International Journal of
AGRICULTURE AND BIOSCIENCES
www.ijagbio.com P-ISSN: 2305-6622 E-ISSN: 2306-3599 editor@ijagbio.com

RESEARCH ARTICLE

Incidence of Various Vibrio Species in Water from Different Sources in Ja'en, Kano State of Nigeria

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ARTICLE INFO

Received: June 23, 2013
Revised: July 22, 2013
Accepted: August 24, 2013

Key words:
Biochemical tests
Isolation
Thiosulphate citrate bile salt sucrose agar
V. cholera
V. parahaemolyticus
V. vulnificus
Vibrio species

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ABSTRACT

Laboratory investigations were carried out on various water samples to ascertain their level of contamination (if any) by *Vibrio* species. A total of 20 samples were collected from different water sources (tap, bore-hole, pond, well and sewage) from Ja'en area, Kumbotso Local Government Area of Kano State, Nigeria. The samples were analyzed over the period of two (2) weeks: Alkaline peptone water was used to enrich the samples which were then cultured on thiosulphate citrate bile salt sucrose (TCBS) agar. The isolates were confirmed using various biochemical tests to species level. Five (5) Species of *Vibrio* were identified: *V. cholerae* (from pond and from well); *V. parahaemolyticus*, *V. vulnificus* (both from pond) and *V. alginolyticus* and *V. hollisae* (from sewage).

Cite This Article as: Shamsuddeen U, RS Abdulkadir and EE Bassey, 2013. Incidence of various Vibrio species in Water from different sources in Ja'en, Kano State of Nigeria. Inter J Agri Biosci, 2(5): 192-195. www.ijagbio.com

INTRODUCTION

Vibrio bacteria are among the most common organisms occurring in surface waters worldwide. They are found in both marine or estuarine and fresh surface water (CDC, 2005). More than 20 *Vibrio* species are known to be pathogenic to man. Among these, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* are most important. Depending on species involved, the clinical manifestations are different ranging from gastroenteritis, septicemia to wound infections (Farmer and Hickman-Brenner, 1992; Oliver and Kaper, 1997; Ulusarac and Carter, 2004). Generally, *Vibrio* infections can be classified into cholera and non-cholera *Vibrio* infections. *Vibrio cholerae* seprogroups 01 and 0139 are the most important of all *Vibrio* species, since they are associated with epidemic and pandemic diarrheal outbreaks in many parts of the world (Centers for Disease Control and Prevention, 1995; Kaper *et al*., 1995). However, other species of *Vibrio* capable of causing diarrheal diseases have received greater attention in the last decades; these include *Vibrio parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. demsela*, *V. fluvialis*, *V. furnissii*, *V. hollisae*, *V. metcshnikovii* and *V. mimicus* (Hoi *et al*., 2005).

This research is aimed at screening the various water samples obtain from Ja'en for presence of *Vibrio* species.

MATERIALS AND METHODS

Study Area

Samples were collected from Ja'en area which is in Kumbotso Local Government Area of Kano state, Nigeria. The main water sources identified in this area were well, tap, bore-hole and pond. Sewage was also included.

Sample Collection

Samples were collected according to the procedure described by Cheesbrough (2005), in sterilized 250ml capacity non-transparent screw caped bottles. The samples were transported to the laboratory in a cold container (a flask containing ice was used for this purpose).

Enrichment of Sample

10ml of each water sample was added to an equal volume of double strength alkaline (pH 8.6) peptone water

and incubated for 24 h at 37^0C (Rhodes *et al*., 1986). Turbidity indicates bacterial growth (Cheesbrough, 2005).

Culture and Isolation

A loopful of inoculum from the enrichment culture was streaked to Thiosulphate citrate bile salt sucrose (TCBS) agar, streaked (quadrant method was adopted) and incubated at 37^0C for 24 h.

Developing yellow, green or green-blue colonies were suspected to be *Vibrio* and were picked; gram stained and viewed microscopically using oil immersion.

Sub-Culture

Colonies that were found to be gram negative bacilli were sub-cultured on non-selective medium, Nutrient Agar (NA) slant. The inoculated NA slants were incubated for at least 4-6 h at 37^0C. The isolates were then tested for oxidase reaction (Gupte, 2006).

Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-p-phenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum (but not nichrome) wire loop and smeared on the wet piece of paper (Holt *et al*., 1994; WHO, 1987). Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test (Cheesbrough, 2005).

Urease test

The isolates were inoculated into liquid urea agar, (which was supplemented with urea supplement) and aseptically dispensed into sterile bijou bottles, and slanted to gel. They were incubated at 37^0C for 24-72 h. Development of a bright pink or red color indicates a positive urea reaction (Cheesbrough, 2005).

Mortility Indole Ornithin (MIO) Test

The MIO media was prepared (in sterilized tubes) adopting the method described by Cheesbrough (2005). Selected colonies were inoculated into the medium using a straight sterilized needle; the needle was used to stab about one-half of the length of the medium. The tubes were incubated at 37^0C for 18-24 h with their caps loosened. Fuzzy growth away from the line of inoculation denotes motility of the organism. Dark turbid purple color denotes positive result for ornithin. After interpreting the result (following the procedure above), few drops of Kovac's reagent were added and observed. Pink to red color denotes positive indole test.

Voges Proskauer (V-P) Test

According to the method described by Hardwood *et al*. (2004), five milliliter of MR-VP broth was inoculated with the test organism and incubated at 37 ^{0}C for 48 to 72 h. 5 drops of 40% potassium hydroxide were added followed by 15 drops of 5% naphthanol in ethanol; the tubes were shaked and the caps were loosened. The tubes were placed in a sloppy position. Development of a red color starting from the liquid-air interface within one hour indicates a V-P positive test.

Test in Triple Sugar Iron (TSI) Agar

TSI agar was prepared by standard method. With a sterile needle, an isolate was obtained from the subculture and streaked on the surface of the slant, and the butt was stabbed 2 to 3 times. The caps of the tubes were loosened and the tubes were incubated for 24 h at 37^0C.

The butt becoming yellow indicates glucose fermentation. If no other sugar is fermented, the slant would be red while the butt is yellow. If in addition to glucose, lactose or sucrose or both are fermented, both the butt and the slant would be yellow (A/A reaction) (Cheesbrough, 2005). If TSI is inoculated with a culture that appears as a non-lactose fermenter but gives an A/A reaction, the chances are that the culture is a sucrose fermenter. If none of the 3 sugars in TSI (glucose, lactose and sucrose) is fermented, the inoculated culture would grow using the peptone present in the medium but no yellow coloration of the butt or the slant would occur (Cheesbrough, 2005).

Lactose Fermentation Test

Lactose solution was prepared using standard procedure and an indicator (methyl red) was added. The test organism was inoculated into the lactose solution and allowed to incubate at 37^0C for 24 h.

Color change to yellow indicates lactose fermentation (Cheesbrough, 2005).

L-Arabinose Fermentation Test

This test was carried out to differentiate *Vibrio fluvialis* (L-arabinose fermenter) from *Vibrio cholerae* and other V. species which do not ferment L-arabinose (Cheesbrough, 2005). Neutral red was added as an indicator. Following inoculation and incubation at 37^0C for 24 h, color change to yellow indicates L-arabinose fermentation.

Cholera Red Test

Pure culture of *Vibrio* was grown in a tube of peptone water for four (4) days at 37^0C. Few drops of concentrated H_2SO_4 were added to each tube and observed. Development of a reddish pink color (due to formation of nitrous-indole) is a characteristic of *Vibrio cholerae* (Hardwood *et al*., 2004).

Salt Tolerance Test

According to the procedure adopted by West and Colwell (1984), different salt concentrations were prepared using peptone water (0, 3, 6, 8 and 10%) and each of the isolates was inoculated into each of the salt concentration and incubated for 24 h at 37^0C. Turbidity following 24 h incubation indicates growth of the tolerant *Vibrio* species (West and Colwell, 1984).

RESULTS

After subjecting the water samples into series of laboratory analyses, the results obtained were summarized in the following table.

DISCUSSION

In this study, no species of *Vibrio* has been isolated from borehole and tap water sources. Isolation of *V.*

Table 1: Characterization of *Vibrio* species from water samples collected from Ja'en

Water source	Growth on TCBC	Gram's Stain	OX	MDW	MOT	IND	ORN	VP	Urea	Acid from Glu	Suc	Lac	Ara	Cholera red test	Growth in NaCl 0%	3%	6%	8%	10%	Organism
Bore hole																				
A	NG																			
B	Y	grm -ve rods	+	+																
C	NG																			
D	NG																			
Pond																				
A	Y	grm -ve rods	+	-	+	+	+	+	-	+	+	-	-	+	+	+	-	-	-	V. *cholerae*
B	GB	grm -ve rods	+	-	+	-	+	-	+	+	-	+	-	-	-	+	+	-	-	V. *vulnificus*
C	G	grm -ve rods	+	-	+	-	+	-	+	+	-	+	-	-	-	+	+	-	-	V. *vulnificus*
D	G	grm -ve rods	+	-	+	-	+	-	-	+	-	-	+	-	-	+	+	+	+	V. *parahaemolyticus*
Sewage																				
A	Y	grm +ve cocci																		
B	Y	grm -ve rods	+	-	+	+	-	-	-	+	+	-	+	-	-	+	+	-	-	V. *hollisae*
C	Y	grm +ve cocci																		
D	GB	grm -ve rods	+	-	+	+	+	+	-	+	+	-	-	-	-	+	+	-	-	V. *alginolyticus*
Tap																				
A	NG																			
B	NG																			
C	NG																			
D	Y	grm –ve rods	+	+																
Well																				
A	Y	grm -ve rods	+	+																
B	NG																			
C	NG																			
D	Y	grm -ve rods	+	-	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	V. *cholerae*

Key: TCBS = Thiosulphate citrate bile salt sucrose agar, OX = Oxidase test, Mdw = Motility in distilled water, NG = No growth, MOT = Motility test, IND=indole test, ORN = Omithin test, VP = Voge's proskauer test, Glu = glucose, G = green, Suc = sucrose, Lac = lactose, Ara = L-arabinose, grm-ve = gram negative, Y = yellow, GB = green-blue, S/N = Serial number.

cholera, V. parahaemolyticus and *V. vulnificus* from the pond could be a sign of danger especially if the pond would be used as alternative source of water for domestic purposes; this is because these species are human pathogens. *V. cholerae* has also been isolated from well. This is another sign of poor quality of the sample.

The outcome of this research is in line with that of Amirmozafari *et al*. (2005) who also reported the highest frequency of occurrence (53%) of *Vibrio vulnificus* (amongst the other *Vibrio species*) in their study: incidence of Pathogenic Vibrios in the Coastal area of Golestan Province in Iran.

However, Adeleye *et al.* (2010) contrarily indicated that *Vibrio alginolyticus* was predominant (31.8%) among all the species found. This is in line with the result of Martha *et al.* (2010) in their study on Occurrence and Control of *Vibrio species* as contaminants of processed marine fish; which also revealed the highest level of occurrence of *Vibrio alginolyticus*. Presence of *V. vulnificus* in this research could be a significant point of concern considering its association with disease outbreaks either with ingestion of contaminated seafoods or association with infectious wounds (Stahr *et. al*., 1989; Dalsgaard and Hoi, 1997; Nascimento *et al*., 2001; Morris, 2003).

Conclusion

Screening the water samples (collected from the various sources) revealed presence of *V. cholera, V. parahaemolyticus, V. vulnificus, V. alginolyticus and V. hollisae* from sewage, pond and well. Samples from borehole and tap were found to be free of any species of *Vibrio*.

Recommendation

This study, along with evidences from the epidemiology of other diarrheal diseases; wound and skin infections (caused by pathogenic *Vibrio* species), suggest improved hygienic practices, including point-of-use chlorination of water, use of safe water vessels as well as hand washing with soap and general sewage treatment. These might be effective in preventing the transmission of cholera and other non-cholera *Vibrio* infections. Antimicrobial treatment of general sewage (by chlorination, Ozone, UV light etc.) should be ensured before it gets into the water ways. Dwellers should avoid open defecation in and around water bodies especially when the water is used for domestic purposes.

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