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Prevalence of *Vibrio cholerae* in different food samples in the city of Dhaka, Bangladesh

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Abstract

Among the food borne disease outbreaks, cholera, caused by *Vibrio cholerae*, is a well known issue in Bangladesh. Present study attempted to randomly isolate *V. cholerae* from 40 types of food samples categorically including meat, fish, vegetables, fruits, street food, bakery shop food, fast food, sweets and dairy products. Cultural and biochemical tests revealed that all the samples harbored *V. cholerae*. The highest frequency of *V. cholerae* was observed in the chicken samples collected from Mailbag Market (4.2×10^7 cfu/g) and the lowest load was observed in the frozen meat sample collected from a super shop (1.1×10^3 cfu/g). Such a bacterial load indicated a high risk to public health. The study of antibiogram of the isolates revealed high resistance against ampicilin, chloramphenicol, nalidixic acid, trimethoprim-sulphamethoxazole, tetracycline, and cefotaxime, whereas sensitivity was observed against ciprofloxacin, neomycin, imipenem and erythromycin. Overall, our study found a significant number of *Vibrio* spp. including the drug resistant ones in the locally available common foods, which might pose serious public health hazard.

Introduction

Vibrio cholerae has long been known to be responsible for the life threatening secretory diarrhoea termed as Asiatic cholera or epidemic cholera (Ryan and Ray, 2004; Faruque and Nair, 2008). The global disease burden has been estimated to be 3-5 million cases and accounts for a total of 100,000-130,000 death per year (WHO, 2010). In Bangladesh, the situation is quite vulnerable. A number of studies have shown that the epidemic outbreaks in Bangladesh usually occur in a manner of bimodal seasonality, particularly during the spring and in the late monsoon. In 1991, a major epidemic caused 210,000 and 235,000 cholera cases and over 8000 deaths (Siddique *et al*., 1992). A recent surveillance carried out in Dhaka, Bangladesh, intriguingly revealed a trend of decreasing fatality rate with increasing infection rate over past 3 decades (Akanda *et al*., 2012). This finding raised doubt about the existing preventive measures taken to subjugate the disease burden.

Although cholera is primarily known as a water-borne disease in the endemic regions including Bangladesh, contamination of foods can also be an imperative mode for cholera transmission (Glass and Black, 1992). Many studies have reported that foods including vegetables, fruits, seafoods, dairy products, poultry and meat products and others can become contaminated with *Vibrio* spp. through improper handling, undercooking, washing with unhygienic water and by the use of untreated night soil (Feachem, 1981; Huq *et al*., 1983; Rabbani and Greenough, 1999). Due to poverty and poor sanitation, cholera is highly prevalent in developing countries like Bangladesh where many outbreaks has been implicated to the consumption of faecally-contaminated foods such as rice, millet gruel, and vegetables (Rabbani and Greenough, 1999).

Formerly, it was assumed that cholera epidemics were unlikely to occur by antibiotic-resistant strains as the bacteria were thought to lack the ability to retain resistance plasmids. However, in late 1970s, it was proved to be incorrect as cholera epidemics occurred by the antibiotic resistant strains in the United Republic of Tanzania and Bangladesh (Sack *et al*., 2001). It has also been reported that due to inappropriate use of antibiotics, different enteric pathogens including *V. cholerae* are becoming increasingly resistant which underlines the pervasiveness of the pressures that lead to the emergence and spread of resistance (Sack

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et al., 2001).

In Bangladesh, no epidemiological survey of *Vibrio* infection due to the general as well as available food ingestion has been carried out. Along these lines, current study was an attempt to find out the level of contamination by *V. cholerae* in different food items consumed by a large number of people in the city of Dhaka, Bangladesh. Also, we focused on the resistance pattern of the *Vibrio* isolates against the commonly used drugs for a better management of *Vibrio* caused food borne diseases in our country.

Materials and Methods

Sample collection, processing, enrichment, cultivation and biochemical identification of V. cholerae isolates

Samples were collected aseptically in the month of September 2011 from different places of Malibag & Mouchak area in the Dhaka city of Bangladesh. 10 gm of each sample was collected and added aseptically in a sterile homogenizing beaker containing 90 ml of sterile normal saline and homogenized. Samples were then subjected to 10 fold serial dilution up to 10^{-5}. From 10^{-2} and 10^{-4} dilutions, 0.1 ml of samples were spread onto Thiosulfate–Citrate-Bile-Salt-Sucrose (TCBS) agar (Difco Laboratories, Detroit, Mich) and incubated at 37°C for 18 to 24 hours. Yellow colonies from TCBS were streaked on Taurocholate Tellurite Gelatin Agar (TTGA) (Difco Laboratories, Detroit, Mich) for further identification of *V. cholerae*. The shape, arrangement & Gram reactions of the isolates were observed under bright field microscope at 1000× magnification (Human, Humascope). Biochemical tests including triple sugar iron (TSI) agar test, motility test, Voges-Proskauer (VP) test and salt tolerance tests were performed to confirm the *V. cholerae* isolates (Cappuccino and Sherman, 1996).

Determination of antimicrobial susceptibility by modified Kirby-Bauer method

All the isolates were tested for antibiotic susceptibility against 10 antibacterial drugs by disc diffusion assay on Mueller-Hinton Agar (Difco, Detroit, MI) with antibiotic discs (Neo-Sensitabs, Rosco, Denmark) including ampicillin (10 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), nalidixic acid (30 µg), trimethoprim-sulphamethoxazole (10 µg), imipenem (10 µg), tetracycline (30 µg), erythromycin (15 µg), neomycin (30 µg), chloramphenicol (30 µg). A single colony was inoculated into 2 ml of Mueller-Hinton broth, and incubated at 37°C for 4 hours. The turbidity of the growing cultures was then adjusted to a 0.5 McFarland standard. A sterile cotton swab was dipped into the adjusted suspension within 15 min and the excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then spread evenly over the entire surface of the LB agar plate to obtain uniform inoculums. Plates were then allowed to dry for 3-5 min. Antibiotics impregnated discs were then applied onto the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. Five discs (four antibiotics discs and one blank disc as control) were placed in each Petri dish. Even distribution of discs and minimum distance of 24 mm from center to center were ensured. Within 15 min of the application of the discs, the plates were inverted and incubated at 37°C. After 16-18 hours of incubation, plates were examined, and the zone diameters of complete inhibition were measured in millimeter scale. The zone diameters of complete inhibition for individual anti-microbial agents were recorded as susceptible, intermediate and resistant categories according to the interpretation table supplied by the manufacturer (Oxoid limited, England) (Table 2).

Results

Colony morphology, phenotypic and biochemical traits of isolates

Following incubation for 24 hours, typical colonies on TCBS & TTGA media were initially considered as *V. cholerae*. Shiny yellow colonies (2-3 mm) appeared on TCBS and grey, flattened colonies (1-2mm) surrounded by opaque zone appeared on TTGA. All the isolates exhibited similar staining patterns. They were found to be Gram negative, endospore less and comma-shaped which is a characteristic trait of V. cholerae. Biochemical characteristics of *V. cholerae* from different samples were also observed. All the isolates gave positive results for TSI, oxidase, methyl red and motility test. The organisms showed their salt tolerance up to 6.5% NaCl.

Prevalence of V. cholerae in different food samples: ***Meat and fish samples***

Chicken samples exhibited higher count (4.2×10^7 cfu/g) than all the other samples (Table 1). The lowest bacterial count (1.1×10^3 cfu/g) among all the food samples was found in a frozen meat sample collected from a super shop. All the fish samples that were included in this study exhibited an elevated bacterial load. The highest number was observed in a frozen fish sample having 1.5×10^7 cfu/g whereas the lowest count was found in a dry fish sample (1.0×10^5 cfu/g).

Table 1. Enumeration of *V. cholerae* in different food samples

Sample	Sandwich	Name of the sampling site	Count (cfu/g or cfu/ml) of *V. cholerae* on TCBS
1	Beef	Mirpur 11 Market	1.3×10^3
2	Mutton	Malibag Market	1.5×10^4
3	Chicken	Malibag Market	4.2×10^7
4	Frozen meat	Super Shop	1.1×10^3
5	Shrimp	Malibag Market	3.7×10^6
6	Shrimp	Super Shop	2.6×10^5
7	Dry Fish	Malibag Market	1.0×10^5
8	Rupchanda Fish	Malibag Market	8.5×10^6
9	Poa Fish	Malibag Market	2.5×10^5
10	Frozen fish	Super Shop	1.5×10^7
11	Cucumber	Malibag Market	5.1×10^6
12	Carrot	Malibag Market	4.1×10^5
13	Tomoto	Malibag Market	4.2×10^5
14	Cauliflower	Malibag Market	1.1×10^7
15	Cabbage	Malibag Market	7.6×10^6
16	kolmisak	Malibag Market	2.5×10^5
17	Helencha sak	Malibag Market	8.25×10^5
18	Pani Fol	Malibag Market	2.1×10^6
19	Guava	Malibag Market	3.3×10^6
20	Kamranga	Malibag Market	1.1×10^5
21	Apple	Malibag Market	4.1×10^5
22	Palm	Malibag Market	2.3×10^6
23	Cherry	Mouchak Market	2.7×10^6
24	Grape	Mouchak Market	1.8×10^6
25	Sauce	Siddeswari	3.75×10^5
26	Fuska water	Siddeswari	5.2×10^4
27	Samucha	Siddeswari	1.1×10^6
28	Singara	Siddeswari	1.1×10^6
29	Potato Chop	Siddeswari	1.6×10^6
30	Bread	Siddeswari	1.1×10^6
31	Cake	Siddeswari	2.6×10^6
32	Cooked Food	Home made	2.3×10^6
33	Burger	Siddeswari	4.6×10^6
34	Pizza	Siddeswari	6.1×10^6
35		Siddeswari	3.8×10^6
36	Milk	Malibag Market	3.1×10^6
37	Milk	Malibag Market	7.7×10^6
38	Yogurt	Retailer store	2.5×10^5
39	Yogurt	Retailer store	3.2×10^6
40	Sweet	Retailer store	1.0×10^5

All experiments were performed three times. One representative set of data is shown

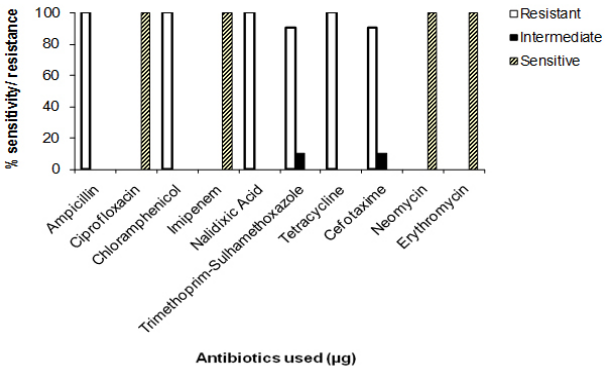

Figure 1. Susceptibility pattern of the isolated *V. cholerae* against different antimicrobial agents were measured *in vitro* by the Kirby-Bauer method as stated in the Materials and Methods section. Commercially available antibiotic discs were used for the assay. Ampicillin (10 µg), ciprofoxacin (5 µg), chloramphenicol (30 µg), imipenem (10 µg), nalidixic acid (30 µg), trimethoprim-sulphamethoxazole (10 µg), tetracycline (30 µg), cefotaxime (30 µg), neomycin (30 µg), and erythromycin (15 µg) were used.

Vegetables

Except kolmisak, high prevalence of *V. cholerae* was observed in all the other vegetables examined in this study. The number of bacteria in ranged from 2.5×10^5-1.1×10^7 cfu/g (Table 1). The maximum load was observed in cauliflower and the minimum in kolmisak. All the fruit samples showed huge bacterial load with the highest count of 3.3×10^6 cfu/g in guava whereas the lowest count was observed in kamranga (1.1×10^5 cfu/g).

Street food samples

The highest load was found in potato chop (1.6×10^6 cfu/g) and the lowest in fuska water (5.2×10^4 cfu/g). Similar result was found in bakery products. Cake (2.6×10^6 cfu/g) and bread (1.1×10^6 cfu/g) collected from a bakery food shop in Siddeswari area showed higher load. In case of fast food samples, the highest bacterial load was observed in pizza (6.1×10^6 cfu/g) while the lowest count was found in a home-made cooked food (2.3×10^6 cfu/g).

Sweets and dairy food samples

Among this category, the highest bacterial count was observed in a milk sample (7.7×10^6 cfu/ml) while sweets collected from a retailer store consisted of the lowest load (1.0×10^5 cfu/g) (Table 1).

Antibiotic susceptibility pattern of V. cholera isolates

Another aspect of our study was to determine the antibiotic susceptibility patterns of the isolates. For this purpose, 40 isolates were tested for their antibiotic susceptibility against 10 commonly prescribed antibiotics belonging to different groups. 36 isolates (90%) were found to be resistant against trimethoprim-sulphamethoxazole (SXT) and cefotaxime. On the other hand, the isolates were found to be sensitive against ciprofloxacin, imipenem, neomycin and erythromycin. Only four isolates (10%) showed intermediate resistance against SXT and cefotaxime. The results are shown in Figure 1.

Discussion

Generally, foods might be suspected together with water in conveying the cholera disease within communities. Foods can be faecally contaminated more frequently during preparation and handling, particularly by infected food handlers in an environment with poor hygienic condition. In this study, various types of food samples were collected from different areas of Dhaka city to examine and compare the load of *V. cholerae* among these samples.

All the samples showed to have high prevalence of *V. cholerae* ranging from 1.1×10^3 cfu/g in frozen meat sample to 4.2×10^7 cfu/g in chicken sample

Table 2. Zone diameter interpretive chart (Oxoid limited, England)

Name of Antibiotics	Disc content (µg)	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Ampicillin (Amp)	10	≤13 n=40	14-16 n=0	≥17 n=0
Ciprofloxacin (CIP)	5	≤15 n=0	16-20 n=0	≥21 n=40
Cefotaxime (CTX)	30	≤15 n=36	16-18 n=4	≥19 n=0
Nalidixic Acid (NA)	30	≤15 n=40	13-18 n=0	≥19 n=0
Trimethoprim-sulphamethozaxole (SXT)	10	≤13 n=36	14-16 n=4	≥17 n=0
Imipenem (IPM)	10	≤13 n=0	14-15 n=0	≥16 n=40
Tetracycline (Te)	30	≤14 n=40	15-18 n=0	≥19 n=0
Erythromycin (E)	15	≤13 n=0	14-16 n=0	≥17 n=40
Neomycin (N)	30	≤11 n=0	12-15 n=0	≥16 n=40
Chloramphenicol (C)	30	≤12 n=40	13-17 n=0	≥ 18 n=0

n = isolate number tested in this study
All experiments were performed three times. One representative set of data is shown.

(Table 1). It has been shown that *V. cholerae* can live and grow on cooked chicken and can increase in load ranging from 10^3 to 10^6 within 16 hours (Kolvin and Roberts, 1982). Another study reported that consumption of undercooked horsemeat was incriminated in a small outbreak of cholera in 1918 (Seligman, 1918). Moreover, fish has also been found to serve as an important vehicle for cholera transmission in the endemic areas (Rabbani and Greenough, 1999). In our study, prevalence of *V. cholerae* in fish samples ranged from 1.0×10^5 cfu/g in dry fish sample to 1.5×10^7 cfu/g in frozen fish sample (Table 1). This outcome suggested that drying or exposure to sunlight might be an effective means of inactivating *V. cholerae* (Pan American Health Organization, 1991).

Similar profile was found in case of vegetable samples with the highest load in cauliflower (1.1×10^7 cfu/g) (Table 1). According to Ackers *et al*. (1997), vibrios contaminating the rind of the fruits will not survive for more than a few days. However, according to Mcdougald and Kjelleberg (2006), vibrios are capable of developing adaptive response to low nutrient condition. The load of vibrios in fruit samples observed in this study is also in agreement with this information.

In endemic areas, transmission of cholera through contaminated foods served by street vendors and restaurants should be considered. In Dhaka, the capital city of Bangladesh, there was two outbreaks of cholera in 1974 and 1975 indicating a substantial association of the infection with foods from restaurants and free food-distribution centers for famine-affected people (Khan *et al*., 1983). In our study, alarming growth was found in all the street food samples. Similar results were observed in case of bakery products. Although fast foods have ever growing popularity among city dwellers, according to our study, the microbiological quality of the fast foods assessed was not up to the standard. Among the sweets and dairy products, a milk sample of a reputed dairy industry (7.7×10^6 cfu/ml) and sweets (1.0×10^5 cfu/g) collected from a retailer store were found to have the highest and lowest count, respectively (Table 1). The detection of *V. cholerae* had brought up the concern that these foods might have the possible risk to public well being upon consumption; and hence highlighting the need for improvement of hygiene and sanitation practice in the settings studied.

Apart from that, our study also revealed the antibiotic susceptibility patterns of the isolates against ten (10) most commonly used antibiotics. High resistance was observed against ampicillin, chloramphenicol, nalidixic acid, trimethoprim-sulphamethoxazole, tetracycline, cefotaxime and all the isolates were found to be sensitive against rest of the antibiotics (Figure 1). In a study conducted in India showed that among 94 isolates, 43 strains of *V. cholerae* contained multiple-drug resistant plasmids and exhibited resistance to ampicillin, neomycin, tetracycline, gentamicin, streptomycin, sulfonamide, furazolidone and chloramphenicol. (Thungapathra *et al*., 2002). Studies suggest that irrational and inappropriate use of antimicrobial agent facilitates the emergence of drug resistance. Several surveys in Bangladesh have shown that peoples are habituated in frequent use of antibiotics than needed which contributes to the emergence of multi-drug resistant pathogens by putting selective pressure for evolution and proliferation of resistance genes (Akond *et al*., 2008).

The risk factors associated with the high prevalence of *Vibrio cholerae* in food samples may pose impact on the cholera disease outbreaks. As stated earlier, cholera has long been a major public health trouble in Bangladesh (Sack *et al*., 2003; icddr,b, 2011). Only icddr,b's Dhaka Hospital and Mirpur Treatment Centre are known to provide care for >150,000 patients with diarrhoeal illness each year (Pietroni *et al*., 2009). A substantial health risk is the antibiotic resistance properties of *V. cholerae* as we found in our study. Antibiotic resistance is a major clinical hindrance in treating infections caused by pathogens, ultimately leading to an increase of morbidity and early mortality not in Bangladesh but also in the other developing countries (Okeke *et al*., 2005; Tenover *et al*., 2006).

Thus, the incidence of cholera causing pathogen

V. cholerae in food samples and their drug resistance pattern found in this study calls for an immediate need to pay attention. The peoples engaged in handling different types of food should follow the rules and guidelines of hygiene strictly. A judicious exploitation of antibiotics both for food items and for treatment of diseases should be followed to combat this drug resistance in pathogenic microbes.

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