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Antibiotic Susceptibility of Coliform and Vibrio Species in Shellfishes from Estuary Chronically Contaminated with Polycyclic Aromatic Hydrocarbon

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Abstract
Polycyclic aromatic hydrocarbon (PAH) levels and antibiotic susceptibility of coliform and *Vibrio* species in swimming crab (*Callinectes latimanus*), mangrove oyster (*Crassostrea tulipa*), and periwinkle (*Tympanotonus fuscatus*) from Qua Iboe River and Cross River estuaries were assessed using standard procedures. Bacterial load in the shellfishes and surface water was 1.2 to 1.4 times higher than the control, but the difference was significant at $p = 0.05$. Total PAH level of 11.36 ± 0.3 mg kg^{-1} in *C. latimanus*, 17.57 ± 0.9 mg kg^{-1} in *C. tulipa*, and 13.88 ± 0.5 mg kg^{-1} in *T. fuscatus* compared to 7.51 ± 0.3 mg L^{-1} in the surface water and indicates 1.06 to 1.57 times higher PAH accumulation than in surface water and was significant ($p = 0.05$). The dominant coliform and *Vibrio* species in the shellfishes were *Serratia marcescens*, *Enterobacter aerogenes, Escherichia coli, Klebsiella*, *Citrobacter diversus*, *and Vibrio alginolyticus, V. estuarianus, V. fischeri, V. fluvialis and V. parahaemolyticus.* A mean of 21.2% difference between two study groups indicates that shellfishes were the main source of gastrointestinal illness with 43% median resistance to commercially available antibiotics. Accumulation of PAH and abundance of an emerging multiple antibiotic resistant bacterial strains is a cause of concern and potential health risk to consumers of the shellfishes.
Keywords: Shellfish, coliform, *Vibrio*, polycyclic aromatic hydrocarbon, antibiotic resistance

1. Introduction

The Qua Iboe River Estuary (QIRE) is an ecologically productive and major hydrodynamic feature in the Niger Delta area of Nigeria. The area receives hydrocarbon derived pollutants through many routes including discharge of produced water and gas flaring from oil exploration and production activities. The input of persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and other compounds of anthropogenic origin especially untreated sewage into the estuary is a major environmental and public health concern (Eduok et al., 2010). Naturally formed and anthropogenic PAHs are ubiquitous compounds found in estuarine environment (Wagener et al., 2012; Chung and King, 2000) although high molecular weight PAHs from anthropogenic sources can reach toxic concentrations that can pose risk to the environment and human health (Menicom et al., 2005). Several PAHs have the potential to bioaccumulate and biomagnify within and along food chain due to their persistence and lipophilic properties, become associated with particulate matter such as clays and humics that are deposited in soils and sediments. Generally environmental contamination with PAH is considered hazardous because of their carcinogenic, mutagenic and teratogenic properties and also implicated in causing reproductive problems (Luch, 2005) for the aquatic biota and consequently in man (Udotong *et al.,* 2008).

The QIRE is comprised of tidal creeks, lagoons, wetlands, and tributaries fringed with mangrove vegetation made up of species of *Avicennia, Rhizophora* and *Nypa*, and harbor an abundance of edible aquatic biota. Indigenes of the Niger Delta are exposed to microbial and petrogenic pollutants through the consumption of the diverse aquatic biota in the ecosystem. The dominant shellfishes in the estuary include mangrove oyster, periwinkle, swimming crab, and mussel. These shellfishes are widely consumed by the coastal and estuarine communities in the Niger Delta as a delicacy and dietary protein supplement. The biota constitutes lower level food chain and contain elevated tissue burden of PAHs and microbial load due to their filter-feeding habit and are potential route for transmission to humans (Eduok *et al*., 2010). For instance, the ingestion of bivalve mollusks has been frequently associated with food-related infectious diseases (Cook *et al.,* 2001).

Reported cases of gastrointestinal distress attributed to seafood harvested from the QIRE are more pronounced in consumers eating partially cooked shellfishes such as the mangrove oyster (Eduok *et al*., 2010). Although some agents of waterborne diseases such as the *Vibrio* species are indigenous aquatic bacteria, others originate from the intestinal tracts of humans and enter the aquatic ecosystem through fecal contamination. The bacterial property frequently used to assess anthropogenic pollution is their response to antibiotics (Oliveira and Pinhata, 2008; Lobova et al., 2002) and several studies have implicated human activities for the increased level of antibiotic resistance (Lin et al., 2004; Biyela et al., 2004; Park et al., 2003). Furthermore, pharmaceutical substances including antibiotics extensively used in the treatment of human and animal diseases are incompletely metabolized by humans and animals. Thus, they can be detected in the urine and feces, and are introduced into the aquatic and terrestrial environment through effluent discharges and the use of sewage digestate for soil

amendment. (Biyela et al., 2004; Costanzo et al., 2005). Once in the aquatic environment, it is reported that their fate vary and are not biodegradable because of the inhibitory effect on aquatic organisms (Tamtam *et al*., 2008).

The emergence of microbial species that are resistant to the commonly used antimicrobial therapy is a significant threat in the healthcare sector. The problem is exacerbated when organisms are exposed to chemicals that can reduce or confer competitive advantage on certain organisms. For instance, pollutants such as PAHs with mutagenic properties in sublethal doses probably can alter the microbial genome or genes located on plasmids and transposons and consequently change their response to antimicrobial agents. Although there are studies on the pollution status of the QIRE (Essien et al., 2011; Eduok et al., 2010; Udotong et al., 2008), to our knowledge, there is a lack of information on the antibiotic susceptibility of microorganisms exposed to PAHs in shellfishes from this ecosystem. This study presents the result of bacteriological assessment, PAHs levels, exposure and accumulation in mass consumer shellfishes from QIRE, the response of the coliform and *Vibrio* species to routinely used antibiotics of clinical significance and correlates the implication of their prevalence in the shellfishes and on public health.

2. Study area, sample collection and processing

The study area was the Qua Iboe River estuary (QIRE), a dominant hydrographic feature in the Niger Delta region of Nigeria (Ekpe *et al*., 1995; Ukpong, 1995) with major oil installations, gas flare sites and artisanal fishing communities. The Cross River estuary (CRE) has no oil exploration and production activities (Oyo-Ita et al., 2010) although is subjected to intense artisanal fishing served as the reference site. Like every estuary in the Niger Delta region, inputs of agricultural waste, untreated sewage through direct defecation are a common feature in both ecosystems. The periwinkle (*Tympanotonus fuscatus*) and swimming crab (*Callinectes latimanus*) samples were hand-picked along the shoreline and the exposed intertidal sediment at low tide. Mangrove oyster (*Crassostrea tulipa*) samples were collected with the aid of machete at different and often exploited natural oyster beds in the QIRE and CRE during the wet season months of June to September, 2014. Samples for microbiological analysis were collected into a sterile isothermal container and samples for chemical analysis were collected separately into Amber glass containers with Teflon-lined screw-cap and transported to the laboratory.

The samples were extensively washed with sterile water and rinsed with normal saline to remove all surface contaminants before shucking. The edible parts were removed with a sterile knife and transferred to a sterile blender for homogenization and serially diluted using phosphate buffered saline. In addition, a randomized trial was conducted for 120 days to examine the risk associated with the consumption of shellfishes and available water in the QIRE compared with conventionally treated water. Out of the 52 households that volunteered to participate, 27 households were designated as the control group and supplied with potable water and instructed on how to maintain general sanitary conditions during harvesting, processing, and preparation of shellfishes before consumption. The presence-absence of gastrointestinal symptoms observed daily in each household were recorded by a designated member of the household. The data were subsequently collated and analyzed.

2.1 Determination of microbial load

One milliter aliquot of appropriate dilution (10^{-4} and 10^{-5}) of each shellfish sample was pour-plated in triplicate onto chromogenic *E. coli*/coliform selective medium (Oxoid) for *E.coli* and total coliforms. Thiosulfate-citrate-bile salts-sucrose (TCBS) and Bushnell-Haas (BH) minimal medium (Sigma-Aldrich) supplemented with 1.5% Nacl was used for the enrichment and isolation of *Vibrio* species. The inoculated plates were incubated at 37 and 44.5 ± 0.2 ^{o}C for mesophilic and thermotolerant *E. coli* respectively for 24 h and thereafter the number of colony-forming units (CFUs) enumerated. Results are expressed as CFU g^{-1} of sample. *E. coli* was differentiated from other coliforms (pink colonies) by their typical purple colonies. The isolates were identified by comparing their cultural, morphological and biochemical characteristics with those of known taxa using the Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994) and Cowan and Steel's manual for the identification of medical bacteria (Barrow and Feltham, 1992).

2.2 Antibiotic susceptibility testing

Pure isolates of each coliform and *Vibrio* species maintained differently in chromogenic *E. coli*/coliform selective medium and TCBS agar respectively were comparatively assayed for antibiotic susceptibility using single disc diffusion method (Kirby-Bauer) according to the NCCLS recommended guidelines. Colonies of test isolate from each plate were transferred into test tubes containing 5 mL nutrient broth and incubated at 35 ^{o}C and 28 ± 2 ^{o}C for 24 hours to reactivate the cells. The reactivated organisms were inoculated unto 5 mL of sterile phosphate buffered saline (PBS) and the optical density adjusted to 0.5 MacFarland standard (containing approximately 1 x 10^{8} CFU mL^{-1}). 25 - 30 mL freshly prepared and cooled Mueller-Hinton agar (MHA) in

triplicate petri dishes were allowed to set. A representative of each batch of MHA plates with pH between 7.2 and 7.4 at room temperature after solidifying was incubated at 35 ± 2°C for 24 h and examined for sterility.

Further to this, the plates were seeded with 1 mL of the test bacterial suspension in PBS, swirled to evenly distribute the inoculum and kept on the bench for 1 hour to dry. Sterile forceps was used to place the individual antibiotics on the seeded agar plates and gently pressed onto the agar surface to ensure firm contact. The inoculated plates were allowed to stand for an hour on the bench for pre-diffusion and incubated at 35 and 28 ± 2 ^{o}C for 24 hours. On the basis of the diameter of clear zone around the paper disc, the degree of resistance or susceptibility was determined. The resulting zones of bacterial inhibition were measured using vernier caliper to the nearest whole millimeter. The average of triplicate determinations were taken as inhibition zones of the bacterial growth for a particular antibiotic. We tested 118 and 96 nonfastidious bacterial strains from QIRE and CRE respectively against 11 antibiotics (with different concentrations in parenthesis) widely used in clinical practice belonging to Penicillin (Ampicillin, SAM, 20 µg), Beta lactam/beta-lactamase inhibitor combination (Amoxicillin-Clavulanic acid, AMC, 30 µg), CEPHEMS (Ceftazidime, CAZ, 30 µg; Cefotaxime, CTX, 30 µg; Ceftriaxone, CRO, 30 µg), Tetracyclines (Tetracycline, Te, 30 µg), Folate pathway inhibitor (Sulphamethoxazole-Trimethoprim, SXT, 25 µg), Phenicols (Chloramphenicol, C, 30 µg), Fluoroquinolones (Ciprofloxacin, CIP, 5 µg) (HARDY Diagnostics, Santa Monica, CA), Ceftazidime-Clavulanic acid (CAC 30/10 µg) and Cefotaxime-Clavulanic acid (CTC 30/10 µg) (Becton Dickson and Company, Sparks, MD).

2.3. Chemical analysis

The samples were extracted and fractionated as described elsewhere (Olajire et al., 2005). 50 g of the dried sample was spiked with pre-deuterated PAH cocktail (anthracene-d_{10}, naphthalene-d_8, acenaphthylene-d_8, acenaphthene-d_{10}, flouranthene-d_{10}, fluorine-d_{10}, pyrene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, benzo[a]pyrene-d_{12}, benzo[a]anthracene-d_{12}, benzo[b]fluoranthene-d_{12}, benzo[ghi]perylene-d_{12}, dibenzo[a,h]anthracene-d_{14}, indeno[1,2,3-cd]pyrene) as internal standard (ES2528, Promochem, Wasel, Germany). The samples were dried with anhydrous Na_2SO_4, extracted with a mixture of dichloromethane (DCM) and acetone at 65 °C for 24 h using a soxhlet extractor. The extracts were dried in sample concentrator and thereafter solvent-exchanged with *n*-hexane. A glass column packed with 30 g of alumina deactivated with 4.5% water was used to fractionate the extracts. 50 mL of hexane/DCM (95/5%, v/v) was used to elute the aromatic and polycyclic aromatic fractions whereas the polar fractions were eluted with DCM. The PAH fractions were concentrated by rotary evaporator, dried under nitrogen and re-dissolved in DCM for gas chromatography–mass spectrometry (GC-MS) analysis. GC/MS of the aromatic fractions was performed on a Hewlett-Packard model 6890 GC coupled to a Hewlett-Packard model 5973 quadrupole MSD. Separation was achieved on a fused silica capillary column coated with DB-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness). The GC operating conditions were as follows: temperature hold at 65 ^{o}C for 2 min, increase from 65 to 300 ^{o}C at a rate of 6 ^{o}C min^{-1}, with a final isothermal hold at 300 ^{o}C for 20 min. Helium was used as carrier gas. The sample was injected in the splitless mode with an injector temperature of 300 ^{o}C. The mass spectrometer operated in the electron impact mode at 70 eV of ionization voltage. PAH concentrations were calculated relative to the pre-deuterated internal standards. The acquired data were processed with the Chemstation software.

2.4 Bioconcentration of PAH in shellfishes

Bioconcentration factor (BCF) is a dimensionless index indicating that net accumulation of pollutant relates to those detected in environmental compartment (Spacie *et al.,* 1995) expressed as: BCF = Corganism/Csource. Where $C_{organism}$ is concentration of PAH in the shellfish as a result of dietary uptake and C_{source} is concentration of PAH in the estuarine water. Usually, BCF values greater than 1 suggests biomagnification of the pollutant may be occurring, and trophic dilution is implicated when the value is less than 1 (Newman, 2010).

2.5 Statistical Analysis

ANOVA and Kruskal-Wallis test was performed using Statistica® software version 11 (Statsoft, Tulsa, OK, USA). Values are presented as mean ± standard deviation represented by error bars with levels of significance maintained at 95% for each test.

3. Results and discussion

Two hundred and fourteen bacterial strains belonging to ten genera were isolated from shellfishes exposed to varying PAH concentrations in QIRE and CRE and assayed for susceptibility to 11 antimicrobial agents. The median distribution of coliform, thermotolerant *E.coli* and *Vibrio* species in the surface water and shellfishes harvested from six locations in the QIRE and control site (CRE) is presented in Figure 1. The bacterial load in the shellfishes was 0.99 to 1.06 and 0.6 to 1.7 times higher than in the surface water from QIRE and CRE respectively, indicating bacterial accumulation as a result of filter-feeding mode of substrate uptake by the bivalve molluscs. The difference in bacterial load between the shellfishes and surface water was low but

significant at $p = 0.05$.

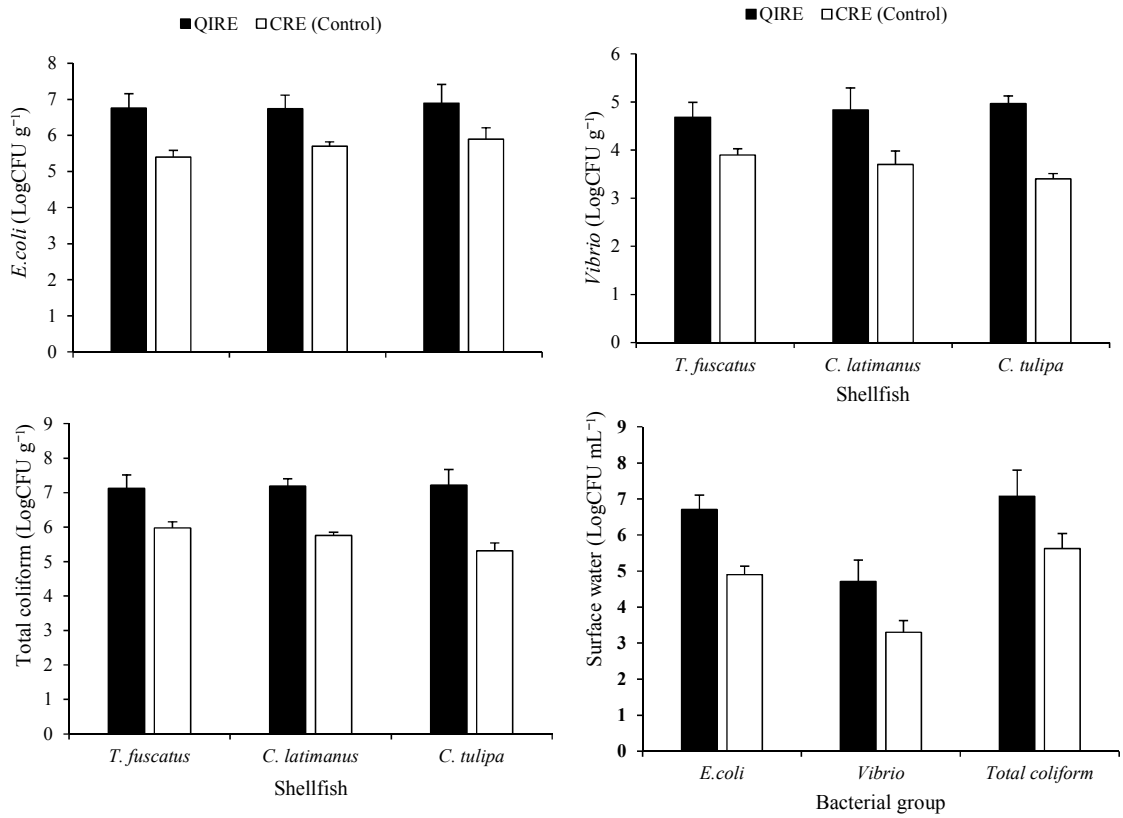

Figure 1 Distribution and levels of microbial load in the surface water and shellfishes harvested from the QIRE and CRE. (Error bars represent standard deviation of triplicate measurements).

The bacterial load in the shellfishes from QIRE was 1.2 to 1.5 times higher than in CRE whereas there was 1.3 to 1.4 times higher load in QIRE than in CRE surface water and the difference was significant (p = 0.05). The differences in microbial load between the QIRE and CRE can be attributed to the low anthropogenic pollution from industrial sources into the aquatic environment. In addition, the habit of defecating directly into the water and the surrounding wetlands by the estuarine inhabitants in the study area and the site used as control contributed to the low difference in the microbial load between QIRE and CRE. The dominant organisms in the surface water and shellfishes were *Serratia marcescens*, *Enterobacter aerogenes, Escherichia coli, Klebsiella sp*, *Citrobacter diversus*, *and Vibrio alginolyticus, V. estuarianus, V. fischeri, V. vulnificus and V. parahaemolyticus.* These organisms are implicated in waterborne, water-washed, foodborne infections (Lipp and Rose, 1997) and infections from molluscan shellfish consumption (Potasman *et al*., 2002; Rippery, 1994).

The polycyclic aromatic hydrocarbon (PAH) concentration in the surface water and shellfishes are presented in Figures 2 and 3 respectively. The individual PAH concentration in the QIRE shellfishes ranged from 0.20 to 4.39 mg kg^{-1} with a total of 11.36 mg kg^{-1} in *C. latimanus*, 17.57 mg kg^{-1} in *C. tulipa*, and 13.88 mg kg^{-1} in *T. fuscatus* compared to 7.51 mg L^{-1} in the background surface water. The PAH concentrations in QIRE samples indicated a difference of 1.5 (*C. latimanus*), 1.8 (*T. fuscatus*) and 2.3 (*C. tulipa*) compared to the surface water and was significant at $p = 0.05$. In contrast, individual PAHs concentration in surface water from CRE ranged from 0.01 to 0.02 ± 0.002 mg L^{-1} whereas 0.02 to 0.04 ± 0.003 mg kg^{-1} was detected in the shellfishes. The total PAH (∑PAH) in the shellfishes from CRE ranged from 0.37± 0.01 to 0.44 ± 0.01 mg kg^{-1} indicating 1.1 to 1.3 times higher concentration than the surface water. The probable reasons for the low PAH concentration in the surface water and shellfishes, in part can be attributed to transformation and volatilization, and in part, sequestration in sediment and transport to the sea (Essien et al., 2011).

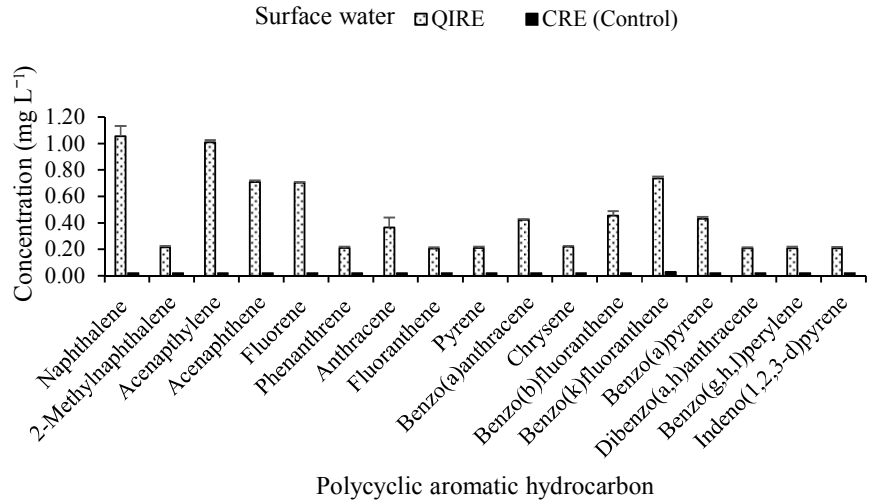

Figure 2. Polycyclic aromatic hydrocarbon concentration in surface water from QIRE and CRE

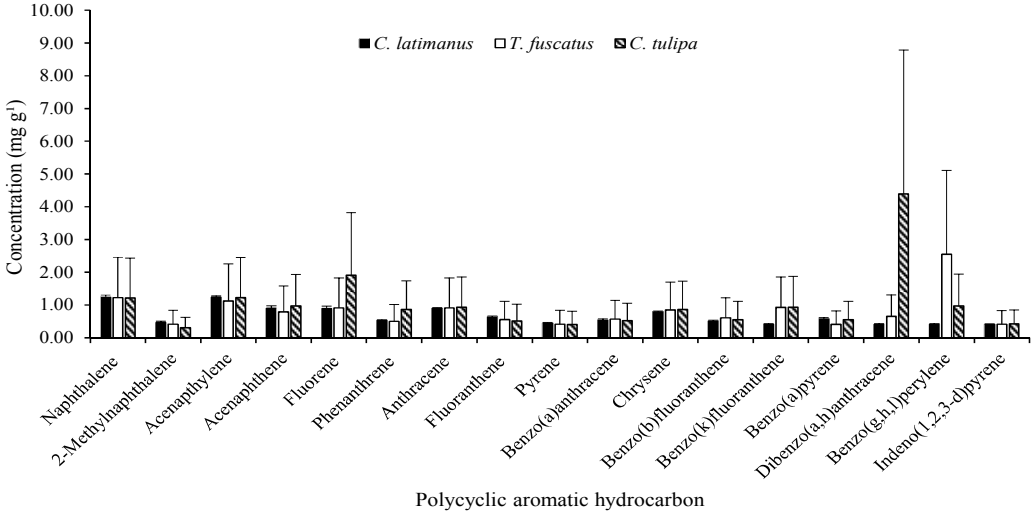

Figure 3. Concentration of individual PAH in the shellfishes from Douglas creek, QIRE. (Error bars represent standard deviation of triplicate measurements)

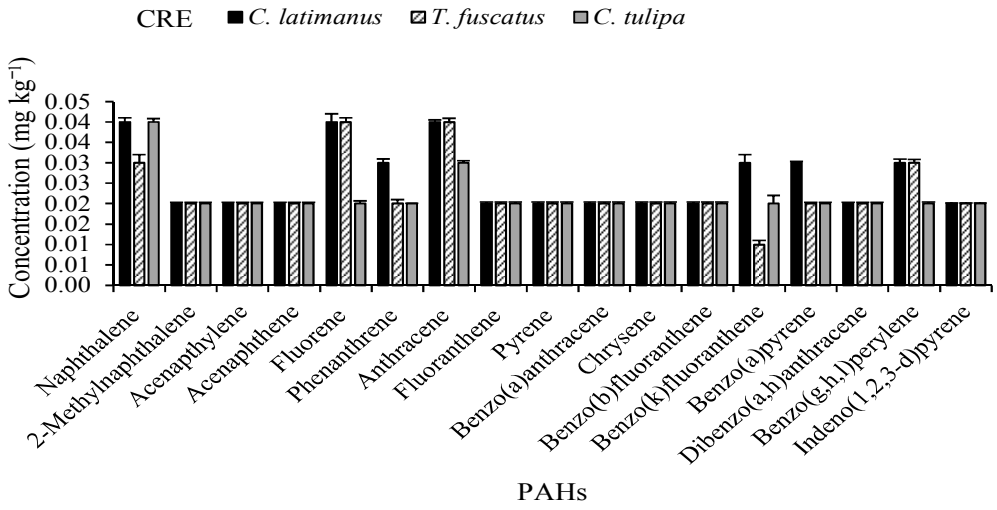

Figure 4 Concentration of individual PAH in the shellfishes from CRE (Error bars represent standard deviation of triplicate measurements)

Overall, the ∑PAH in QIRE samples was 23 times higher in surface water than in CRE whereas in *C. latimanus, T. fuscatus* and *C. tulipa*, 26, 36 and 48 times higher difference respectively was observed. The result suggests that PAH accumulated in the shellfishes above the background concentration in the surface water from QIRE compared to that in the CRE (Figure 4). Although uptake of PAHs by the shellfishes can be through water and food, for numerical expediency only the surface water as the main source of constant concentration was determined. Thus, the bioconcentration factor of PAH in the shellfishes (Figure 5) suggests that anthropogenic pollution of surface water in the ecosystem primarily through oil exploration and production activities, gas flaring, industrial and sewage discharges was more pronounced in QIRE than in the CRE.

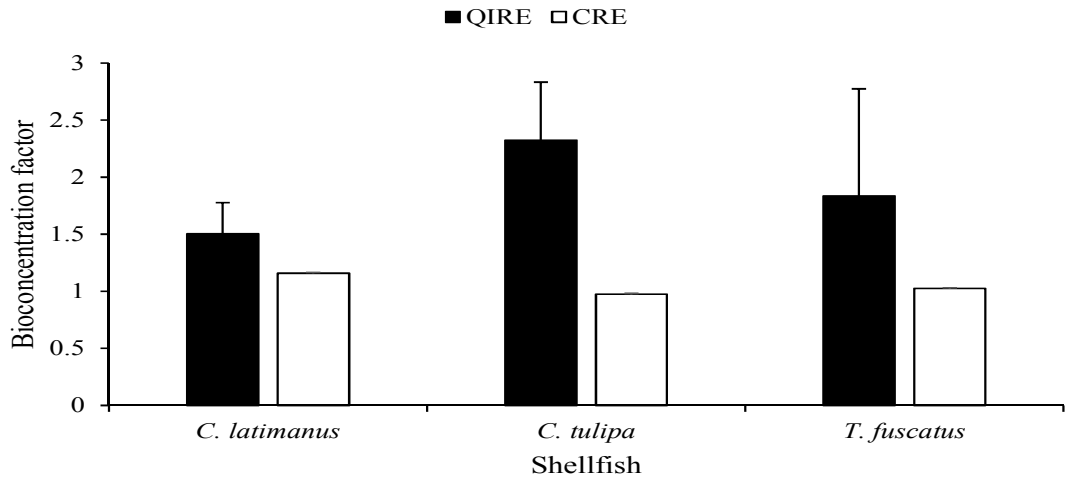

Figure 5 Levels of PAH accumulated in shellfishes from QIRE and CRE. Values were normalized against the concentration in surface water

Episodic outbreaks of gastrointestinal illness is a common occurrence in the QIRE fishing communities, in part as a result of the consumption of poorly prepared and/or inadequately cooked shellfishes, and in part because of poor hygienic conditions and lack of potable water (Eduok *et al*., 2010). Generally, mild to severe abdominal cramps, vomiting, stooling were the symptoms with a 21% higher rate of gastrointestinal symptoms observed in 25 study households consuming surface water from the ecosystem and without proper shellfish processing or depuration in relation to the control group (Figure 6). The difference between the two treatment groups was significant (p = 0.5) and provides a good index that water-borne and water-washed infections in the QIRE communities was primarily due to the low literacy level and lack of potable water. Although, culture-dependent methods were used in the study, the generally observed low level occurrence of foodborne infection and inability to completely minimize or eliminate gastrointestinal symptoms in the control group suggests that some of the infectious agents were viable but nonculturable pathogens and plausibly include protozoans and enteric viruses. In addition, some pathogens such as *V. vulnificus* that colonize oysters are difficult to remove from the shellfishes by depuration suggesting that most organisms are not merely accumulated in the oyster during feeding but likely interact by forming biofilm (Webb *et al*., 2007).

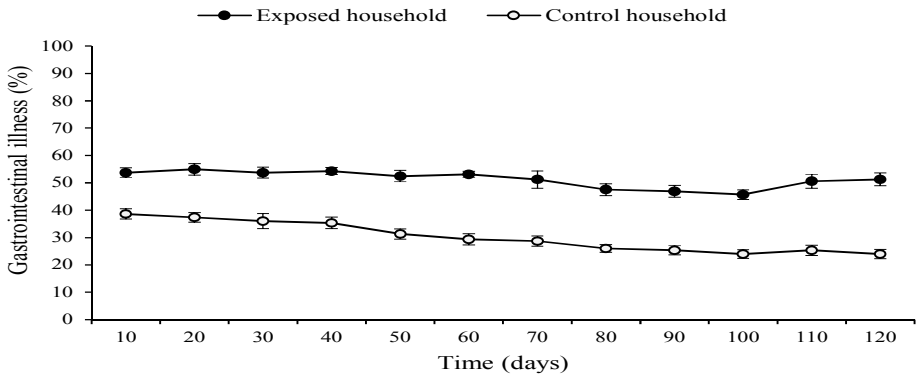

Figure 6 Gastrointestinal illness associated with consumption of water and shellfishes in households from Qua Iboe River estuarine community.

The response of bacterial strains in shellfishes from QIRE to the commonly used antimicrobial agents in clinical practice is presented in Figure 7. The results of the susceptibility assay indicated the prevalence of resistant coliform and *Vibrio* species harbored by the shellfishes from QIRE in relation to similar but susceptible bacterial strains from CRE. The percentage resistance among the bacterial strains from QIRE was 8.2 to 16 higher in relation to CRE whereas the intermediate zone response was 1.5 to 2.8 times higher for QIRE than CRE. The susceptibility pattern of the organisms indicate interspecies differences, for instance, the zones of inhibition for *E. coli* ranged from 4 ± 0.3 to 24 ± 0.5 mm in relation to *V. aestaurianus* with 12 ± 0.2 to 26 ± 0.3 mm (Figure 7) and suggest that members of the coliform and *Vibrio* species responded differently to the antimicrobial agents. The 42% (*E. aerogenes*) to 52% (*E. coli*) resistance of coliforms as indicators of fecal contamination in the shellfishes is a cause of concern due to their implication in foodborne infection and intoxication.

Pre-exposure of the coliforms to antimicrobial agents and the effect of PAH as the dominant pollutant in the ecosystem are the probably reasons for the observed difference in their response. A mean response of 41% (resistant), 24% (intermediate) and 35% (susceptible) was observed for all bacterial strains from QIRE. However, 70 and 72% of the bacterial strains from QIRE were resistant to AMP and Te (Figure 8) whereas 81 and 100% were susceptible to CTC and CAC respectively. Although the reason for this is unclear at the moment, we perceive it can be the effect of the antimicrobial combinations on the bacterial chromosome, plasmid and transposon-encoded resistant genes.

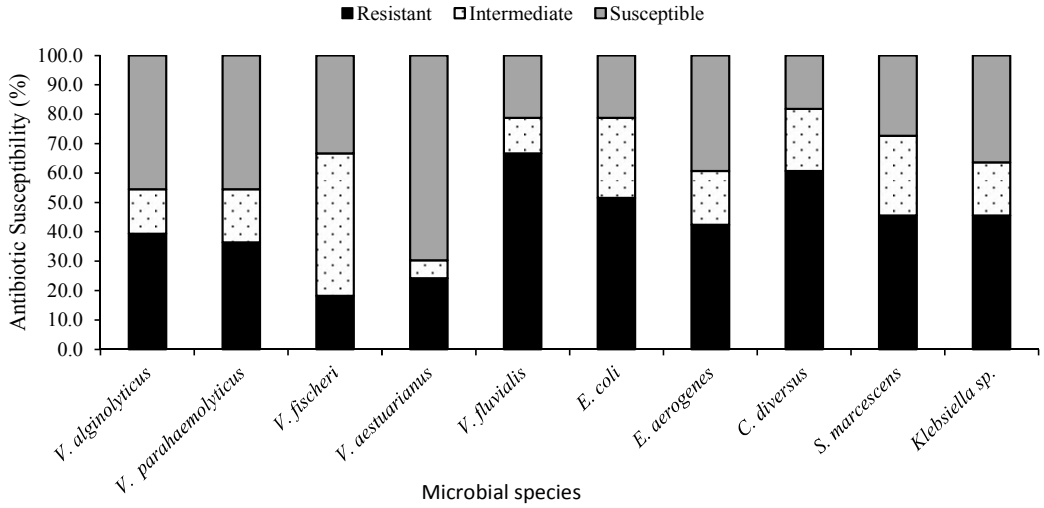

Figure 7 Susceptibility pattern of bacterial strains in shellfishes from QIRE to antibiotics routinely used in clinical practice. (Cumulative response of 3 - 5 members of the bacterial strain from each of the shellfishes, n = 118).

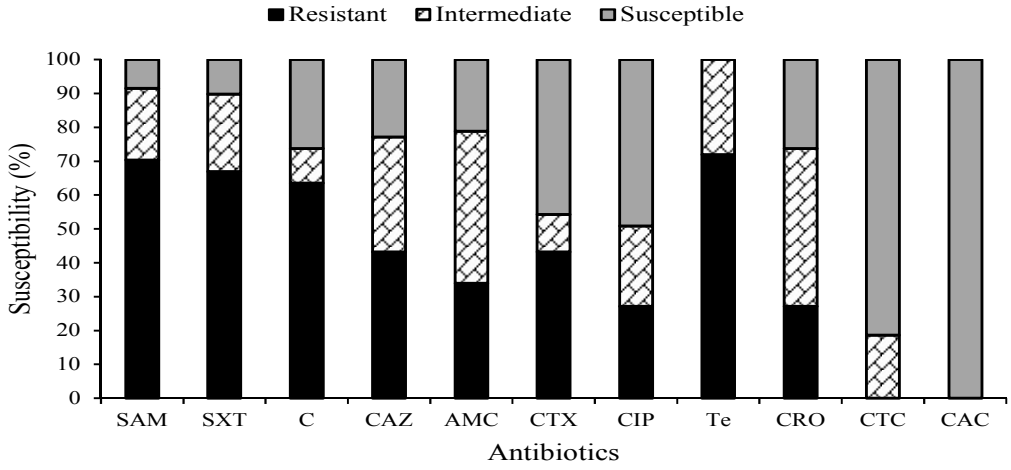

Figure 8 Multiple antibiotic resistance pattern of bacterial strains in shellfishes from QIRE (Cumulative response of bacterial strain from the shellfishes, n = 118).

Generally, the inhibitory zones of organisms in shellfishes from CRE ranged from 16 ± 0.4 to 28 ± 0.3 mm indicating that 72 to 75% were susceptible in relation to a range of 34 to 39% in QIRE (Figure 9). Thus, 1.8 to 2.2 times higher number of susceptible bacterial strains were obtained from CRE compared with QIRE and further demonstrate that there was a significant difference ($p = 0.05$) in the resistant pattern of bacterial strains in shellfishes from the polluted QIRE ecosystem. Therefore, a key environmental contributor to the pronounced antibiotic-resistant bacteria in the shellfishes from QIRE is the persistent exposure to low-level cytotoxic and mutagenic PAH of pyrogenic origin through anthropogenic pollution of the ecosystem.

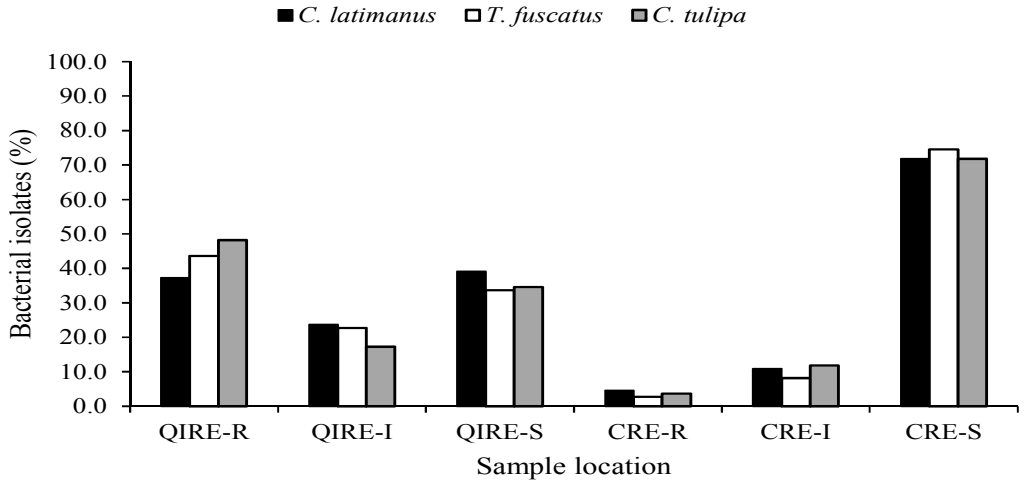

Figure 9 Susceptibility pattern of bacterial strains in shellfishes from QIRE and CRE to the routinely used antibiotics (R = resistant, I = intermediate, S = susceptible)

It is plausible that multiple antibiotic resistant microorganisms present in sewage can horizontally transfer the resistant gene to other organisms through cell to cell interaction in the aquatic ecosystem (Aminov and Mackie, 2007) which resulted in the observed antibiotic resistance among the QIRE bacterial strains. Our result is consistent with other studies (Choi et al., 2003; Herwig et al., 1997) in which pronounced antibiotic resistance was observed in polluted areas having high human impact in relation to unpolluted sites in aquatic environment. Thus, PAHs accumulated in the shellfishes and response of the organisms to the antimicrobial agents was positively correlated and imply that there is a causal link between bacterial resistance induced by available PAHs concentrations in the surface water and shellfishes. In addition, our result indicates that the QIRE is contaminated by persistent organic pollutants of anthropogenic origin that has contributed to emergence of antibiotic resistant bacterial strains in shellfishes harvested from the ecosystem. Antibiotic resistance of the bacterial strains induced through environmental pollution and exacerbated by the deplorable living conditions has shifted the weight of argument in favor of herbal remedies to minimize the health risk associated with the consumption of shellfishes from the estuarine communities.

4. Conclusion

Overall, pollution of the QIRE is primarily from crude oil exploration and production activities, deposition of pollutants from gas flaring and sewage discharge. The shellfishes harvested from the QIRE to serves as dietary protein supplement accumulated PAH which can biomagnify along the food chain. Furthermore, the shellfishes from QIRE in part, are passive carriers of human enteric pathogens harboring some of the agents implicated in gastrointestinal disorders in the estuarine communities, and in part, are reservoirs of antibiotic resistant bacterial species. The increase in multidrug-resistant bacterial strains in the QIRE was enhanced by anthropogenic pollution of the ecosystem especially with PAHs. Consequently, PAH-induced antibiotic resistant bacterial strains appear to be widespread in shellfishes harvested from the QIRE ecosystem and can poses a serious challenge to clinical practice in the Niger Delta communities with serious health consequences on the inhabitants.

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