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Metal contamination and coexistence of metal and antibiotic resistance in *Vibrio* species recovered from aquaculture ponds with and without history of antibiotic usage in Southwest Nigeria

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Abstract

Background: The use of metal-containing and antimicrobial compounds is a common practice in aquaculture operations in Nigeria. Only a few studies have focused on the impact of these chemicals on metal and antibiotic resistance development in the bacterial flora of aquaculture. This study therefore sets out to fill this gap.

Results: Five metals, namely copper (Cu), zinc (Zn), lead (Pb), nickel (Ni) and chromium (Cr), were detected at different concentrations in the pond water samples, with most values obtained exceeding the maximum recommended national standard for fisheries and recreation water quality criteria set by the National Environmental Standards and Regulations Enforcement Agency (NESREA) in Nigeria. A total of 121 *Vibrio* spp. were recovered from the aquaculture pond water with *Vibrio cholerae* having the highest frequency (37) followed by *Vibrio parahaemolyticus* (36), *Vibrio vulnificus* (36) and *Vibrio alginolyticus* (12). Seventy-five isolates were recovered from ponds with history of antibiotic use, while the ponds with no recognized history of antibiotic use had forty-six isolates. The percentage resistance to antibiotics in the isolates was: ampicillin (88.5%), amoxicillin–clavulanate (44.3%), tetracycline (41%), trimethoprim–sulfamethoxazole (22.1%), chloramphenicol (15.6%), ciprofloxacin (11.5%), imipenem (5.7%), ceftazidime (4.1%) and gentamicin (0%). All the isolates tolerated all the metals to 400 µg/mL concentration while only the trio of Pb, Ni and Cr were tolerated beyond the concentration. Zn and Cu appeared to be the most toxic metals on *Vibrio* spp., with no isolate surviving beyond the 400 µg/mL concentration mark, while 57 of the 121 isolates survived Cr at the highest concentration (3200 µg/mL), making it the most tolerated of the metals.

Conclusion: This study reports a high level of metal contamination in the aquaculture ponds studied. The concentration of the metals exceeded the maximum permissible limits in Nigeria. Also, there was coexistence of metal and antibiotic resistance in the bacteria recovered from the aquaculture ponds. There is a need to regulate the use of chemicals, especially antibiotics and some biocidal compounds, to clamp down on the introduction of metals and antibiotics into the aquaculture systems, as these chemicals could facilitate the development of resistance traits in aquaculture bacteria by creating a selective pressure in the aquaculture environment.

Keywords: Antibiotic resistance, Antibiotic usage, Aquaculture ponds, *Clarias gariepinus*, Heavy metal contamination, Metal resistance, *Vibrio* spp.

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Background

Nowadays, aquaculture is regarded as one of the major contributors to food security in agriculture worldwide. It ranks as one of the fastest growing sectors in the agricultural industry in some countries of the world, with freshwater aquaculture dominating the total aquaculture production. Aquaculture serves as a source of quality food supply at low cost to Africans, generates income for farming and fishing households and plays a key role in the economy of many nations (Kitessa et al. 2014; Pradeepkiran 2019).

Currently, there has been a great loss in the aquaculture industry as a result of the occurrence of diseases, including vibriosis caused by *Vibrio* spp. which is one of the most common diseases that affect shelled organisms: molluscs and crustaceans. *Vibrio* belong to the family Vibrionaceae. They are Gram-negative, comma- or rod-shaped, facultative anaerobes (CDC 2014). They are commonly found in the aquatic habitats where they constitute a part of the natural microflora (Maheshwari et al. 2011). Most *Vibrio* species are pathogenic to human and have been linked with food-borne diseases such as diarrhea and cholera.

In order to prevent disease outbreak and loss in the aquaculture industry, the use of antibiotics has been employed. However, it has been established that the high rate of antibiotic-resistant bacteria in the aquatic environment is linked to the undue and unrestricted usage of antibiotics in aquaculture farming (Hui-Gao et al. 2018), resulting in the onset of resistance (Narendrakumar et al. 2019). Bacterial resistance to antibiotics is a genetic phenomenon caused by genes within the organisms that encode different biochemical mechanisms and prevent the action of these drugs, and these are known as antibiotic resistance genes (ARG) (Gonzalez-Candelas 2011). Resistance to antibiotics in the aquatic environment is even more serious because some bacteria have the ability to transfer their genes to others. There is a linkage between antibiotic and metal resistance in the environment, as it has been observed in some cases that the presence of heavy metals in the environment could trigger the resistance of microorganisms to antibiotics since both resistance genes are frequently located on the same mobile genetic elements (Hatha et al. 1993; McIntosh et al. 2008; Fang et al. 2019).

Metals occur in the natural environment and also as products of different anthropogenic activities. However, they can also be present as contaminants in fish feed, a scenario that has been reported by several authors. Kundu et al. (2017) reported the presence of elevated concentration of Cd, Fe, Na and Pb in fish feed obtained in Bangladesh, while the occurrence of Pb, Cr and Cd in fish feed was confirmed by the report of Sabbir et al.

(2018). Bacteria have developed very efficient and different mechanisms for tolerating heavy metal stress through either the presence of genes or other mobile genetic elements.

The use of antibiotics in aquaculture as growth promoters and for therapeutic purposes coupled with the presence of metals and antibiotics in fish feed could facilitate the development of antibiotic and metal resistance in bacteria resident in aquaculture ponds. Therefore, there is a need to investigate aquaculture ponds for the occurrence of metal- and antibiotic-resistant bacteria because of their predisposition to both metals and antibiotics, vis-à-vis determining the metal concentration of the selected ponds. This study aimed at isolating and characterizing *Vibrio* spp. from aquaculture ponds water samples and determining their resistance to both metals and selected antibiotics.

Methods

Description of study site, aquaculture ponds and sample collection

This study was carried out in Awotan, a suburban area in Ido Local Government Area, Ibadan, Oyo State, Nigeria. A total of twenty-six (26) earthen and concrete aquaculture ponds were sampled from five representative commercial fish farms in the area. The fish farms are located at Alakuta, Trailer park, Kelanko and Flash areas of the Awotan community and the species of fish being reared on all the farms is *Clarias gariepinus*. The pond type, water sources and location are given in Table 1. There is a history of antibiotic usage in some of the aquaculture ponds, and the main antibiotics used in their operation are ampicillin, streptomycin, enrofloxacin, tetracycline and chloramphenicol. In some of the aquaculture ponds, no information on antibiotic usage was provided at the time this study was executed and as a result, they were classified as ponds without a recognized or reported history of antibiotic usage. Water samples were collected from the aquaculture ponds in pre-cleaned sterile sample bottles weekly for a period of three months. The water samples were transported to Environmental Microbiology and Biotechnology laboratory, Department of Microbiology, University of Ibadan, Nigeria, in ice packs for processing. Samples were analyzed within three hours of collection.

Metal composition of the aquaculture water samples

The concentration of selected metals in the water samples was determined using the atomic absorption spectrophotometer (AAS) (UNICAM 929, London Atomic Absorption Spectrophotometer powered by SOLAAR software). Standards of Cu, Zn, Cd, Pb and Ni, with range of calibration from 0.2 to 1.0 mg/L, were prepared from

Table 1 Description of the aquaculture ponds used in this study

Aquaculture ponds	Pond type	Water source	Location	Antibiotic usage
Aq ponds 1–3	Concrete	Borehole, water well	Alakuta	No
Aq ponds 4–7	Concrete	River, stream	Alakuta	No
Aq ponds 8	Earthen	River	Alakuta	No
Aq ponds 9–12	Earthen	Stream	Trailer park	No
Aq ponds 13–19	Earthen	Stream	Kelanko	Yes
Aq ponds 20–26	Earthen	Stream	Flash	Yes

Aq: Aquaculture; Yes: recognized history of antibiotic use; None: no recognized history of antibiotic use

1000 mg/L stock solution analytes of the respective metal. The detection limit for each metal was 0.0001 mg/L. The cathode lamp of each metal was used in the analysis of the respective metal concentration in the standards (used as a control) and the sample filtrates (Hseu 2004).

Isolation of *Vibrio* spp.

The streak plate method was employed in the isolation of *Vibrio* spp. from the water samples on CHROMagar *Vibrio* (CHROMagar, France), a chromogenic medium, for the detection and isolation of *Vibrio* spp. Colonies presumptive of *Vibrio* spp. were selected and repeatedly subcultured to obtain pure isolates which were further confirmed using conventional methods. The working cultures were maintained on agar slant under refrigeration condition while the cultures for long-term storage were stored in 15% glycerol kept at -80°C .

Antibiotic susceptibility testing

The in-vitro susceptibility of the *Vibrio* isolates to nine selected classes of antibiotics was carried out using the disk diffusion method (CLSI 2018). The antibiotics used were gentamicin (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), tetracycline (30 μg), amoxicillin-clavulanate (30 μg), ceftazidime (30 μg), imipenem (30 μg), trimethoprim-sulfamethoxazole (23.75/1.25 μg) and ampicillin (30 μg). The zones of inhibition were measured and interpreted using the CLSI (2018) standard. Sensitive, intermediate and/or resistant phenotypes were reported according to the established breakpoints for *Vibrio* spp. Isolates showing resistance to three or more classes of antibiotics were classified as multidrug-resistant as described by Magiorakos et al. (2012).

Determination of minimum inhibitory concentration (MIC) of metals on the isolates

The determination of the MIC of selected metals on the isolates was carried out using the doubling concentration method. Appropriate volume of filter-sterilized solutions of prepared metals was incorporated into sterilized,

molten nutrient agar, mixed gently and poured into sterile plates. An 18–24 h old culture of the isolates was streaked on the metal-incorporated medium and incubated at $35 \pm 2^{\circ}\text{C}$. The plates were observed for growth for 48 h. The culture growing on a particular concentration was then transferred to the next higher concentration until no growth was observed. The concentration at which growth stopped was recorded as the MIC. The concentration of each heavy metal was doubled each time from an initial concentration of 6.25 $\mu\text{g/mL}$ to a final concentration of 3200 $\mu\text{g/mL}$ (Yu et al. 2016). The metal salts used in this study were NiCl_2 , CrCl_3 , PbCl_2 , CuSO_4 and ZnSO_4 .

Results

Metal concentration in aquaculture pond water samples

The metal concentration of water samples obtained from ten of the total twenty-six aquaculture ponds sampled in this study is shown in Table 2. With the exception of ponds 1–4 with no detectable amount of Ni, all the other metals assayed for were detected at different concentrations in the aquaculture ponds. The concentration of Zn was highest in pond 10 (2.82 mg/L), with ponds 9 and 10 having the highest Pb concentration of 0.29 mg/L. Pond 7 had the highest level of Cr at 2.11 mg/L, with Cu being highest at pond 8 (0.09 mg/L). Nickel was highest at pond 6 (0.80 mg/L). The concentration of Cu in the water samples ranged from 0.01 to 0.09 mg/L.

Frequency and distribution of *Vibrio* spp. in the aquaculture ponds

A total of one hundred and twenty-one isolates was obtained from the aquaculture ponds. The frequency of *Vibrio* species is as follows: *Vibrio cholerae* (37), *Vibrio parahaemolyticus* (36), *Vibrio vulnificus* (36) and *Vibrio alginolyticus* (12). The distribution of the *Vibrio* spp. according to the ponds with or without history of antibiotic usage is shown in Table 3. A total of 75 isolates were obtained from the ponds with confirmed history of antibiotic usage and 46 from the ponds without history

Table 2 Metal composition of water samples of ten selected aquaculture ponds (mg/L)

Metal	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6	Pond 7	Pond 8	Pond 9	Pond 10	NESREA limit
Cu	0.01 ± 0.00	0.01 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.09 ± 0.01	0.06 ± 0.01	0.01 ± 0.00	0.001
Zn	0.19 ± 0.04	0.22 ± 0.02	0.48 ± 0.04	0.15 ± 0.01	4.58 ± 0.03	0.89 ± 0.06	2.31 ± 0.05	1.96 ± 0.01	1.07 ± 0.03	2.82 ± 0.04	0.01
Pb	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.07 ± 0.01	0.19 ± 0.02	0.26 ± 0.02	0.19 ± 0.00	0.29 ± 0.02	0.29 ± 0.02	0.01
Ni	ND	ND	ND	ND	0.47 ± 0.03	0.80 ± 0.01	0.22 ± 0.00	0.28 ± 0.03	0.17 ± 0.02	0.39 ± 0.01	0.01
Cr	0.35 ± 0.00	0.87 ± 0.03	1.36 ± 0.11	1.14 ± 0.04	1.43 ± 0.02	1.14 ± 0.05	2.11 ± 0.13	2.63 ± 0.03	2.57 ± 0.00	1.79 ± 0.03	0.001 (Cr ⁶⁺)

Note: Each value is a mean of three replicate samplings. Ponds 1–5: ponds with history of antibiotic usage; Ponds 6–10: ponds without history of antibiotic usage

Cu copper, Zn zinc, Pb lead, Ni nickel, Cr chromium, ND Not detected, NESREA National Environmental Standards and Regulations Enforcement Agency

Table 3 Frequency of occurrence of *Vibrio* species in the aquaculture ponds

<i>Vibrio</i> spp.	Ponds with antibiotic usage	Ponds with no antibiotic history
<i>V. vulnificus</i>	21	15
<i>V. parahaemolyticus</i>	19	18
<i>V. cholerae</i>	23	13
<i>V. alginolyticus</i>	12	0
Total	75	46

of antibiotic usage. No *Vibrio alginolyticus* was isolated from the pond without history of antibiotic use.

Survival of the *Vibrio* spp. at increasing concentration of heavy metals

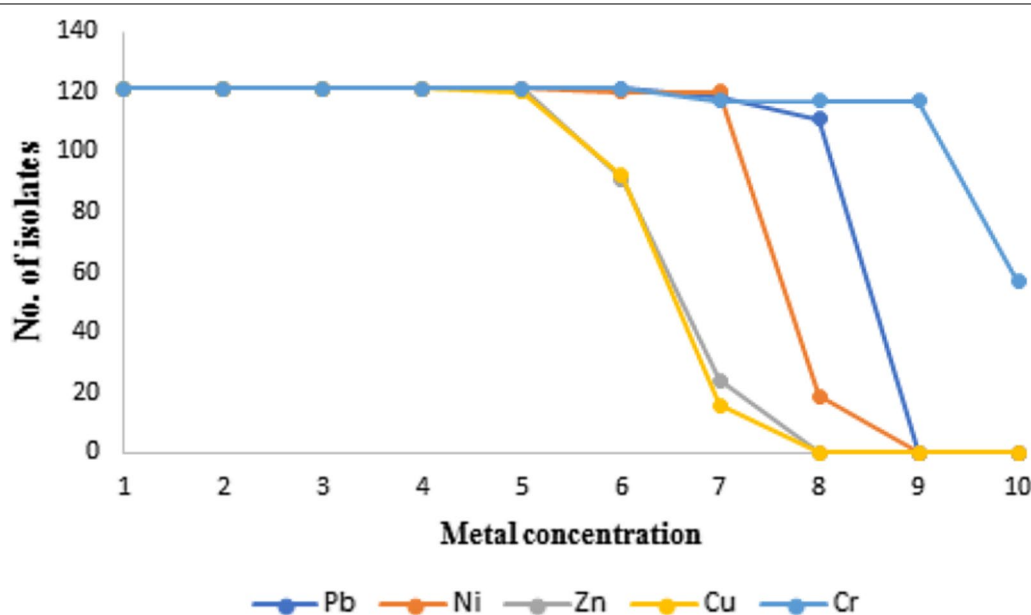
The survival of the *Vibrio* spp. isolated from aquaculture ponds on increasing concentration of five selected metals is shown in Fig. 1. There was 100% survival of organisms at 6.25 µg/mL to 50 µg/mL concentration of the metals. However, there was reduction in the rate of survival as the concentration increased. At 400 µg/mL concentration, 118 of the total 121 isolates were still surviving on Pb, 120 isolates on Ni, 117 isolates on Cr, 24 isolates and 16 isolates surviving, respectively, on Zn and Cu. Zn and Cu are the most toxic metals to the *Vibrio* spp. with none of the isolates surviving at 800 µg/mL concentration of the two metals, while Cr was the least toxic of the metals to the isolates, with 57 of the total 121 isolates surviving

at the highest concentration used in this study (3200 µg/mL).

Resistance of the *Vibrio* spp. to tested antibiotics

The resistance of the *Vibrio* spp. isolated from this study to the tested antibiotics is shown in Fig. 2. Of the total isolates, 88.5% were resistant to ampicillin and 44.3% showed resistance to amoxicillin–clavulanate. There was 41% resistance to tetracycline and 22.1% resistance to trimethoprim-sulfamethoxazole, while 15.6% of the total isolates showed resistance to chloramphenicol. No resistance was observed to gentamicin, and 11.5%, 5.7% and 4.1% of the total isolates showed resistance to ciprofloxacin, imipenem and ceftazidime, respectively.

The resistance to antibiotics in *Vibrio* spp. isolated from ponds with history of antibiotic usage and the ponds without a recognized history of the use of antibiotics is shown in Fig. 3. There was no resistance to gentamicin from isolates obtained from both groups, whereas there was 40% resistance to tetracycline in ponds with usage of antibiotics as against 52.2% in ponds without the use of antibiotics. There was 61.3%, 14.7%, 8% and 21.3% resistance to ampicillin, imipenem, ciprofloxacin and trimethoprim-sulfamethoxazole, respectively, in the ponds with history of antibiotic usage as against 73.9%, 6.5%, 21.7% and 23.9% resistance to the same sets of antibiotics in ponds without antibiotic history. To ceftazidime, chloramphenicol and amoxicillin–clavulanate, there was 5.3%, 9.3% and 46.7% resistance in ponds with antibiotic

**Fig. 1** Survival of isolated *Vibrio* spp. at increasing concentration of metals

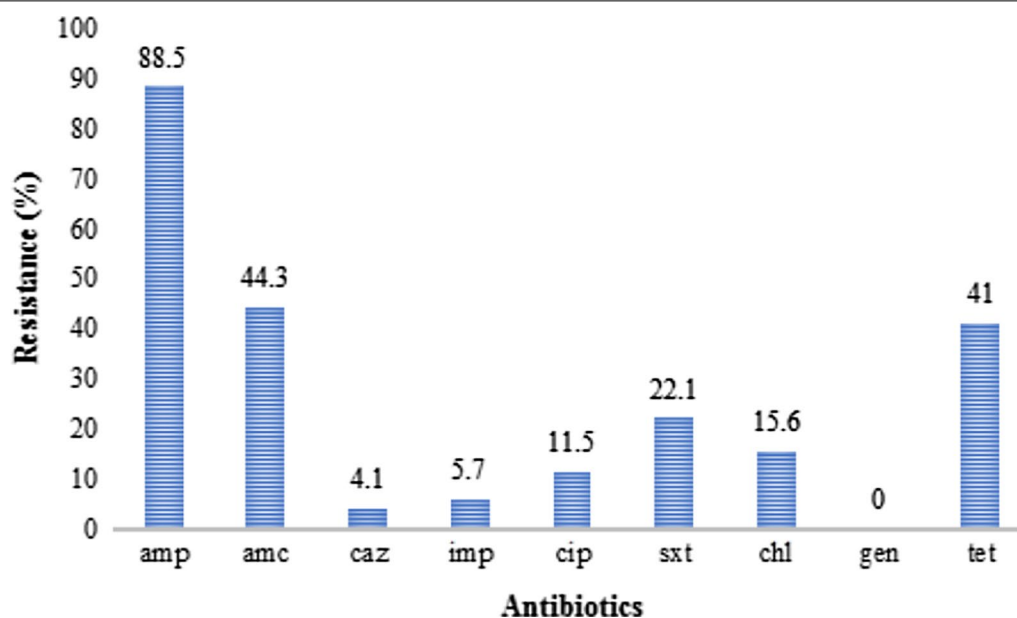


Fig. 2 Resistance of the *Vibrio* spp. to selected antibiotics. KEY: amp: ampicillin; amc: amoxicillin-clavulanate; caz: ceftazidime; imp: imipenem; cip: ciprofloxacin; sxt: trimethoprim-sulfamethoxazole; chl: chloramphenicol; gen: gentamicin; tet: tetracycline

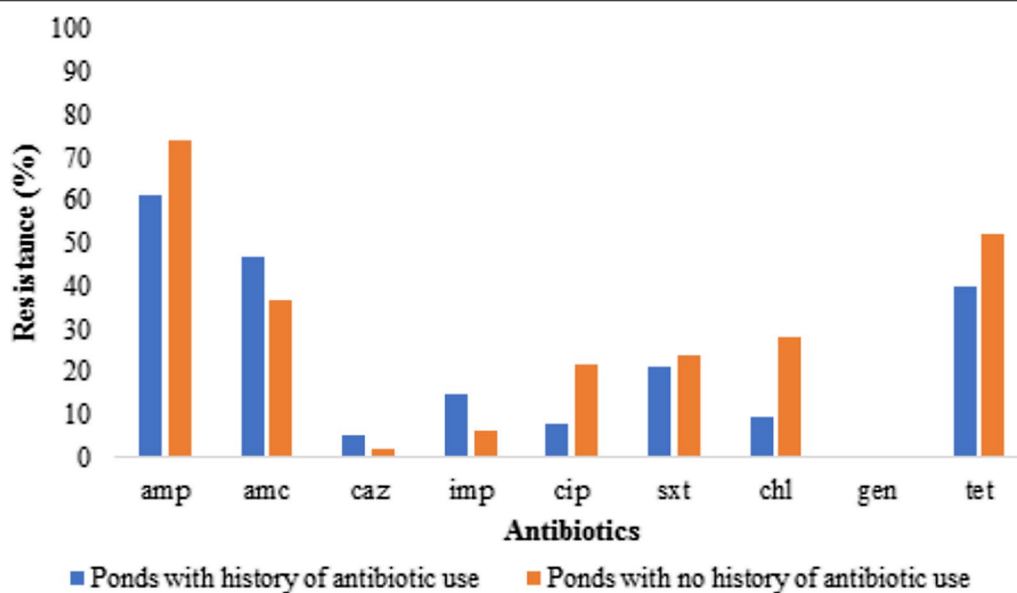


Fig. 3 Resistance of *Vibrio* spp. from ponds with and without history of antibiotic usage to selected antibiotics. KEY: amp: ampicillin; amc: amoxicillin-clavulanate; caz: ceftazidime; imp: imipenem; cip: ciprofloxacin; sxt: trimethoprim-sulfamethoxazole; chl: chloramphenicol; gen: gentamicin; tet: tetracycline

usage as against the ponds without the use of antibiotics with 2.2%, 28.3% and 36.9% resistance. The resistance to imipenem, ceftazidime, chloramphenicol and amoxicillin-clavulanate was higher in the ponds with

history of antibiotic use while resistance to tetracycline, ampicillin, ciprofloxacin and trimethoprim-sulfamethoxazole was higher in ponds without a history of antibiotic usage.

Discussion

The use of antibiotics in aquaculture practices for therapeutic purpose and also as growth promoters in some instances has predisposed the microbial flora of the aquaculture environment to antibiotics and hence the need to develop mechanisms to cope with the selective pressure exerted on them by these antimicrobial agents. Metals on the other hand are important components of the natural water systems and are also present in some quantities in aquaculture feeds, and this has led to the evolution of microbes with highly adaptive resistance mechanisms to heavy metals (Kundu et al. 2017; Hui-Gao et al. 2018; Landrigan et al. 2020).

In the present study, a total of one hundred and twenty-one *Vibrio* isolates consisting of *Vibrio cholerae* (37), *Vibrio parahaemolyticus* (36), *Vibrio vulnificus* (36) and *Vibrio alginolyticus* (12) were obtained from the water samples obtained from the aquaculture ponds. The isolation of *Vibrio* spp. from aquaculture resources and even some aquatic animals has been reported by several authors. He et al. (2016) reported the isolation of *V. parahaemolyticus* from fresh shrimps in China, while Wei et al. (2014), in their study reported the isolation of *V. alginolyticus* from fish and water samples of a fish hatchery in Asian Seabass. This is in addition to the work of Hu and Chen (2016), who isolated species of *V. parahaemolyticus* from crustaceans and shellfish in Shanghai, China. The only major dissimilarity in these studies is the type of fishes and other aquatic animals being reared. In this study, the predominant species of fish being raised is *Clarias gariepinus*.

The metal composition of the ten selected aquaculture ponds showed the presence of Cu, Zn, Pb, Ni and Cr in varying concentration. The Zn composition of the ponds was between the ranges of 0.19–2.82 mg/L and this does not corroborate the study of Adeyemi and Ugah (2017) who reported a range of 0.0067–0.0473 mg/L for Zn while studying the concentration of some heavy metals in water, soil and fish from some ponds in Abuja, Nigeria. The concentration of Zn in this study is higher than the recommended national standard of 0.01 mg/L set by NESREA (2011), implying that the aquaculture ponds are highly contaminated by Zn. The Cu concentration of the ponds in this study is higher than the range of 0.01–0.10 mg/L reported by Mustapha and Agunloye (2016) in a study on four different aquaculture ponds. These values are still significantly higher than the recommended national standard of 0.001 mg/L in some of the ponds in this study.

The Pb composition of the aquaculture pond water ranged from 0.01 to 0.29 mg/L. With the exception of ponds 1, 2 and 3, whose values were within the accepted quality standards for fisheries and recreation surface

water in Nigeria, the Pb concentration of the water from the remaining seven ponds were well beyond the permissible limits set by NESREA (2011). Ni was not detected in four of the aquaculture ponds, while the range for the remaining six ponds was 0.17–0.47 mg/L, which were well beyond the maximum permissible or acceptable limit in Nigeria as shown in Table 3. The Cr concentration for all the selected aquaculture ponds was higher than the minimum permissible limit set by NESREA for Cr^{6+} suggesting a high level of contamination from the metal (NESREA 2011).

All the *Vibrio* species isolated in this study survived in the presence of all the metals at a concentration of 6.25 µg/mL to a concentration of 50 µg/mL; however, the isolates exposed to Cu started declining at a concentration of 100 µg/mL. There was also a rapid decline in the frequency of isolates as the concentration of Ni and Zn reached 200 µg/L and also to Pb and Cr at a concentration of 400 µg/mL. At a metal concentration of 800 µg/mL, Zn and Cu seemed to be toxic to the isolates as they could no longer survive. However, this does not agree with the findings of Yu et al. (2016) who studied the antibiotic and heavy metal resistance of *Vibrio parahaemolyticus* isolated from fresh shrimps and reported 93.3% resistance to copper at a concentration of 3200 µg/mL. In addition, no growth was observed at a concentration of 1600 µg/L in the presence of Pb and Ni. However, 97.5% of the isolates could grow in the presence of Pb at a concentration of 400 µg/mL and 91.7% at a concentration 800 mg/mL; with only 19 isolates (15.7%) surviving in the presence of Ni at 800 mg/mL concentration. This implies that there is no relationship between the level of resistance to metals by the isolates and the concentration of metals in the aquaculture ponds, the only notable exception being Cr, to which the two parameters showed a comparable level of concordance. The level of resistance to metals by the isolates in this study slightly varies when compared with the report of Yu et al. (2016) who isolated *V. parahaemolyticus* from shrimps and reported that 87.8% were highly resistant to Pb and only 19 (6.7%) of their isolates survived in the presence of Ni at a concentration of 3200 µg/mL. There was a high resistance to Cr in this study, as 117 (96.7%) of the total *Vibrio* isolates survived at a concentration of 1600 µg/mL and 57 (47.1%) survived at the highest concentration of 3200 µg/mL. This observation is corroborated by the work of Yu et al. (2016) who also reported a high resistance to Cr. The high level of resistance to Cr in this study could be attributed to the elevated concentration of the metal in all the aquaculture ponds studied, which could have facilitated an adaptive mechanism to the metal by the resident bacterial flora of the ponds.

The *Vibrio* spp. in this study showed a high level of resistance to ampicillin (88.5%) in comparison with other antibiotics. The high incidence of resistance to ampicillin aligns with previous studies such as the report of Lim and Kasing (2013) and Heenatigala and Fernando et al. (2016), who reported 100% resistance to ampicillin in *Vibrio* isolated from shrimp pond culture. The high level of resistance to ampicillin could be because the antibiotic is one of those being used on the studied aquaculture ponds based on the information obtained from the farm owners, and there might be a high level of adaptation to the antibiotic by the isolates, as a result of prior exposure. This is coupled with the fact that resistance to ampicillin and other β -lactam antibiotics in *Vibrio* spp. has been on the increase over the years, as corroborated by the reports of Devi et al. (2009), Ottaviani et al. (2013) and Dahanayake et al. (2019). Of all the isolates obtained in this study, none resisted gentamicin. This observation showed the relative effectiveness of the antibiotic in inhibiting the organisms. This observation is, however, not in concordance with the report of Lim and Kasing (2013) who reported 100% resistance to gentamicin in their report on antibiotic susceptibility profile of *Vibrio parahaemolyticus* isolated from Tiger shrimp aquaculture. This could be because gentamicin is rarely used in aquaculture farming and hence low adaptation culminating in a low level of resistance to the antibiotic. This phenomenon was also corroborated by the findings of Apenteng et al. (2017) while studying microbial isolates from some fish ponds in Ghana. The percentage resistance of the *Vibrio* isolates to amoxicillin–clavulanate and imipenem in this study were 44.3% and 5.7%, respectively, which is lower than what was reported by Saifedeen et al. (2016) in their study. The resistance to tetracycline in this study was 41%. The obtained resistance is higher than what was reported by Akinbowale et al. (2006) and Amiri et al. (2014), who reported a 5% and 20.7% resistance to tetracycline in *Vibrio* spp. isolated from aquaculture ponds in their respective studies. However, the observed resistance to tetracycline in this study is lower than the 60% resistance reported by Lim and Kasing (2013) in their study. The resistances of the isolates to trimethoprim–sulfamethoxazole and chloramphenicol in this study were 22.1% and 15.6%, respectively, which do not correspond with the study of Heenatigala and Fernando (2016) who reported 100% resistance to chloramphenicol in the *Vibrio* isolated in their study, same as He et al. (2016). The disparity in the resistance observed in this study and other studies could be due to the volume of antibiotic usage and antibiotic types used in the aquaculture ponds and also the fact that antibiotic usage in the ponds could have placed a selective pressure on the isolates, thus stimulating their resistance to other antibiotics or closely related ones.

There seems to be no correlation between antibiotic resistance in the isolates obtained from the ponds with recognized history of antibiotic usage and the ones without, as there was higher level of resistance to tetracycline, ampicillin, ciprofloxacin, trimethoprim–sulfamethoxazole and chloramphenicol in the isolates obtained from the pond with no recognized history of antibiotic use. With the exception of gentamicin, with no resistance observed in isolates from both categories of ponds, there was higher resistance to imipenem, ceftazidime and amoxicillin–clavulanate in the isolates from ponds with history of antibiotic use. It should be well noted that some studies such as that of Witte (1998), Aarestrup (2012), FAO (2016) and Chen et al. (2021) have suggested a strong association between antibiotics use in agriculture and the onset of antibiotic resistance. In this study, however, the trend might be a little confusing as most aquaculture farm operators do not attest to the use of antibiotics in their farming activities due several underlining factors and the fear of being penalized by certain regulatory authorities.

It should be noted, however, that the presence of heavy metals in aquaculture pond water vis-à-vis the use of antibiotics in the operations of some of the ponds could be a major underlining factor in the development of metal and antibiotic resistance in bacteria from the aquaculture environment. Several studies have reported that the presence of metals could facilitate the phenotypic and genotypic development of resistance to certain antibiotics in a dose-dependent manner (Berg et al. 2010; Knapp et al. 2011, 2017; Bernier and Surette 2013; Dickinson et al. 2019), although the studies of Stepanauskas et al. (2005), Tuckfield and McArthur (2008) and Hölzel et al. (2012) did not agree with that particular assertion. It should be highlighted that Zn and Cu have been implicated as major drivers of antibiotic resistance in most bacteria, especially Gram-negative strains (Poole 2017).

Conclusion

This study has shown the occurrence of heavy metals at concentrations well beyond the maximum permissible limit for fisheries and aquaculture operations in Nigeria. In addition, this study has highlighted the presence of metal- and antibiotic-resistant *Vibrio* spp. in the aquaculture ponds studied, and this is totally not connected with the prior use of antibiotics in the aquaculture operations. Further studies could be geared toward the detection of other bacterial groups and the molecular characterization of virulent and antibiotic resistance genes in isolated strains.

Abbreviations

Zn: Zinc; Cu: Copper; Pb: Lead; Cr: Chromium; Ni: Nickel; CLSI: Clinical and Laboratory Standards Institute; AAS: Atomic absorption spectrophotometer; ARG: Antibiotic resistance gene; FAO: Food and Agriculture Organization.

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Authors' contributions

AOA conceived and designed the study. AOA and OGO carried out the laboratory work. AOA and AVO wrote the first draft of the manuscript and did the article formatting. All authors read and approved the final manuscript for publication.

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Availability of data and materials

The datasets used and/or analyzed during the current study are included in the article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no competing interests as regards the design and execution of this study.

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