Microbiological Quality of Fresh Water Prawns (macrobrachium Spp) from the Volta River and its Possible Health Implications

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SUMMARY

The microbiological profile of food for human consumption is as important as its nutritional status. A knowledge of the resident microflora of fresh water prawns can help control and prevent diseases of microbiological origin. This paper reports the microbiological quality of fresh water prawns harvested from the Volta River from 1988 to early 1989. The handling and processing habits were also examined as a background information for formulating improved processing methods.

The bacterial species isolated from the river water and the prawns were enteric non-spore forming ones namely Aeromonas sobria, Enterobacter agglomerans, E. aerogenes, E. cloaca, Citrobacter freundii, Klebsiella pneumoniae, Plesiomonas shigelloides, Serratia liquefaciens, S. marinoruba, S. fonticola and Morganella morgani. M. morgani and Pleisomonas shigelloides are being recorded for the first time in fresh water prawns in Ghana. These are potentially pathogenic microorganisms.

The public health implications of these finding are discussed and further work suggested.

INTRODUCTION

Supplying man with food is becoming an increasing problem because of the rapidly expanding population and growing desire of the developing nations for a greater variety of nutrients the year round[1]. Although meat and meat products are possible rich source of protein for humans they are in short supply in developing countries like Ghana. Fish, crustaceans, shell-fish have become candidate protein supplements for human consumption.

Fish and crustaceans harvested from offshore marine waters are generally expected to be free of potentially pathogenic bacteria normally associated with warm-blooded land animals and humans [2,3].

However, when caught from estaurine waters, in rivers and lakes, their microflora may reflect the degree of contamination in the environment.

Water is one of the chief vehicles of gastro-intestinal disease because water frequently washes soil bacteria and sewage microorganisms during heavy rains into large bodies of water. The main diseases carried by water are enteric fever, dysentry, cholera, infectious hepatitis and gastro-enteritis [4]. Prawns and other shell-fish may become infected when caught in polluted water. Table 1 summarises principal groups of bacteria pathogenic for humans. Living crustaceans harvested from fresh water carry populations of predominantly gram-negative psychrotrophic bacteria on their external surfaces [5]. Those caught in polluted waters may carry bacteria derived from human and animal sources.

TABLE 1

Selected Principal groups of gram-ve bactria that include species pathogenic for humans

Genera of medical importance	Disease(s) caused in humans
Bacillus	meningitis, endocarditis, endophthalmitis, conuc- tivitis, acute gastero- enteritis.
Clostridium	botulism, tetanus, gas gangrene dysphonia profuse diarrhoea.
Streptococcus	Erysipelas, peurperal fever, some throat meningitis
Corynobacterium	Urinary tract infection, diphtheria (wound and skin).
Bacillus	Meningitis, endocarditis, endophthalmitis, conjucti- vitis, acute gastroenteritis.
Plesiomonas	diarrhoea
Serratia	opportunistic infection causing pneumonia, bacteremia, endocar- ditis.
Klebsiella	Gastroenteritis, infection of respiratory and urinary tract.
Morganella	arthritis, urinary tract infection, aerobic
Enterobacter	Urinary tract infection, sepsis.
Proteus	Urinary tract infection, bacteremia, pneumonia.
Yersinia (Pasteurella)	Febrile diarrhoea, bacteremia or endocarditis, severe abdominal pain.
Campylobacter	fever, diarrhoeal disease, enteritis.
Escherichia	abrupt onset of diarrhoea.
Pseudomonas	sepsis, heat-labile exotoxins
Vibrio	cholera, sepsis or enteritis
Clostridium	botulism, tetanus, gas gangrene, dysphonia

The chief importance of prawns from the public health point of view is their ability to give rise to enteric fever. Enteric bacteria are commensals or pathogens in the intestinal tract of vertebrates eg. *E. coli* or in soil and water eg. *Klebsiella, Enterobacter, Aeromonas, Citrobacter, Aerobacter, Serratia, Proteus* and *Vibrio* [6].

Insanitary processing conditions could bring about an increase in population of the bacteria and may also lead to recontamination of the product with potentially harmful bacteria.

There is hardly any information in the literature on the microbiological quality of prawns harvested from the Volta River in Ghana although prawns from the river from Ada and Kpong are cherished good protein supplement for Ghanaians. We began preliminary examination of the microflora of prawns in the Volta River to see if microorganisms resident in the crustacean represent a major health hazard at their normal level of occurrence.

MATERIALS AND METHODS

Fresh water prawns *(Macrobrachium spp)* were obtained from the river and from the market at Ada (5° 47N 0° 38E). The samples were kept on ice and brought immediately to the laboratory for microbiological examination. Water samples from the river were also analysed for total heterotrophic, coliform and total aerobic bacterial loads.

Total Heterotrophic Bacterial Count

The multiple tube dilution technique for determining Most Probable Number, MPN, was followed [7]. Aliquots of test samples were plated in Tryptone Soya Broth (TSB) and then incubated at 35°C for 24h.

Coliform Organisms:

De-headed and de-shelled prawns were macerated (10g samples) and diluted nine times its weight with 0.1% peptone as diluent to obtain 0.1g fresh wt/ml as above. Coliform organisms in river water and prawns were determined in Macconkey Broth after incubation at 37°C for up to 48h.

Total Aerobic Bacteria, Tab:

Total aerobic bacterial loads in the water and prawns were estimated by inoculating plates containing Plate Count Agar (PCA) and then incubated at 35°C for 5 days. Typical colonies appearing were enumerated and population expressed as $\log_{10}$ CFU/g sample.

Confirmatory Biochemical Test:

Colonies were picked and plated on Nutrient Agar. Further confirmatory biochemical tests were carried out using Biotest biochemical identification test kit (Denka Seiken Limited, Tokyo, Japan). Biochemical and fermentation characteristics of *Morganella morgani* have been reported by Amoah et al [8]. Characteristic positive reaction for cytochrome exidase test, ability to ferment carbohydrates (inositol), positive ornithine decarboxylase test and non haemolytic activity on blood agar plate (in addition to others) were used to identify one isolate as *Plesiomonas shigelloides*[9].

RESULTS

Total Heterotrophic Bacterial Count:

Results obtained are presented in Table 2. Total heterotrophic bacterial counts in prawns *(Macrobrachium spp.)* purchased from the river side were between 3.8 - 5.4 $\log_{10}$ MPN/g (Table 2). Analysis of variance showed that there was no statistical difference ($p > 0.05$) between heterotrophic bacteria on prawns which were either surface-sterilized or non surface-sterilized prior to plating on nutrient agar. During the two penultimate sampling periods only, total heterotrophic bacteria load obtained in the market samples was about 1.6 - 1.9 log cycles more than what existed in the prawn samples purchased from the river side. Total

heterotrophic bacteria resident in water sample was between 3.2 -4.2 log MPN/ml (Table 2).

TABLE 2

Microbiological population profile of prawns (Macrobrachium sp.) harvested from the Volta River at Ada (2 months intervals)

General heterotrophic bacteria log_{10} MPN/ml or/g					Coliform bacteria (log_{10} MPN/ml or/g)					Total aerobic bacteria (log_{10} CFU/g or ml)				
R		M		W	R		M		W	R		M		W
a	b	a	b		a	b	a	b		a	b	a	b	
5.2	5.2	5.2	5.4	3.5	5.2	5.2	5.2	5.3	1.4	3.1	3.9	5.5	4.3	3.2
5.4	5.4	5.2	5.2	3.4	5.2	5.2	5.2	5.2	1.6	4.7	4.3	5.3	4.1	3.4
3.8	3.8	5.4	5.2	3.2	3.4	3.2	3.5	3.9	2.7	4.7	4.4	5.6	4.9	3.6
3.9	4.2	5.4	5.2	3.9	4.3	4.2	3.8	3.7	1.5	4.3	4.1	5.8	4.3	3.2
3.9	4.1	5.8	5.4	4.2	4.4	4.3	3.9	3.8	1.8	3.3	3.0	5.5	4.4	3.5

R: Prawns obtained from river side
M: Prawns purchased from the market
W: Water samples
a: Non-surface sterilised
b: Surface sterilized with 70% Alcohol.

Coliform Bacteria:

Coliform bacteria population in fresh prawns obtained from the river side at Ada was between 3.4 - 5.2 log_{10} MPN/g; corresponding market samples of prawns were contaminated with about the same level of population of coliforms (3.5 - 5.2 log MPN/g) (Table 2). Surface-sterilized and non-surface-sterilized prawns from either the river side or the market did not differ significantly ($P > 0.05$) in coliform bacterial population level after plating. A lower level of coliforms (1.4 - 2.7 log_{10} MPN/ml) were detected in the water sample (Table 2).

Total Aerobic Bacteria, TAB:

Table 2 summarises results obtained. TAB count in fresh prawns harvested from the river was between 3.1 - 4.7 log_{10} CFU/g. Market samples were 0.6 - 2.4 log cycles higher than what existed in prawns obtained from the river side (Table 2). The river water had a TAB count of 3.2 - 3.6 log_{10} CFU/ml.

Confirmatory Biochemical Test:

The predominant microflora resident in the prawns purchased from the river side and the market were enteric non-spore forming gra-negative ones.

Species encountered were *Aeromonas sobria, Enterobacter agglomerans, E. Aerogenes, E. cloaca, Citrobacter freundii, Klebsiella pneumoniae, Plesiomonas shigelloides, serratia liquefaciens, S. marinoruba, S. fonticola* and *Morganella morgani*. The water samples contained *Escherichia coli* and *Pseudomonas spp* as well as those species named above. *M. morgani* and *Plesiomonas shigelloides* are being recorded for the first time in fresh water prawns in Ghana.

DISCUSSION

Bacterial infections are important causes of food-borne infections and other diseases in humans particularly in developing countries where strict adherence to microbiological specifications for foods is virtually non-existent or where regulations in existence are not enforced. The Volta river in Ghana is used extensively for fishing; prawns *(Macrobrachium spp)* are one of the major shell fishes harvested for human consumption.

Living crustaceans harvested from fresh water carry population of 10^2 - 10^3/g of predominantly gram negative psychrotrophic bacteria on their external surfaces [5]). the number in the alimentary canal is 102 - 10^7g depending on the amount of food present). Aquatic animals like prawns may accumulate bacteria from the environment because of their filter feeding habit [5]. We encountered the following range of bacterial populations in de-headed, de shelled prawns: general heterotrophic (3.9 - 5.8 log10 MPN/g); coliform bacteria (3.9 - 5.2 log10 MPN/g); total aerobic counts (3.1 - 5.8 $\log_{10}$ CFU/g). This data agrees with that stated by Speci[5]. The microflora of shrimp *(Pandalus platyceros)* consisted of 68% gram positive and 32% gram negative bacteria. The gra negative was composed of *Flavobacterium, Pseudomonas* and *Enterobacteriaceae*[10].

Microflora encountered in the prawns *(Aeromonas sobria, Enterobacter agglomerans, E. aerogenes, E. cloaca, Citrobacter freundii, Klebsiella pneumoniae, Plesiomonas shigelloides, Serratia liquefaciens, S. marinoruba, S. fonticola* and *Morganella morgani)* were predominantly enteric non-spore forming bacteria. The water samples from the Volta river contained *Esherichia coli* and *Pseudomonas spp* as well as *A. sobria, E. agglomerans, E. aerogenes, P. Shigelloides* and three *Serratia spp.* Isolation of the above named enteric bacteria from both the river water and prawns is an indication of environmental pollution. Coliform counts of more than 100 per ml of water in some areas of the Volta river estuary has been reported[11]. Our present data confirms their findings as well as identifying the individual species responsible for the pollution of the water and the prawns.

The raicroflora encountered in prawns from Ada contain many potentially pathogenic bacteria (Table 1). Members of the Enterobacteriaceae are common in soil, water etc. as parasites and pathogens of man, other animals and plants. The genera in soil or water includes *Citrobacter, Edwardsiella, Aerobacter, Aeromonas, Enterobacter, Erwinia, Escherichia, Hafnia, Klebsiella, Morganella, Proteus, Serratia, Shigella, Vibrio* and *Yersinia* [6]. We encountered seven of these genera in the river water and in prawns from Ada. Water is one of the chief vehicles of gastro-intestinal disease because water frequently washes soil bacteria and sewage microorganisms during heavy rains into large bodies of water. The main diseases carried by water are enteric fever, dysentry, cholera, infectious hepatitis and gastroenteritis[4].

Aeromonas spp cause opportunistic nosocomial infections; septicaemia, meningitis and pneumonia. They may also produce several exotoxins and enterotoxic activity[12]. Other reports have associated haemolysin production by the *Aeromonas* with cytotoxicity [13,14] *Serratia spp* are sometimes associated with gastroenteritis and are also known to cause nosocomial infections[15]. *Enterobacter aerogenes* may be found causing urinary tract infection and in sepsis. Although K. pneumonia was

originally known as a respiratory pathogen, it is now commonly encountered in hospital infections of the respiratory and urinary tracts and it produces a heat-stable enterotoxin that gives rise to diarrhoea similar to those produced by *Escherichia coli* [16]. This organism is also responsible for nosocomial infection of wounds[15]. *Plesiomonas shigelloides* causes diarrhoea and may produce an enterotoxin which may, functionally and immunologically be related to the prototype cholera enterotoxin choleragen[17]. *Morganella morgani* is an opportunistic organism infrequently isolated from human faeces. It is responsible for septic infections notably urinary tract infection, bacteraemia and wound infections. *M. morgani* is also a causative agent of diarrhoea[18]. Recently Schonwetter and Orson[19] reported that *M. morgani* caused chronic arthritis in an elderly patient.

Although coliforms encountered in the market and river side samples did not differ significantly ($p > 0.05$), TAB counts on market samples were 0.6 - 2.4 log cycles higher than what existed in the prawns bought from the river side. Handling frequently brings about an increase in population of bacteria. Insanitary selling shelves and fish processing paraphernalia may lead to contamination of the product with potentially harmful bacteria and the microflora may also change during spoilage. We will follow up with a detailed study of the enteropathogenic characteristics of these species in selected animal models.

It is also essential to examine the prawns for other possible microorganisms in the head-on, shell-on, storage conditions to see if the microflora may change significantly during extended marketing condition.

In recent times gamma irradiation has taken a leading role in the preservation of world food supplies. Future studies will examine the effect of gamma irradiation on the shelf-life of Ghanaian prawns harvested from the Volta River.

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Chairman: Thank you Mrs. Amoah. Now the floor is open for questions.

Orleans Aggrey: I would like to say that it is mentally consoling to hear from the last speaker that the sort of bacteria that exist in the prawns that we eat could be said to be in fairly acceptable limits. But I am not too sure that I heard the previous speaker making a point about the sort of high incidence of bacteria in the coastal waters and then the high catch of fish. What I want to know is, is there any correlation between the incidence of high bacteria in the coastal waters and the high catch of fish during the high peak season for the fishermen? Because it seems to me, when we begin to talk about science, we are a little more interested in finding the extent to which such scientific research findings could be related to our every day life. So I would want to suggest that our attention is drawn to this very important factor because most of us who live along the coast keep on eating fish and we are not too sure about the extent to which the bacteria that exist in the coastal waters constitute a sort of health hazard to us. Thank you.

Speaker: When you talk about bacteria in a water environment, you are talking about the normal bacterial flora in the water as well as the pollution bacteria. The bacterial flora increases as and when there are rains. Normally when there are a lot of rains, there is an up build of bacteria, and this includes the natural bacterial flora of the lake. However, with our type of exercise, we are monitoring pollution

bacteria and though they also play a part in the ecology of the lake, we are mostly concerned with its implications so far as health is concerned. Does that answer your question?

Mr. Orleans: Yes it does except that if the Gentleman wouldn't mind being a little more explicit on this particular issue. Namely, bacteria pollution in the coastal waters and the fish that we catch along the coast.

Mr. Aidoo: I said in my paper that we are monitoring bacteria that cause pollution. The paper I presented was basically on the bacteria that we isolated from the sea water. We are yet to go on to do one which involves isolation of these organisms from sea foods. That is yet to be done; but so far, what we have done is concerned with the bacteria that we isolated in the sea water.

Dr. Odamtten: Just to contribute to the discussion; the shell fishes in the rivers as well as in the sea are what we call filter feeders. The water passes through the alimentary canal of the fish or whatever, and in so doing, the animal will concentrate the bacteria within itself. So then you are looking at what has been concentrated within the fish or the prawn etc. The fact that we have a lot of bacteria in the water does not connote that when we look at the fish, we are going to get an equal amount of the same bacteria inside the fish. There may not be any correlation.

Comment: Well, I don't want to disappoint you, but you can get food poisoning from eating infected fish and clams. Work has been done on some types of fish, sea clams and shrimps and if these are infected, you may get the infection from them if they are not properly cooked.

Comment: My contribution is just to indicate that apart from isolation, one sometimes needs to determine whether the organism in question is able to elicit some of the pathogenic mechanisms associated with that class of organism, particularly elaboration of enterotoxins and the presence of factors which enable it to attach, and this is important particularly in the case of *E. Coli*, we know of enterotoxigenic and enteropathogenic *E. Coli*. So my suggestion is, in cases like this, it will be useful to go further on and characterize these organisms before we can specifically say that they are capable of causing disease in man.

Comment: I will make a comment first and then ask a question. My comment is, from the ongoing, it appears we want to say that there are foods in which we find some microorganisms, then we are going to say they are going to cause food poisoning. But I think if you look at our food habits around here in Ghana, we do a lot of cooking. And as one of my professors said, it is not by accident but something we have acquired through age-old habits, seeing that foods eaten raw brings disease. So I think the main trust should be preventive, i.e., the way we handle these foods; fish, crabs or prawns etc. Because if we go to the market places once all these foods are there, they have been handled, they will give a high count of microorganisms. Therefore, I think in management terms, we are going to look at the handling as it is done in cases of "eggs". Now my question is, in your talk, Madame Speaker you did mention that you are going to go on and determine the antibiogram i.e. the sensitivities. This to me shows that you are thinking in terms of using antibiotics for these, but I think that will be an error because in cases of food poisoning it is advised that most times it is self limiting and therefore encouragement of antibiotics will not be in order.

Speaker: The use of the antibiogram is just to ascertain the fact that what we have identified (i.e. the local isolate) is either susceptible or resistant to antibiotics. So that when the person has the infection, one knows exactly how to deal with it. The other point is on the fact that we cook most of our food. Yes we do, but some of these organisms produce heat stable toxins which even after cooking, are still within the food and that is when we have to be ab-

solutely sure. The handling, as with prawns which we have fried and then put in polythene bags and sold, this handling process can lead to a lot of contamination though the food has been cooked, it is eaten as it is purchased and that can also bring in contamination and then cause disease. In this case it can be the consumption of something which is heat stable or the organism itself.

Chairman's Closing Remarks: I would like to congratulate the Noguchi Memorial Institute for Medical Research because I think that it is playing the expected role in the control of communicable diseases and malnutrition in the country. I will say that its efforts will be supported both from official circles as well as from the general public. And I do hope that the future, for the Institute will be very bright. Thank you.