Brief Original Article

Occurrence of *Vibrio cholerae* in fish and water from a reservoir and a neighboring channel in Ouagadougou, Burkina Faso

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Abstract

Introduction: *Vibrio cholerae* is a human pathogen and natural inhabitant of aquatic environments. In this study, we surveyed the occurrence of *V. cholerae* in fish harvested from a reservoir that receives discharges from the population in Ouagadougou through several channels.
Methodology: A total of 238 fish and 80 water samples were analyzed for the presence of *V. cholerae*.
Results: Altogether, 13 *V. cholerae* strains were isolated. They were all identified as non-O1/non-O139 *V. cholerae* without the *ctxA* gene. The strains were mostly susceptible to the antimicrobials tested.
Conclusion: Although no strains of epidemic *V. cholerae* serotypes were encountered, it is important to monitor the microbiological quality of this extensively used water resource and its fish.

Key words: channel; fish; Ouagadougou; reservoir; *Vibrio cholerae*; water.

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Introduction

Human infections caused by microorganisms transmitted by fish or an aquatic environment are quite common, depending on the season, the patient's contact with fish or the environment, dietary habits, and the immune system status of the exposed individual [1]. Infection rates often peak after storms, floods, or sewage spills that transfer contaminated effluent to aquatic ecosystems [2]. Vibrios are abundant in aquatic environments, where they are found free-living in water or in association with plankton. Vibrios favor higher water temperature; consequently, the outbreaks are more frequent during the warmer season [3,4]. One of the most dangerous gastroenteric infections is cholera. Epidemic cholera is caused by enterotoxin-producing *V. cholerae* of serogroups O1 and O139. Cholera vibrios are commonly transmitted to humans through ingestion of contaminated food or water, and the disease occurs mainly in developing countries with inadequate sanitation [5-7]. Studies have suggested that fish may act as reservoirs and vectors of *V. cholerae* [8]. While an estuarine environment represents an ideal setting for the survival and persistence of *V. cholerae*, cholera has also become endemic in arid and inland areas of Africa [9] such as Burkina Faso [10]. *V. cholerae* strains of non-O1/non-O139 serotypes can cause diarrheal symptoms and extraintestinal infections in humans [11]. Therefore, environmental monitoring for the presence of *V. cholerae* and other *Vibrio* species with pathogenic potential is important, as it can identify the potential sources of infections.

In Ouagadougou, the capital of Burkina Faso, many channels have been built to drain water off during the rainy season. People living next to the channels use them as a bin, throwing in a great deal of rubbish, which decreases the hygienic quality of the channel water. Yet, these water sources are used for fishing, bathing, other domestic activities, and irrigation of vegetables, thus increasing the risk of human infections. Local fishing activities provide income and are increasing due to the construction of

many new reservoirs. In this study, we concentrated on one of the reservoirs located in the middle of the city of Ouagadougou, Barrage de Tanghin, and on one of the channels running close to it. The reservoir's water capacity is approximately 7 million m^3 and it receives discharges of the city's untreated sewage, human waste, and runoff, which increases during the rainy season. Also, the hospital of Ouagadougou is located near the reservoir; the channel into which its wastewater flows is less than 50 m from the reservoir. During the rainy season, the channels often overflow and the water spreads to larger areas, posing a potential health risk to the city's inhabitants. In this study, we report on the discovery of *V. cholerae* in the water and fish from the Tanghin Reservoir and its neighboring channel.

Methodology

Sampling

The most important fish species in the reservoir is tilapia (*Oreochromis niloticus*). A total of 238 tilapias, with an average length of 12 cm, were collected within a period of six months (March to August 2010), 228 from the Tanghin Reservoir and 10 from a channel. All the fish were purchased alive from fishermen. Fish were transported to the laboratory and processed within four hours of collection. The outer surface of the killed fish was disinfected by wiping it with 70% ethyl alcohol for two minutes, washing it three times with sterile water. The intestine was removed aseptically by using a scalpel and was ground with a sterile mortar and pestle. A total of 80 water samples of 250 mL each were taken from the reservoir and the channel, using a disinfected ladle fixed to a long stick. Water samples were transported to the laboratory on ice and analyzed within two hours of collection. The water temperature was measured at the sampling site with a pH meter with a temperature probe (WTW PH330; Aqua Merik, St-Nicolas, Canada).

Microbiological analyses

Broth enrichment technique was used to enhance detection of *Vibrio* spp. First, 1 g of each crushed intestine sample was added to 9 mL of alkaline peptone water (APW; Liofilchem, Roseto degli Abruzzi, Italy) and incubated at 37°C for 24 hours. Water samples were highly turbid and thus 1 mL of sample was inoculated directly into 9 mL of alkaline peptone water and incubated at 37°C for 24 hours. In both cases, a loopful of enriched broth was then streaked on thiosulfate-citrate-bile salts-sucrose agar (TCBS; Oxoid Ltd., Basingstoke, England) and incubated at 37°C for 24 hours. Presumptive *V. cholerae* colonies on TCBS were flat, circular, yellow, and sucrose-fermenting. Strains grown from typical colonies were cultured on nutrient agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hours and then submitted to oxidase and catalase tests. Strains positive in these tests were confirmed to be *V. cholerae* by API 20E (BioMerieux, Marcy l'Etoile, France). The strains were conserved in heart infusion with 15% glycerol in the freezer and sent to Finland for further characterization. Serotyping was done using glass agglutination and the antisera against O1 polyvalent (both Inaba and Ogawa), O1 Inaba, O1 Ogawa, and O139 (Bengal) (Denka Seiken Co., Tokyo, Japan). For detection of hemolysis, the strains were grown on sheep blood agar plates incubated at 36°C overnight.

DNA extraction and polymerase chain reaction (PCR) for ctxA detection

To extract DNA, one or two colonies were suspended in 0.9% NaCl solution and centrifuged at 3000 g for 5 minutes. The pellet was suspended in 100 µL of sterile water, boiled for 10 minutes, and centrifuged at 10,000 g for 1 minute. The supernatant was used as template in the PCR reactions. Each 25 µL PCR reaction contained 15.45 µL of water, 2.5 µLl of *Taq* buffer (Applied Biosystems, Foster City, California, USA), 2.5 µL of $MgCl_2$ (25 mM), 1.25 µL of dNTP (5 mM), 2.5 µL of primer ctxA-F and 2.5 µL of primer ctxA-R, 0.3 µL of *Taq* DNA-polymerase (5 U/µL) (Applied Biosystems, Foster City, California, USA), and 1 µL of template. The PCR program consisted of 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Before the first cycle, the reaction mixture was heated at 94°C for 5 minutes; after the last cycle, the final extension was performed at 72°C for 10 minutes. The control strains were *V. cholerae* RHV 5578 O1 Inaba El Tor (UK NEQAS 8032), *V. cholerae* RHV 5822 O1 Ogawa El Tor (UK NEQAS 9668), *V. cholerae* RHV 5654 non-O1/non-O139 (UK NEQAS 9018), and *Escherichia coli* RHE 6715 (ATCC 25922) as a negative control. PCR products were run on 1.5% (wt/vol) agarose gel to resolve the amplified products, which were visualized under UV light after ethidium bromide staining.

Antimicrobial susceptibility testing

Antimicrobial susceptibility patterns were tested with an epidemiologic antimicrobial panel of ampicillin (10 µg), chloramphenicol (30 µg),

streptomycin (10 µg), sulphonamide (300 µg), tetracycline (30 µg), trimethoprim (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), cefotaxime (5 µg), mecillinam (10 µg), and imipenem (10 µg) (Oxoid Ltd., Basingstoke, Hampshire, UK) using the disk diffusion method and following the EUCAST guidelines (http://www.eucast.org). *E. coli* ATCC 25922 was used as a control.

Results

A total of 238 fish, 25 reservoir water, and 55 channel water samples were collected and processed. From these, 78 sucrose-fermenting bacterial colonies were obtained, of which 18 were confirmed to be *V. cholerae* by the API test (Table 1). Of the *V. cholerae* strains, 15 were isolated – mainly from fish – between March and May, which corresponds to the warm season. During the raining season, only 3 strains were isolated in August from the channel water. The measured water temperature did not vary according to the season, being on average 30.5°C (± 1.5°C).

Thirteen of the strains remained alive for further characterization. None of them reacted with O1 or O139 antisera and they also lacked the *ctxA* gene tested for by PCR. However, all of them were hemolysis positive on blood agar. Six fish isolates and one water isolate were resistant to ampicillin, one fish isolate was intermediate to streptomycin, and one fish isolate was intermediate to sulphonamide, but otherwise the isolates were sensitive to the tested antimicrobials.

Discussion

V. cholerae was isolated from 18 of the 318 (6%) fish and water samples we studied. This prevalence may actually be an underestimate, because upon exposure to an unfavourable environment, vibrios persist by entering a viable but nonculturable (VBNC) state [12]. All the *V. cholerae* strains we serotyped were non-O1/non-O139, and none of them contained the *ctxA* gene coding for the cholera toxin A subunit. Although organisms of the O1 serogroup are frequently isolated from aquatic environments, most of the environmental O1 isolates do not produce cholera toxin, to which the clinical state of cholera is principally attributed [13]. The absence of *V. cholerae* O1 in our samples may be due to the high prevalence of non-O1/non-O139 strains, which are believed to be indigenous members of the aquatic ecosystem and which can prevent isolation of the truly pathogenic strains [14]. Holding samples from freshwater ecosystems at an ambient temperature ranging from 31°C to 35°C for 20 hours before processing has been found to selectively increase the number of culturable vibrios [15].

Although the *Vibrio* strains isolated in our study were not of the serotypes causing cholera, they may still cause sporadic cases of watery diarrhea and inflammatory enterocolitis. There is also a risk for skin infections or life-threatening systemic infections, especially in children or in other subjects with immune-compromising factors, from swimming in the water [11]. Different *Vibrio* species have been

Table 1. Isolation of *Vibrio cholerae* from fish, Barrage de Tanghin reservoir and channel water between March and August 2010

Period	Type of sample	No. samples n = 318	No. *V. cholerae* isolates (%) n = 18	API profiles
March	Fish	29	5 (17%)	5347124
	Reservoir water	10	1 (10%)	5347124
April	Fish	30	3 (10%)	5147124*, 5307124
May	Fish	47	5 (11%)	5346124**, 5347124
	Reservoir water	5	0	-
	Channel water	15	1 (6%)	5347124
June	Fish	31	0	-
	Reservoir water	5	0	-
	Channel water	15	0	-
July	Fish	24	0	-
August	Fish	77	0	-
	Reservoir water	5	0	-
	Channel water	25	3 (12%)	5347124

* For two isolates; ** For four isolates

detected in seafood meant for human consumption [16]. In Israel, *V. cholerae* non-O1/non-O139 were isolated from the intestines of most of the fish species sampled [8].

Temperature and salinity of the environment have been reported to be the major parameters influencing the dynamics of *V. cholerae* [17]. In our study, *V. cholerae* was isolated more often during the warm period, which included March, April and May, although no apparent temperature change in the water was detected. Unlike most vibrios, *V. cholerae* does not require NaCl to grow [9], which probably enhances its survival and competitiveness against other vibrios in freshwater environments such as the Tanghin Reservoir. In the future, global warming might create new favourable environments for *V. cholerae* and increase its incidence in vulnerable areas [3].

The high prevalence of *V. cholerae* in the channel water in Ouagadougou is not surprising, since channels receive sewage from local settlements and from the hospital, and thus contain human pathogens. That *V. cholerae* was also isolated from the reservoir water is worrisome, because this water is used for irrigation of vegetables among other uses. Many similar reservoirs are used as a source of potable water in other towns in Burkina Faso. In a recent study in Nigeria, animal manure was found to contain *V. cholerae* O1 [18]. Especially during heavy rains, the pathogens can be washed to the rivers and contaminate the drinking water used in the countryside as well as the irrigation water. For instance, contaminated river water was claimed to have transported cholera vibrios from the outbreaks or sporadic cases in neighboring countries to Azerbaijan [7].

Alarmingly, *V. cholerae* O1 variant with reduced susceptibility to ciprofloxacin has been spreading in western Africa in recent years [19], but the environmental vibrios we isolated were largely sensitive. Half of our *V. cholerae* isolates were resistant to ampicillin. This is in agreement with a study recently conducted in South Africa, where ampicillin resistance was a common finding [20].

Conclusions

Our work demonstrates that fish and reservoir and channel water in Ouagadougou, Burkina Faso, harbor *V. cholerae*. No cholera vibrio serotypes were isolated in our study; however, other vibrios can potentially cause sporadic gastroenteritis and serious diseases. Their presence indicates a need for quality microbiological surveillance of fish products and water, which is used for various human activities and for irrigation of fresh vegetables.

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