



Vibrio parahaemolyticus: A Potential Diarrheagenic Pathogen on Gombe Fresh Vegetables

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ABSTRACT

Vibrio parahaemolyticus are considered one of the main causes for bacterial gastroenteritis worldwide. Research conducted reports outbreaks involving raw fruits and vegetables in other part of Nigeria, however, little is known on the occurrence of this pathogen in Gombe especially on vegetables. In the light of these, this work aimed at determining the presence of this pathogen in fresh vegetables sold in Gombe metropolis. Three types of vegetables were sampled, namely cabbage (*Bressica oleracea*) 20 samples, tomato (*Solanum lycopersicum*) 20 samples and lettuce (*Lactuca sativa*) 20 samples. Sample were processed by enrichment in alkaline peptone water (APW) and cultured on thiosulfate-citrate-bile salt sucrose agar (TCBS). Suspected growth, especially that appeared green on TCBS were subculture on nutrient agar (NA) and subjected to biochemical tests, which include Triple Sugar Iron (TSI), Oxidase test, arginine dihydrolase test, Lysine and ornithine decarboxylase, Moeller decarboxylase base medium (Difco) amended with an amino acid at a concentration of 1%, Cells grown in the presence of 0, 6, 8 and 10 % (wt/vol) NaCl in nutrient broth, Acid production from 1% arabinose, sucrose and lactose fermentation, Methyl red and Voges-Proskauer reaction and Urease test. Of the sixty 60 samples collected 46.67% growth was observed TCBS and only 1.67% of samples harbored *V. parahaemolyticus*. In tomato, lettuce cabbage samples had 45%, 55% and 40% *Vibrio* growth was observed respectively. Ten 10% (equivalent to 1.67 of all samples collected) was confirmed to be growth of *V. parahaemolyticus* from cabbage sample collected at Shongo market.

KEYWORDS: Gastroenteritis, Foodborn, Halophilic

BACKGROUND

V. parahaemolyticus can survive well in aquatic environment due to its ability to endure halophilic condition. Others report that *V. parahaemolyticus* can be isolated from seafood such as fish, shellfish and crustacean as well as sea water [1]. *Vibrio parahaemolyticus* is a common foodborne pathogen in Asia [2,3]. It is a gram-negative halophilic bacterium that lives in marine and estuarine environments around the world [4]. It causes watery diarrhea often with abdominal cramp, nausea, vomiting fever and chills [5]. Outbreaks of foodborne disease caused by *V. parahaemolyticus* are generally associated with contaminated raw or undercooked seafood [6,7]. Fruits and vegetables, particularly those eaten raw and without peeling, have been demonstrated to be the transmission vehicle for many microorganisms [8].

There are several gastroenteritis cases involving contamination of *V. parahaemolyticus* and other pathogenic bacteria in fresh vegetables all over the world. Gopal et al. [9] stated that *V. parahaemolyticus* is the cause of 20 - 30% of food poisoning in Japan. Chang and Chen [10] stated that fruits and vegetables can act as vehicles to transmit food borne diseases apart from other food products in Taiwan and Western countries. Another latest outbreak involving *V. parahaemolyticus* was reported in Singapore in the April 2009. In Gombe Nigeria, vegetables are often eat raw/fresh

as salad, some time with other food, mostly rice and couscous, it is in view of these the research wish to determine the occurrence *Vibrio parahaemolyticus* in fresh Lettuce, cabbages and tomatoes sold in Gombe metropolis isolating and biochemical identify the isolates

METHODS

Study Area Description

Gombe state is one of the six northern states of Nigeria. It is located between latitude 9° 30' N and 12° 30' N and longitudes 8° 45' E and 11° 45' E of green which meridian. Gombe state borders with yobe and borno to the north and east, and taraba and adamawa to the west and bauchi to the west. Gombe state occupies a total land area of about 20,265sq.km.

Sample Collection

The study was conducted between August and September. Sixty (60) vegetable samples (tomato lettuce and cabbage) were collected from two market in Gombe metropolis, Gombe main market and Shongo market. Twenty (20) tomatoes (10 Gombe main market and 10 shongo market), 20 lettuce (10 Gombe main market and 10 shongo market), 20 cabbage (10 Gombe main market and 10 shongo market). Each sample was inserted in to white polythene to avoid mixing with other samples and transported directly to biology laboratory federal university Kashere.

Sample Processing

Vegetable samples were processed based on the method described by Farjana and Rashed with little modification, thus, 1gram of vegetable sample was homogenized in 9ml peptone water and incubates at 37 for 18- 24 hours to enrich *Vibrio parahaemolyticus* species specifically.

Isolation and Identification

Isolation and identification was based on method described by Mohammadjavad and Konneman [11]. Growth from alkaline peptone water was sub cultured on selective media TCBS, plates were inverted and incubated for 24 hours at 37. Colony morphology was examined and recorded. In each TCBS agar plate, 1ml of culture from alkaline peptone water (APW) was poured. The petri dishes were numbered and incubated at 37°C for 24 hours. After 24 hours, green colonies appeared on TCBS agar plates. For the isolation of *Vibrio parahaemolyticus*, green colonies were taken and subjected for biochemical test as described by Mohammadjavad (2013) and Konneman et al, (1994).

Biochemical Tests

Biochemical tests carried out include Triple Sugar Iron (TSI) was prepared as recommended by the manufacturer and results were read after incubation at 37°C for 24 h. Oxidase test. The method used for the arginine dihydrolase test was conducted in accordance with Thornley. Lysine and ornithine decarboxylase assays were performed by using Moeller decarboxylase base medium (Difco) amended with an amino acid at a concentration of 1% (wt/vol). Cells grown in the presence of 0, 6, 8 and 10 % (wt/vol) NaCl in nutrient broth was also conducted and turbidity measured as described by Barrow and Feltham [13] and Kaysner and DePaola [14]. Acid production from 1% arabinose, sucrose and lactose fermentation were determined. The methyl red and Voges-Proskauer reaction were conducted by using MR-VP medium and urease test in accordance with Konneman et al, [12] incubated at 37°C for 48 h.

RESULTS

Out of the sixty (60) samples of vegetables collected and analyzed, 46.67% (28) growth on thiosulfate citrate bile salt sucrose agar (TCBS) was observed. However, only one (1.67) isolate was confirmed to be *V. parahaemolyticus* based on the biochemical test conducted. In samples collected and analyses from tomato and lettuce samples, 45%, 55% growth was observed respectively. Similarly, in the cabbage samples, there was 40% growth of other species of *Vibrio*, out of which 10% (1) (equivalent to 1.67 of al samples collected) of the isolates was confirmed to be growth of *V. parahaemolyticus* from sample collected at Shongo market as shown in table 1 below.

Table 1: Showing summary of results of samples analysis in respect to place of collection and types of sample
Key: % = percentage, TCBS = thiosulfate-citrate-bile salt sucrose agar

Source	No. of samples collected	No.(%) of growth on TCBS	No. of positively identified	Sample type
Shongo market	10	3 (30.00)	1 (10.00)	Cabbage
Main market	10	5 (50.00)	0	Cabbage
Shongo market	10	5 (50.00)	0	Lettuce
Main market	10	6 (60.00)	0	Lettuce
Shongo market	10	7 (100)	0	Tomato
Main market	10	2 (20.00)	0	Tomato
Total	60	28 (46.67)	1 (1.70)	

DISCUSSION

From the epidemiological stand point of view, the low rate of percentage isolation could be attributed to environmental factors, such as survival in halophilic aquatic environment as previously reported by Drake [1]. Similarly, seawater salinity exerts a strong influence on the survival of *Vibrio* spp. Low salinity may favor *V. vulnificus* growth in shellfish, while *V. parahemolyticus* tolerates higher salinity values [16]. Previous studies, had attributed the prevalence of pathogenic *Vibriosis* in general, appears to be influenced by temperature. Panicker et al., [17] reported that, seasonal variation and cycle are considered to correlate with water temperature that affects the abundance of *V. parahemolyticus*.

Fruits and vegetables were reported to exhibit potent antibacteria substances, especially the non greenish vegetables because of their phenolics and organic acids contents. For example, the antimicrobial activity in *Capsicum* was reported to be due to the phenolic compound and 3-hydroxycinnamic acid (coumaric acid) [15]. Lee and his colleagues studies the antibacterial activity of group of fruits and vegetables including: aronianberry, asparagus, bell pepper, beet, blackberry, blueberry, broccoli, carrot, cucumber, cherry, cranberry, garlic, ginger, grape, red onion, red cabbage, rhubarb, rutabaga, raspberry, pomegranate, spinach, strawberry, and green tea and reported that all green vegetables have no antibacterial activity on *Staphylococcus epidermidis* and *Klebsiella pneumonia* whereas all purple and red vegetable and fruit juices have showed antibacterial activities [18], so the isolation of one *V. paraheamolyticus* from Shongo market could be supported by the types of cabbage analyses, which coincidentally is not red cabbage that was previously eported to contain antibacterial substance. Although the isolation of *V. paraheamolyticus* from Shongo market could be a coincidence, however by inference, cabbage sold in Shongo constitute more public health risk than once sold at Gombe main market.

Owning to the fact that Chang and Chen [10] stated that fruits and vegetables can act as vehicles to transmit food borne diseases apart from other food products and the isolation *V. parahaemolyticus* from fresh vegetables, the research found it important to conclude that state ministry for Health should always be on alert, develop and use active surveillance strategy, whenever a gastrointestinal illness is reported, to determine the potential pathogen involved, other than the traditionally sought of.

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