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Original Article **Open Access**

Bacteriological assessment of crab (*Pachycheles pubescens*) and dog whelk (*Nucella lapillus*) shellfishes from mesotidal estuarine ecosystem

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Abstract:

Background: Shellfishes are susceptible to a wide variety of bacterial pathogens, most of which are capable of causing disease in humans but are considered to be saprophytic in nature. Bacteriological diversity of shellfishes depends on the fishing grounds, habitats and environmental factors around them. This study assessed the bacteria associated with shellfishes, *Pachycheles pubescens* (crab) and *Nucella lapillus* (dog whelk) harvested from mesotidal estuarine ecosystem.
Methodology: The bacteriological assessment of crab (*Pachycheles pubescens*) and dog whelk (*Nucella lapillus*) harvested from Okwano Obolo estuary in Eastern Obolo local government area (LGA), Akwa Ibom was evaluated. The density of heterotrophic and potential pathogens was determined using standard analytical procedures. The pure bacterial isolates were grouped into recognizable taxonomic units and characterized to their generic level.
Results: The mean (and range) total heterotrophic bacterial count (THBC), total coliform count (TCC), faecal coliform count (FCC), *Salmonella-Shigella* count (SSC) and total *Vibrio* count (TVC) of the crab samples (log10 cfu/g) for the crab samples are; 4.281±0.085 (4.18-4.39); 4.187±0.078 (4.11-4.30); 4.115±0.081 (4.00-4.20); 4.076±0.058 (4.00-4.14); and 4.114±0.085 (4.00-4.23) respectively (p=0.003915). For the dog whelk samples, the mean (and range) THBC, TCC, FCC, SSC and TVC are 4.232±0.095 (4.11-4.36); 4.185±0.095 (4.04-4.28); 4.082±0.068 (4.00-4.18); 4.062±0.055 (4.00-4.15) and 5.155±0.062 (4.08-4.23) respectively (p=0.028856. Bacterial species isolated from the crab and dog whelk samples included *Salmonella*, *Bacillus*, *Shigella*, *Corynebacterium*, *Pseudomonas aeruginosa* and *Vibrio* (which was the most frequently isolated bacteria pathogen from both samples in 80%).
Conclusion: Some of the bacteria species especially *Vibrio*, *Salmonella* and *Shigella* isolated from the crab and dog whelk samples are known human pathogens, that can pose serious health risk if these seafoods are not properly cooked before consumption.

Keywords: Bacteria; *Pachycheles pubescens*; *Nucella lapillus*; estuary

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Évaluation bactériologique des mollusques et crustacés du crabe (*Pachycheles pubescens*) et du buccin (*Nucella lapillus*) de l'écosystème estuarien mésotidal

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Résumé:

Contexte: Les mollusques et crustacés sont sensibles à une grande variété de pathogènes bactériens, dont la plupart sont capables de causer des maladies chez les humains, mais sont considérés comme étant de nature saprophyte. La diversité bactériologique des coquillages dépend des zones de pêche, des habitats et des facteurs environnementaux qui les entourent. Cette étude a évalué les bactéries associées aux coquillages, *Pachycheles pubescens* (crabe) et *Nucella lapillus* (buccin) récoltées dans l'écosystème estuarien mésotidal.
Méthodologie: L'évaluation bactériologique du crabe (Pachycheles pubescens) et du buccin (*Nucella lapillus*) récoltés dans l'estuaire d'Okwano Obolo dans la zone de gouvernement local d'Obolo oriental (LGA), Akwa Ibom a été évaluée. La densité des pathogènes hétérotrophes et potentiels a été déterminée à l'aide de procédures analytiques standard. Les isolats bactériens purs ont été regroupés en unités taxonomiques reconnaissables et caractérisés à leur niveau générique.
Résultats: La moyenne (et la plage) du nombre total de bactéries hétérotrophes (THBC), du nombre de coliformes totaux (TCC), du nombre de coliformes fécaux (FCC), du nombre de *Salmonella-Shigella* (SSC) et du nombre total de *Vibrio* (TVC) des échantillons de crabe (log10 ufc/g) pour les échantillons de crabe sont; 4,281±0,085 (4,18-4,39); 4,187±0,078 (4,11-4,30); 4,115±0,081 (4,00-4,20); 4,076±0,058 (4,00-4,14); et 4,114±0,085 (4,00-4,23) respectivement (p=0,003915). Pour les échantillons de buccins, la moyenne (et la plage) THBC, TCC, FCC, SSC et TVC sont de 4,232±0,095 (4,11-4,36); 4,185±0,095 (4,04-4,28); 4,082±0,068 (4,00-4,18); 4,062±0,055 (4,00-4,15) et 5,155±0,062 (4,08-4,23) respectivement (p=0,028856). Les espèces bactériennes isolées des échantillons de crabe et de bulot comprenaient *Salmonella, Bacillus, Shigella, Corynebacterium, Pseudomonas aeruginosa* et *Vibrio* (qui était la bactérie pathogène les plus fréquemment isolées des deux échantillons dans 80%).
Conclusion: Certaines des espèces de bactéries, en particulier Vibrio, Salmonella et Shigella isolées des échantillons de crabe et de buccin, sont des agents pathogènes humains connus, qui peuvent poser de graves risques pour la santé si ces fruits de mer ne sont pas correctement cuits avant consommation.

Mots clés: Bactéries; *Pachycheles pubescens*; *Nucelle lapillus*; estuaire

Introduction:

Shellfish comprises a variety of exoskeleton-bearing aquatic invertebrates including crustaceans such as lobsters, prawn crabs, and shrimps. Shellfish, in general, contains appreciable quantities of digestible proteins, essential amino acids, bioactive peptides, long chain polyunsaturated fatty acids, vitamin B and minerals, including copper, zinc, inorganic phosphate, sodium, potassium, selenium, iodine, and also other nutrients, which offer health benefits to the consumers. Bacteria, ubiquitous in the marine environment, constitute an important component of the coastal microbial communities. They play unique role in nutrient cycling, trophic dynamics of aquatic food webs and acts as a disease agent in human (1).

Estuaries provide good breeding sites for shellfishes and other fishes. Environmental parameters such as temperature, salinity, dissolved oxygen, and turbidity play a role in the filtration activity, thereby affects the retention of bacteria by bivalves (2). These factors determine the distribution and composition of bacterial communities. During the past decades, outbreaks of seafood associated infections have caused illness and death (3). The most prevalent microorganisms that contaminate the shell fishes are bacteria introduced into water bodies through human activities. Janina et al., (1) reported that shellfishes are able to harbor microorganisms in their environment.

Shellfishes are filter feeder organisms that selectively strain small particles of phytoplankton, zooplankton, and inorganic matters and accumulate a diversity of other contaminants from surrounding waters (4). The number and type of pathogenic bacteria present in marine or estuarine water depends on seasonal, climatic and anthropogenic factors (5). Bivalve shellfish have been known as bio-indicators of aquatic contamination with heavy metal and pesticides for decades (6).

Bacterial pollution in shellfishes is a common challenge in almost all the coastal areas of developing countries. Shellfish-borne infectious diseases are generally transmitted through faeco-oral route, as such, the shellfish must be examined to ensure that pathogenic bacteria are not present (7*).* The main hazard associated with consumption of shellfishes arises from contamination of their habitat water, especially if eaten raw or lightly cooked. These circumstances make the shellfish an important vector of food borne diseases, thereby presenting a significant human health risk (8). A study (9) showed that consumption of raw or undercooked seafood is recognized as health risk to consumers. Faecal pollution enhances the occurrence of shellfish diseases and the population at risk include individuals with immunocompromised disorders such as patients with cancers, chronic liver and kidney diseases (10,11). Shellfishes have high nutritive base and serve as a good medium for the growth of microorganisms that can lead to food poisoning such as salmonellosis and vibriosis, when con-

sumed (12).

There has been a significant concern about the safety of shellfishes for human consumption. Despite extensive efforts to assure the safety supply of shellfishes, the incidence of infection and mortality have been increasing (13). Therefore, bacteria associated with shellfish are of paramount importance due to the widespread consumption of shellfishes by the populace at large. This study assesses the bacteria pathogens associated with shellfishes.

Materials and method:

Study setting

The study location is Okwano-Obolo in Eastern Obolo, a coastal settlement in Akwa Ibom State of the Niger Delta region of Nigeria. The region lies within latitude 4° 44′ N, longitude 8° 41′ E and latitude 4° 42′ N, longitude 8° 42′ E bordering the Atlantic Ocean. It is a fishing settlement characterized by different shellfishes including soft, dark, mudflats, crabs, dog whelks, and periwinkles. The ecosystem is equally used for petroleum and exploration activities (14).

Sample collection

Edible shellfishes; crabs (*Pachycheles pubescens)* and dog whelks *(Nucella Lapillus)* were randomly obtained from the local fishermen in Etekwun, a fishing settlement in Okwano-Obolo of Eastern Obolo, Akwa Ibom State, Nigeria. Five samples (30g each) of each shellfish were collected and placed in ice-cooled chest and transported to the microbiology laboratory of the University of Uyo for analysis.

Isolation and enumeration of bacterial isolates

Isolation and enumeration of heterotrophic bacteria and potential pathogens were determined using standard analytical techniques as described by Harrigan and McCance (15). The samples were first macerated to paste using sterile ceramic mortar and pestle. Serial dilution of the samples was done to achieve a reduction in microbial population in the sample by weighing 1g of each sample and transferring into test tubes containing 9ml sterile water. A ten-fold serial dilution was carried out aseptically on each of the sample as previously described (16).

Heterotrophic bacterial counts in the samples were determined by the pour plate technique (15) on nutrient agar plates. The isolation and enumeration of potential pathogens was carried out using three different media; thiosulfate-citrate-bile salt-sucrose agar (TCBS) for *Vibrio* species, mannitol salt agar (MSA) for *Staphylococcus aureus* and eosin methylene blue agar (EMBA) for faecal coliforms. Hetero trophic bacteria plates were incubated at 28± 2°C for 24 hours, TCBS and MSA plates at 37^0C for 48 hours, and EMBA plates at 35^0C for 4 hours, followed by 14 hours at 45^0C. Discrete colonies on the culture plates were enumerated and recorded as colony forming units per gram (CFU/g) of the sample.

Identification of bacterial isolates

Discrete colonies were sub-cultured in nutrient agar plates to obtain pure cultures. Bacteria isolates were identified based on cultural, morphological and biochemical characteristics. Relevant conventional biochemical tests included catalase, coagulase, oxidase, citrate utilization, sugar fermentation, motility, methyl red and Voges Proskauer tests as described in Bergey's Manual of Determinative Bacteriology (17).

Statistical analysis

Data obtained from the microbiological assessment of the samples were analysed on SPSS package version 2.0 by one-way analysis of variance (ANOVA) with Duncan multiple range test for post-hoc determinations of significant differences ($\alpha < 0.05$).

Results:

Table 1 shows the mean total heterotrophic bacterial count (THBC), total coliform count (TCC), faecal coliform count (FCC), *Salmonella-Shigella* count (SSC) and total *Vibrio* count (TVC) of the crab samples (log10) of 4.281±0.085 (range 4.18-4.39); 4.187±0.078 (range 4.11-4.30); 4.115±0.081 (range 4.00-4.20); 4.076±0.058 (range 4.00-4.14); and 4.114±0.085 (range 4.00-4.23) respectively (p =0.003915 ANOVA). For the dog whelk samples, the mean THBC, TCC, FCC, SSC and TVC are 4.232±0.095 (range 4.11-4.36); 4.185± 0.095 (range 4.04-4.28); 4.082±0.068 (range 4.00-4.18); 4.062±0.055 (range 4.00-4.15) and 5.155±0.062 (range 4.08-4.23) respectively (p=0.028856 ANOVA) (Table 2).

The isolated bacteria pathogens from the shellfish samples are *Pseudomonas aeruginosa, Vibrio cholerae, Salmonella* Typhi*, Shigella* sp, *Corynebacterium* sp, *Escherichia coli, Bacillus subtilis, Staphylococcus aureu*s and *Proteus vulgaris* (Table 3). The most frequently isolated bacteria pathogen from the crabs is *Vibrio cholerae* in 80% of the samples while the least frequently isolated bacteria were *Bacillus* sp, *S. aureus* and *E. coli* in 20% of the samples. *Pseudomonas aeruginosa, Shigella* sp and

Salmonella sp were isolated in 60% while *Corynebacterium* sp and *Proteus vulgaris* in 40% of the crab samples. The most frequent bacterial isolate from the dog whelk samples is also *V. cholerae* in 80%, and the least frequent are *Proteus vulgaris*, *Corynebacterium* sp, *S. aureus* and *E. coli* in 20%. *Shigella* sp and *Salmonella* sp were isolated in 60% while *P. aeruginosa* and *Bacillus* sp in 40% of the samples (Table 3).

Table 1: Bacterial loads of crab shellfish (*Pachycheles pubescens*)

Sample	THBC (Log_{10} CFU/g)	TCC (Log_{10} CFU/g)	FCC (Log_{10} CFU/g)	SSC (Log_{10} CFU/g)	TVC (Log_{10} CFU/g)
Crab 1	4.398	4.301	4.079	4.146	4.230
Crab 2	4.322	4.204	4.176	4.079	4.114
Crab 3	4.279	4.114	4.000	4.000	4.079
Crab 4	4.230	4.114	4.114	4.114	4.146
Crab 5	4.176	4.204	4.204	4.041	4.000
Mean	4.281	4.187	4.115	4.076	4.114
SD	0.085	0.078	0.081	0.058	0.085

THBC = Total Heterotrophic Bacterial Count; TCC = Total Coliform Count; FCC = Faecal Coliform Count; SSC = *Salmonella/Shigella* Count, TVC = Total *Vibrio* Count. CFU/g = Colony forming unit/gram, SD = Standard Deviation

Table 2: Bacterial loads of dog whelk shellfish (*Nucella lapillus)*

Sample	THBC (Log_{10} CFU/g)	TCC (Log_{10} CFU/g)	FCC (Log_{10} CFU/g)	SSC (Log_{10} CFU/g)	TVC (Log_{10} CFU/g)
Dog whelk 1	4.362	4.279	4.114	4.146	4.230
Dog whelk 2	4.279	4.255	4.176	4.041	4.146
Dog whelk 3	4.230	4.204	4.000	4.079	4.114
Dog whelk 4	4.176	4.146	4.079	4.041	4.204
Dog whelk 5	4.114	4.041	4.041	4.000	4.079
Mean	4.232	4.185	4.082	4.062	4.155
SD	0.095	0.095	0.068	0.055	0.062

THBC = Total Heterotrophic Bacterial Count; TCC = Total Coliform Count; FCC = Faecal Coliform Count; SSC = *Salmonella/Shigella* Count; TVC = Total *Vibrio* Count; CFU/g = Colony forming unit/gram; SD = Standard Deviation

Table 3: Frequency of bacteria isolates from shellfish (crab and dog whelk) samples

Bacterial isolates	Crab samples (n=5)	Dog whelk samples (n=5)
Pseudomonas aeruginosa	3 (60.0)	2 (40.0)
Shigella sp	3 (60.0)	3 (60.0)
Vibrio cholerae	4 (80.0)	4 (80.0)
Salmonella sp	3 (60.0)	3 (60.0)
Bacillus sp	1 (20.0)	2 (40.0)
Corynebacterium sp	2 (40.0)	1 (20.0)
Esherichia coli	1 (20.0)	1 (20.0)
Staphylococcus aureus	1 (20.0)	1 (20.0)
Proteus vulgaris	2 (40.0)	1 (20.0)

Discussion:

Crab and dog whelk are highly nutritious, but susceptible to contamination and spoilage. Bacteriological assessment of samples of crab and dog whelk shellfishes from Okwan-Obolo estuary of Anambra State, Nigeria, shows that the shellfishes harbor various bacterial pathogens, which may have arisen from the contamination and faecal pollution in the ecosystem. Our findings align with those of Leight et al., (18) who reported that pathogenic and indicator bacteria such as the family *Enterobacteriaceae* in aquatic resources originates from the contamination of the water due to human waste products.

Food-borne diseases constitute a serious public health problem at a global level, and seafoods such as crabs are mainly related to outbreaks and cases of diseases including food poisoning. In our study, a high frequency of isolation of *Vibrio* sp was recorded in crab and dog whelk shellfishes. *Vibrio cholerae* is an important microorganism in human, clinical and food safety. The bacterium is capable of developing resistance to antimicrobials and producing toxins thereby generating different disease types including food poisoning from consumption of contaminated seafood (19). *Vibrio cholerae* and *S. aureus* are considered pathogens of cosmopolitan distribution and their main reservoir is human beings (19).

Different measures have been developed and established for the control and prevention of food contamination such as implementation of good hygienic practices, quality assurance system and microbiological criteria for the acceptance of products for consumption with emphasis on the hygiene of food handlers and storage conditions. *Escherichia coli* in seafood is considered a sanitary case and is a risk to consumers, if related to pathogenic strains especially diarrheagenic *E. coli* (20). However, the presence of non-pathogenic *E. coli* in shellfish is of public health interest since this bacterium is recognized as an indicator organism of faecal contamination suggesting the presence of other enteric pathogens. To ensure that consumed seafood does not harbor *E. coli*, some key measures have to be put in place such as maintaining the microbiological water quality, adequate hygiene conditions in handling process and bacteriological safety during all processes. The isolation of *E. coli* from the crab samples in this study points to the unsanitary status of the seashore where the samples were collected. The isolation of *Vibrio* sp from the samples is in agreement with the study of Vogan et al., (21) who reiterated that crabs such as Dungeness crab (*Cancer magister*), rock crab (*Cancer irroratus)* and tanner crab (*Chinoecetes opilio*) have been found to harbor a variety of Gram-negative and Gram-positive bacteria, and that gills of the fishes were the most common reservoirs of these organisms, including food-borne pathogens such as *Vibrio*, *Aeromonas*, *Acinetobacter*, *Klebsiella* and *Pseudomona*s species.

Another bacterium of public health importance is *Salmonella* specie, the causative agent of salmonellosis, a disease that is characterized by enteric (or typhoid) fever along with gastroenteritis, abdominal cramps and diarrhoea (22). The predominance of *Salmonella*

in crab in this study is associated with human and animal actions in the beach being an enteric bacterium from flora of both humans and animals (23,24). *Staphylococcus aureus*, *Shigella* and *Salmonella* are pathogens carried by human, and their occurrence in the crab and dog whelk shellfishes can be attributed to improper handling and cross contamination by humans. Considering the large consumption of shellfishes, it is important to create awareness to the public on the health risk of improperly cooked shellfishes. The pathogenic organisms isolated are known to be responsible for many illnesses including infantile diarrhoea, blood stream infections and meningitis by *E. coli* and *S. aureus*, salmonella food poisoning and acute gastroenteritis caused by *Salmonella* species (25). Some of these bacteria are also indigenous bacteria flora in water body, hence are common and widely distributed in the aquatic environment. Maintenance of bacteriological standard for the aquatic ecosystem, good sanitary practices and further processing (preservation) before consumption is necessary to improve the quality of edible shellfishes.

Conclusion:

Crabs and dog whelk from Okwano-Obolo estuary of Akwa Ibom State, Nigeria, harbor pathogenic bacteria including *Vibrio*, *Salmonella* and *Shigella* implicated in many disease conditions in humans, especially in persons with underlying medical or immunocompromising conditions. It is therefore important that shellfishes harvested from this estuary be properly processed before consumption. A better estuarine management should be framed to prevent transmission of pathogenic bacteria in water and the distributed shellfishes. Proper hygiene should be encouraged and toilet facilities provided in order to reduce contamination of the crab and dog whelk with bacteria associated with human faecal deposits at the seashore.

Contributions of authors:

AN and NA conceptualized the study; AN, NA, CU, DU and EU designed the labora tory methods; SA, AN, and EU collected the data; SA, AN, NA, CU and DU performed laboratory analysis of samples; AN, NA, CU, DU, EU and SA analysed the data; AN, NA, CU, DU, EU performed data validation; AN and SA curated the data and wrote the initial manuscript draft; AN, NA and CU revised the manuscript, and AN supervised the project. All authors agreed to the final manuscript.

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Conflict of interest:

Authors declared no conflict of interest

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