. These ponds were selected because of regular administration of Oxytetracycline in control and prevention of bacterial infections in fish. A pond where antibiotic was not being used as at the time of the study was selected as a control. The control pond is also located at another area of Offatedo.

BRIEF HISTORY OF THE FISH FARM SITE

The study farm started operation in March, 2001 with two ponds. The site is located in a secluded area of Offatedo along Osogbo road. The control farm started operation in September, 2006 with one large pond and small pond created close to the residential house occupied by the farm owner. The site is located at the interior part of Offatedo town along Osogbo, Osun State.

COLLECTION OF SEDIMENT AND WATER SAMPLES

Sediment and water samples were collected with sterile sample bottle which was placed against the movement of the water current. Similarly, sediment sample was obtained from the bottom of the pond by immersion of the sterile sample bottle into the bottom of the pond.

ISOLATION AND CHARACTERIZATION OF BACTERIA

One milliliter of pond water and 1g of pond sediment was suspended into 9ml of sterile distilled water and serially diluted up to 10^{-6}. Muller Hinton Agar (MHA) was prepared in two 250ml conical flasks and each were supplemented separately with 20 $\mu g/ml$ of Oxytetracycline and Doxycycline respectively, Aliquot of 0.5ml each of serially diluted pond water and sediment was plated out in four replicates and incubated at 35^0c for 24hours. Distinct colonies of tetracycline resistant bacteria growing on each plate were selected and purified by sub culturing on fresh MHA plates. The pure cultures were then stored on MHA slants at 4^oc in the refrigerator. The isolated bacteria were then sent for Identification at the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State.

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DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

Following Identification, minimum inhibitory concentration (MICs) were determined for all Isolates using agar dilution method as described by the European Committee for Antimicrobial Susceptibility Testing (EUCAST, 2000) and results interpreted by the MIC breakpoint for Enterobacteriacea

200ml of Muller Hinton Agar were prepared in six different conical flasks, sterilized at 121^0c for 15 minutes cooled to 40^oc in a water bath and then supplemented with the following range of concentration of oxytetracycline viz: 32µg/ml, 64µg/ml, 128µg/ml, 256µg/ml, 512µg/ml, and 1024µg/ml respectively.

Similarly, six 200ml of MHA were prepared, sterilized at 121^0c for 15 minutes and supplemented with the following range of concentration of Doxycycline viz: 32µg/ml, 64µg/ml, 128µg/ml, 256µg/ml, 512µg/ml, and 1024µg/ml respectively. The above prepared MHA supplemented with a concentration of oxytetracycline and Doxycycline were then poured into Petri dishes and allowed to solidify. Each of the Isolates was streaked on MHA plates with each concentration of test antibiotics. The MIC for each Isolates was determined as the lowest concentration of the antimicrobial agent to inhibit bacteria growth.

ANTIBIOTIC SUSCEPTIBILITY TEST

The susceptibility of the bacteria Isolates was assayed using disc diffusion method as described by the British Society for Antimicrobial Chemotherapy (BSAC) (Andrews, 2008). A suspension of each Isolate in normal saline was compared with 0.5 McFarland standards to standardize the inoculums. The suspension was used to Inoculate MHA Plates using sterile swabs sticks and antibiotics disc containing septrin (30µg), Chloramphenicol (30µg), Augmentin (25µg), Gentamycin (10µg), Pefloxacin (10µg), Tarivid (30µg) and Streptomycin (10µg) was aseptically layered on the surface of the plates. The plates were incubated at 35^0c for 24 hours. After incubation, the zone of growth inhibition around each disc was measured and used to classify the organisms as sensitive or resistant to an antibiotic according to the interpretive standard of the Clinical and Laboratory Standard Institute (CLSI, 2005).

RESULTS AND DISCUSSION

Results of tetracycline resistant bacteria count in cfu/ml on plates supplements with Oxytetracycline and Doxycycline and their standard deviation are presented in Table 1.The high level of total antibiotic resistant bacteria (TARBC) in water and sediment of control and

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experimental pond may be as a result of selective pressure exerted by an antimicrobial agent which may favor the emergence and spread of resistance among aquatic bacteria (Gordon *et al*; 2006).

A total of 42 bacteria isolated from the study pond were identified by biochemical tests, in Table 2, it can be seen that 12 of the isolates were *Pseudomonas aeruginosa* , *Proteus vulgaris* had 5 strains, *Klebsiellaedwardsii* had 8 strains *Morganellamorganii* had 16 strains and *Proteus mirabilis* had one strain respectively

The results of the minimum Inhibitory concentration of Oxytetracycline and Doxycycline are presented in Tables 3 and 4. Among bacteria isolated from the experimental pond, the least MIC was <32 μg/ml while the highest is 1024 μg/ml and 512 μg/ml for Oxytetracycline and Doxycycline respectively. However, among the isolates from the control pond, the MIC ranged between <32 μg/ml and 1024 μg/ml and 512 μg/ml for Oxytetracycline respectively.

The result of antimicrobial susceptibility test of 42 isolates from both experimental pond and control pond are presented in Table 5. It showed that 54.7% of the total isolates were resistant to Streptomycin, 57.1% were resistant to Amoxicillin, 42.8% were resistant to Chloramphenicol, 40.4% were resistant to Sparfloxacin, Ciprofloxacin, Augmentin, Pefloxacin and Tarivid while 38.9% are 19% were resistant to Septrin and Gentamycin respectively.

Table 1: Total tetracycline resistant bacteria count in cfu/ml on plates supplemented with Oxytetracycline and Doxycycline

Antibiotics	Samples	Experimental pond	Control pond
Oxytetracycline	Water	1.51×10^8 (10.5)	9.5×10^6 (3.5)
	Sediment	5.45×10^7 (17.5)	1.75×10^7 (0.5)
Doxycycline	Water	9.1×10^7(15.0)	3.5×10^6 (0.5)
	Sediment	3.0×10^6(3.0)	1.5×10^6 (0.5)

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Table 2: Biochemical characterization of the bacterial isolates and their identification

ISOLATE CODE	CELL SHAPE	GRAM REACTION	CATALASE	TSI REACTION	SIM REACTION	CITRATE UTILIZATION	MR	VP	GLUCOSE	MALTOSE	MANNITOL	SUCROSE	LACTOSE	O-F Or H&L	NITRATE REDUCTION	POSSIBLE IDENTIFICATION ORGANISM
P1	SR	-	++	NC NCNC	-+-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Pseudomona aeruginosa*
P2	MLR	-	+++	YG NC NC	+++	-	+	+	YG	YG	NC	YG	NC	F	+	*Proteus vulgaris*
P3	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P4	SR	-	++	NC NCNC	-+-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Ps. aeruginosa*
P5	SR	-	++	NC NCNC	-+-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Ps. aeruginosa*
P6	LR	-	+++	Y NC NC	---	-	-	+	Y	NC	NC	NC	NC	F	+	*Klebsiellaedwardsii*
P7	SR	-	+	NC NCNC	-+-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Ps. aeruginosa*
P8	SR	-	++	NC NCNC	-+-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Ps. aeruginosa*
P9	LR	-	+++	Y NC NC	---	-	-	+	Y	NC	NC	NC	NC	F	+	*Kl. edwardsii*
P10	SR	-	++	NC NCNC	-+-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Ps. aeruginosa*
P11	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P12	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P13	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P14	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P15	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*

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P16	SR	-	++	NC NCNC	-+-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Ps. aeruginosa*
P17	LR	-	++	Y NC NC	---	-	-	+	Y	NC	NC	NC	NC	F	+	*Kl. edwardsii*
P18	LR	-	+++	Y NC NC	---	-	+	+	Y	NC	NC	NC	NC	F	+	*Kl. edwardsii*
P19	LR	-	++	Y NC NC	---	-	+	+	Y	NC	NC	NC	NC	F	+	*Kl edwardsii*
P20	SR	-	++	NC NCNC	-+-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Ps. aeruginosa*
P21	SR	-	+	NC NCNC	-+-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Ps. aeruginosa*
P22	MLR	-	+	Y NC +	-++	-	+	+	Y	NC	NC	Y	NC	F	+	*Pr. mirabilis*
P23	MLR	-	+++	YG NC +	+++	-	+	+	YG	YG	NC	YG	NC	F	+	*Pr. vulgaris*
P24	SR	-	++	NC NCNC	++-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Ps. aeruginosa*
P25	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Pr. morganella*
P26	LR	-	+++	Y NC NC	---	-	-	+	Y	NC	NC	NC	NC	F	+	*Kl. edwardsii*
P27	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P28	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P29	SR	-	++	NC NCNC	++-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Ps. aeruginosa*
P30	LR	-	+++	Y NC NC	---	-	-	+	Y	NC	NC	NC	NC	F	+	*Kl. edwardsii*
P31	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P32	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P33	MLR	-	+++	YG NC +	+++	-	+	+	YG	YG	NC	YG	NC	F	+	*Pr. Vulgaris*
P34	LR	-	++	Y NC NC	---	-	+	+	Y	NC	NC	NC	NC	F	+	*Kl. Edwardsii*

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P35	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P36	MLR	-	+++	YG NC +	+++	-	+	+	YG	YG	NC	YG	NC	F	+	*Pr. Vulgaris*
P37	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P38	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P39	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P40	MLR	-	++	YG NC NC	++-	-	+	+	YG	NC	NC	NC	NC	F	+	*Morganellamorgani*
P41	SR	-	+	NC NCNC	-+-	-	-	-	NC	NC	NC	NC	NC	OX	+	*Ps. aeruginosa*
P42	MLR	-	+++	YG NC +	+++	-	+	+	YG	YG	NC	YG	NC	F	+	*Pr. Vulgaris*

LEGEND

SR-   **short rod**   **MLR** **-**   **medium long rod**   **LR-**   **long rod**   **SIM -**   **+**

YG-   **acid and gas production**   **Y-acid production only**   **NC-** **no change**   **TSI-**   **+++**

F-   **Fermentative**   **OX-**   **oxidative**

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Table 3: Minimum inhibitory concentration (MIC in μg/ml) of organisms from the Experimental pond.

Isolate Code	Oxytetracycline	Doxycycline
P1 *Pseudomonasaeruginosa*	512	<32
P2 *Proteusvulgaris*	1024	64
P3. *Morganellamorganii*	512	64
P4. *Ps. aeruginosa*	512	64
P5. *Ps. aeruginosa*	512	64
P6. *Klebsiellaeduwardsii*	512	<32
P7. *Ps. aeruginosa*	64	<32
P8. *Ps. aeruginosa*	256	<32
P9. *Kl. edwardsii*	<32	<32
P10. *Ps. aeruginosa*	256	<32
P11. *Morganellamorganii*	512	128
P12. *Morganellamorganii*	256	<32
P13. *Morganellamorganii*	512	<32
P14. *Morganellamorganii*	512	<32
P15. *Morganellamorganii*	256	64
P16. *Ps. aeruginosa*	512	512
P17. *Kl. edwardsii*	<32	<32
P18. *Kl. edwardsii*	512	64
P19. *Kl. edwardsii*	512	512
P20. *Ps. aeruginosa*	512	512
P21. *Ps. aeruginosa*	128	512
P22. *Pr.mirabilis*	<32	<32
P23. *Pr. vulgaris*	<32	<32

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Table 4; Minimum Inhibitory concentration (MIC in μg/ml) of organisms from control pond.

Isolate Code	Oxytetracycline	Doxycycline
P24 *Ps. aeruginosa*	512	<32
P25 *Morganellamorganii*	256	64
P26 *Kl. edwardsii*	<32	<32
P27 *Morganellamorganii*	1024	64
P28 *Morganellamorganii*	128	<32
P29 *Ps. aeruginosa*	512	64
P30 *Kl. edwardsii*	128	<32
P31 *Morganellamorganii*	512	128
P32 *Morganellamorganii*	1024	128
P33 *Pr. vulgaris*	1024	128
P34 *Kl. edwardsii*	64	64
P35 *Morganellamorganii*	256	256
P36 *Pr. vulgaris*	64	<32
P37 *Morganellamorganii*	1024	128
P38 *Morganellamorganii*	256	<32
P39 *Morganellamorganii*	512	128
P40 *Morganellamorganii*	64	64
P41 *Ps. aeruginosa*	<32	<32
P42 *Pr. vulgaris*	512	128

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Table 5: Antimicrobial susceptibility of the isolated bacteria

Antibiotic	Susceptibility of bacteria Isolate		
	R	**I**	**S**
SXT	16 (38.9%)	6 (14.2%)	20(47.69%)
CH	18 (42.8%)	2 (4.7%)	22 (52.3%)
SP	17 (40.4%)	-	25 (59.5%)
CPX	17 (40.4%)	1 (2.3%)	24 (57.1%)
AM	24 (57.1%)	6 (14.2%)	12 (28.5%)
AU	17 (40.4%)	3 (7.1%)	22 (52.3%)
GN	8 (19%)	-	34 (80.9%)
PEF	17 (40.4%)	-	25 (59.5%)
OFX	17 (40.4%)	-	25 (59.5%)
S	23 (54.7%)	-	19 (45.2%)

R: Resistant, I: Intermediate, S: Sensitive, n: Total number of Isolates, SXT; Septrin, CH: Chloramphenicol, SP: Sparfloxacin, CPX: Ciprofloxacin, AM: Amoxacilin, GN: Gentamycin, PEF: Pefloxacin, OFX: Tarivid, S: Streptomycin, Au: Augmentin.

DISCUSSION

In Tables 2, the total antibiotic resistant bacteria counts in experimental pond water sample was 1.51×10^8 cfu/ml (Oxytetracycline) with the standard deviation (SD) of 10.5 and 9.1×10^7 cfu/ml (Doxycycline) with S.D of 15.0. Similarly, the antibiotic resistant bacteria counts in the sediment are 5.45×10^7 cfu/ml (Oxytetracycline) with SD of 17.5 and 3.0×10^6 cfu/ml (Doxycycline) with SD of 3.0.

In the same vein, the in control pond water sample was 9.5×10^6 cfu/ml (Oxytetracycline) with SD of 3.5 and 3.5×10^6 cfu/ml (Doxycycline) with SD of 0.5 respectively. Likewise, the in control sediment sample was 1.75×10^6 cfu/ml (Oxytetracycline) with SD 0.5 and 1.5×10^6 cfu/ml (Doxycycline with SD 0.5 respectively. The high level of in water and sediment of pond treated with antibiotic may be as a result of selective pressure exerted by the antibiotics used in the pond. This is in line with the report of Gordon *et al.*, (2006) that depending on the concentration,

contaminating antimicrobials may exert a selection pressure and may favor the emergence and spread of resistance among aquatic bacteria.

Antibiotic resistant bacteria isolates were sent for identification and the results of the identification are shown in Table 4.It can be seen that nine (9) isolates were *Ps. aeruginosa*, two (2) were *Pr. vulgaris,* five (5) *Kl. edwardsii,* six (6) *M. Morganii* and one (1) is *Pr. mirabilis,* all of which were isolated from the experimental pond. Similarly, isolates from the control pond were identified as *Ps. aeruginosa* (3), *Pr. vulgaris* (3), *Kl. edwardsii* (3), and *M. Morganii* (10) respectively. Except for *Pr. mirabilis* is found among isolates from the experimental pond, species composition of bacteria from control and experimental ponds have found to be similar. This result is in conformity with the report of Sudeshna and Timothy (2007) who reported that bacteria belonging to the family of Enterobacteriaceae were isolated from fish farms. Other bacteria isolates that have been reported to be isolated from aquatic environment, especially from fish pond environment, includes *Salmonella* spp, *Streptococcus inine, Vibrovulnificus, Vibro cholera, Escherichia coli*, (Akinbowale*et al*., 2006).

The results obtained in the determination of minimum inhibitory concentration (MIC) of Oxytetracycline and Doxycycline showed that MIC of Oxytetracycline of 23 isolates from experimental pond ranged between <32µg/ml to 1024µg/ml while the MIC for Doxycycline ranged from <32µg/ml to 512µg/ml. Similarly, in Table 5, the result of MIC of Oxytetracycline of 19 isolates from control pond ranged from <32µg/ml to 1024µg/ml while the MIC for Doxycycline of 19 isolates from the same pond ranges from <32µg/ml to 256µg/ml respectively. This high-level resistance to the two antimicrobial agents used in this study may be as a result of the adaptation of the bacteria species to the stress imposed by exposure to antimicrobial agents either used in the ponds or produced by other bacteria in a pond ecosystem.

The accumulation of surplus antimicrobial and antimicrobial residues may occur in integrated fish farms where the ponds are only rarely emptied at the time of fish harvest, such an accumulation has been reported to establish selective pressure favoring selection and growth of antimicrobial resistant bacteria (Andreas *et al*., 2002). This may be the reason why some of the bacteria isolates used for this study were able to resist all the tested antibiotics.

In the same vein, the use of antimicrobial as growth promoters in animal husbandry has been linked to certain antimicrobial resistance patterns among human bacteria, suggesting that there is a possible flow of antimicrobial resistance genes between animal and human pathogens.

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Potential transfer of resistant bacteria and resistance genes from aquaculture environment to human can occur through direct consumption of antimicrobial resistant bacteria present in fish and associated products (Andreas *et al.,*2002) and this can lead to an increase in the number of infections, an increased frequency of treatment failure and increased severity of infection. Apparently, the most effective means to prevent and control the development and spread of antimicrobial resistance is to reduce the use of antimicrobial agents in aquaculture, to arrive at effective prevention and control of the use of antimicrobial agents in aquaculture, similar elements are needed in aquaculture as in another area of animal production.

CONCLUSION

The isolates obtained from the experimental pond and control pond reveal that there is a high population of tetracycline resistant bacteria (TRB) in water and sediment of both ponds. This may be as a result of the use of antibiotics in the pond which creates a selective pressure on the bacteria flora of the pond. The TRB isolated from the ponds were identified as *Pseudomonas aeruginosa* (n=12), *Morganella morganii (n=16)*, *Klebsiellaedwardsii (n=8)*, *Proteus vulgaris (n=5)* and *Proteus mirabilis* (n=1) respectively.

Minimum inhibitory concentration (MIC) of Oxytetracycline and Doxycycline is quite high. Among the 23 isolates of Oxytetracycline from the experimental pond, MIC ranged between<32µg/ml to 1024µg/ml while the MIC of Doxycycline ranged from <32 µg/ml to 512 µg/ml. Similarly, the MIC of Oxytetracycline from control pond ranged from <32 µg/ml to1024 µg/ml while the MIC for Doxycycline from the same pond ranged from <32µg/ml to 256 µg/ml respectively. The high level of resistance to the two antibiotics used in this study may be as a result of the adaptation of the bacteria species to stress imposed by antimicrobial agents.

The isolates from both ponds exhibited multiple resistances to other antibiotic. For example, *Pr. vulgaris* (P_2, P_{33}, P_{40}) exhibited resistance to Septrin (30µg), Chloramphenicol (30µg), Sparfloxacin (10µg), Ciprofloxacin (10µg), Amoxicillin (30µg), pefloxacin (30µg), Ofloxacin (10µg) and Streptomycin (30µg). Similarly, *M. morganii* (P_3, P_{11}, P_{13}, P_{27}, P_{31}, P_{32}, P_{37}) showed resistance to Septrin (30µg), Chloramphenicol (30µg) Sparfloxacin(10µg). Ciprofloxacin (10µg), Amoxacillin (30µg), Pefloxacin (30µg), Ofloxacin (10µg) and Streptomycin (30µg), *Ps. aeruginosa* (P_{16}, P_{20} & P_{21}) were resistant to all the ten antibiotic used in this study. *Kl. edwardsii*(p_{19}) also exhibited resistant to all antibiotics while *Pr. mirabilis* was sensitive to all the antibiotics.

RECOMMENDATION

In the light of this study, to control and prevent effectively the development and spread of antimicrobial resistance from fish farms in Nigeria and environmental hazards associated with residues of antibiotics used in fish production.

1 There should be a reduction in the use of the antimicrobial agent in aquaculture production.

2 There should be a regulatory framework at the national level to ensure registration, approval, monitoring and control the use of the antimicrobial agent in aquaculture for public health safety.

REFERENCES

o Adelowo O. O. and Fagade O. E. (2009): The tetracycline resistance gene test 39 is present in both Gram – negative and Gram – positive bacteria from a polluted river, southwestern Nigeria.

o Adelowo O. O, Ojo F.A and Fagade O.E (2009); Prevalence of multiple Antibiotic resistance among bacterial isolates from selected poultry waste dumps in Southwestern Nigeria. World j Microbiol Biotechnol, 25; 713-719.

o Akinbowale O. L. Peng H and Barton M. D. (2006): Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia j Appl. Microbial 100:1103.

• Andrew J.M (2008); BSAC standardized disc Susceptibility testing method (version 7). J. Antimicrobio. Chemother.62, 256-278.

o Anna M F, Veruscka M, ElisabethaSuffredini, Loredana C0221 and Luciana Croci (2008): Evaluation of antibacterial resistance in vibro strains Isolated from imported, seafood and Italian aquaculture settings 1:164-170.

• Cabello, F.C. (2004): Antibiotics and aquaculture in ehile;[implications for human and animal health. Rev Med chi 132: 1001-1006.

• Clinical and laboratory standards institute (CLSI), 2005; Performance standards for antimicrobial Susceptibility testing; Fifteenth international supplement M100-S15. CLSI, Wayne, PA, USA.

• European committee for antimicrobial susceptibility testing (2000); Determination of minimum inhibitory concentration (MICs) of antibacterial Agents by Agar Dilution. Clinical

Bolaji A. S. *et. al., Jour. Sci. Res. A. Sci.*3, No.1, (2017): 01-15

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Microbiology and infection 6(9); 509-515.

- Gordon, L, E Giraud, J. P Ganiere, F. arman, A, Bouju-albert, N. DE Placotte, C, Mangion, and H, Le Bris, (2006); Antimicrobial resistance survey a river receiving effluents from freshwater fish farms. J. Appl. Microbiol. 102,1167-1176.

- Lihehaug, A. Lunestard, B.T., and Grave , K. (2003): Epidemiology of bacterial diseases in Norwegian aquaculture a description based on antibiotic prescription data for the ten year period 1991 to 2000. Dis Aquatic Org. 53:115 - 125.

- Ole, E., Heuer, Hilde kruse, Kari grave, P. Collignon, IddyaKarunasagar, and Fredarick I. Angulo (2009): Human Health consequences of use of Antimicrobial agents in aquaculture: 1248. :49.

o Sudenshna Ghosh and Timothy M. Lapara (2007):The effect of sub therapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria is not journal (2007), 1-3.