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(RESEARCH ARTICLE)

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Identification of bacteria of the genus *Vibrio* in some water sources in the prefectures of Télimélé and Boké (Republic of Guinea).

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Abstract

For a long time, the main problem with water intended for human consumption has been health, and this problem stems from the existence of micro-organisms (bacteria, viruses, protozoa, parasites) that transmit many dangerous infections to humans. Vibrio cholerae is one of these micro-organisms. Cholera is a severe acute diarrhoeal disease caused by Vibrio cholerae, a Gram-negative bacterium that colonises warm, saline and alkaline surface waters, often in association with phytoplankton or zooplankton. The aim of this study was to identify bacteria of the Vibrio genus in the waters of the Télimélé and Boké prefectures. The study was carried out from the beginning of May to the end of September 2021. The samples were analysed in the bacteriology laboratory of the Institut de Recherche en Biologie Appliquée de Guinée (IRBAG) in Kindia, using conventional identification methods. The results of the analysis proved the existence of the bacterial strain in the water analysed. During the analyses, we encountered two types of Vibrio: parahaemolyticus with 1.28% in Télimélé and alginolyticus with 2.56% in Boké. These germs were found in wells and lakes, but Vibrio spp was not found. In addition to bacteria of the species *Vibrio parahaemolyticus* and *Vibrio alginolyticus*, we encountered two (2) other types of microorganisms: *Enterococcus faecalis* observed in ponds and streams and Salmonella spp. found in taps, boreholes and wells.

Keywords: Vibrio; Water sources; Télimélé; Boké.

1. Introduction

Water is a natural resource, a driving force, an indispensable need and an essential component of human life, fauna and flora. However, if left untreated, it can damage people's health by causing serious illness. Water-related health problems are on the increase. Indeed, this resource is considered to be the world's leading cause of death. The composition of pathogenic micro-organisms in water varies enormously. These microorganisms have a variety of effects on health, ranging from mild infections such as gastro-enteritis to fatal diseases such as cholera [1].

Cholera is a highly contagious disease caused by the ingestion of food or water contaminated by the bacterium Vibrio cholerae. This bacterium produces an enterotoxin that causes diarrhoea and can lead to severe dehydration and death if left untreated [2, 3].

They are small bacilli, often with a curved shape known as a 'comma'. The best-known species of *Vibrio* is *Vibrio* cholerae, the agent responsible for cholera. *V. cholerae* appears under the microscope as Gram-negative, round-ended bacilli with a comma shape 1.5 to 2.4 µm long and 0.3 to 0.4 µm wide. [4, 5].

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They are chemoorganotrophic, facultative aerobic-anaerobic and have a respiratory and fermentative metabolism. They are halophilic bacteria, isolated from the aquatic environment and generally able to grow on Thiosulphate Citrate Bile Sucrose medium [6].

Every year, the number of these species increases as a result of new discoveries made through taxonomic research. Some of these species, such as *V. cholerae*, *V. parahaemolyticus, V. vulnificus* and *V. alginolyticus*, are pathogenic and pose the greatest risks to public health, while others, such as *V. aerogenes*, are non-pathogenic [7].

It colonises the small intestine and its main symptoms are spontaneous vomiting and sudden, profuse, watery diarrhoea leading to extreme dehydration, loss of electrolytes and increased blood acidity. The stools are liquid, afecial, colourless and odourless [8].

Many countries are regularly hit by devastating outbreaks of the disease, such as Zimbabwe in 2009 and Nigeria in 2010, with case-fatality rates well above the acceptable threshold of 1% [9].

Every year, the WHO estimates that between 1.3 and 4 million people are infected with cholera, with between 28 and 14,2000 deaths worldwide. In 2013, the same institution reported that 47 countries had reported a total of 129064 cases, including 2102 deaths. In 2014, 42 countries reported a cumulative total of 190549 cases with 2231 deaths, with 5 countries alone accounting for 84% of cases: Afghanistan, Ghana, Haiti, Nigeria and the Democratic Republic of Congo [10].

In Guinea, the results found in 2016 in water gave the following distribution of positive cases: Matam 1 case, Kaloum 1 case, Dixinn 2 cases and Ratoma 1 case, while no positive cases were found in Matoto. In 2010, Bertrand Sudre and Didier B. gave the following breakdown: Ratoma (36.9%) and Matoto (38.3%), mentioning that in 2009 there were 6 cases in Conakry without death (Communes of Dixinn 1 case, Matam 1 case; Matoto 3 cases and Ratoma 1 case. [11].

In the prefecture of Kindia, in June 2004, 93 cases were recorded out of the 183 notified by the central reference laboratory (Ministry of Health). Six (6) months after the first case of cholera in Forécariah in 2012, the prefecture as a whole recorded 87 cases, including four deaths, according to the prefectural authorities. Four (4) suspected cases of cholera with one death were reported by the same authorities as at 31 July 2012. [11].

Since 1990, major cholera epidemics have been recorded in Guinea. The incidence of this disease in Guinea from its appearance in 1970 to the present day and its periodic persistence have prompted the present study, the aim of which is to identify bacteria of the Vibrio genus in water from the prefectures of Télimélé and Boké in the Republic of Guinea.

2. Materials and methods

2.1. Our research was carried out in Télimélé and Boké.

Télimélé is one of the prefectures in the Kindia region of the Republic of Guinea, in the west of the country. Télimélé is located 263 km from Conakry and 126 km from Kindia, covering a total area of 9,000 km^2 with a population density of 34 inhabitants per km^2. Its geographical coordinates are: latitude 10o54 north; longitude 13o1 west, with a population of 304,060 in 2016. It is bordered to the north by the Republic of Mali and Senegal, to the east by the prefecture of Dinguiraye, to the south by Mamou and Télimélé, and to the west by the Boké region [12].

Boké is located on Guinea's maritime coast. The prefecture of Boké is located in the north-western part of Guinea, known as maritime Guinea, and more specifically in Baga country. It is about 270 km from Conakry and covers a total area of 11,053 km^2, with a population density of 44 inhabitants per km^2. Its geographical coordinates are 10o55 North and 17o25 West, with a population of 481,007 in 2016. It is bordered to the north by Guinea Bissau, to the north-west by the prefecture of Gaoual, to the west by the prefecture of Télémilé, to the south by the prefecture of Boffa and to the west by the Atlantic Ocean. [13].

Our analyses were carried out in the bacteriology laboratory of the Institut de Recherche en Biologie Appliquée de Kindia (IRBAG).

2-1 Equipment: Sampling was carried out during the winter season, from the beginning of May to the end of September of the year 2021. In the course of the research, we took 78 samples from various water sources: taps, boreholes, wells, streams and marshes, bathing water, lakes and rivers. The following equipment was used to carry out this work: Blades

and slides, Pasteur pipettes, Binocular electric microscope, Autoclave, 37°C ovens, racks, Bunsen burner, platinum loop, tweezers, Pasteur oven, water bath, sterile test tubes, sterile flasks, and culture media (Hyperalkaline Peptone Water, Nutrient Agar, Alkaline Nutrient Agar (ANG), TCBS medium (Thiosulphate Citrate Bile Sucrose), Mueller-Hinton Agar, Nutrient Broth, Nitrate Broth, Kligler-Hajna (KIA) Medium, Mannitol-Mobility Medium, Sterile Distilled Water, Urea-Indole Medium).

2.2. Method: We used the conventional identification method with the following steps:

Vibrio bacteria are rare in the environment and are generally isolated by enrichment in order to increase the probability of the bacterium's existence.

Water samples of 200 ml were enriched in hypersaline alkaline peptone water (10% of the sample) for 24 hours at 37°C.

Microscopic examination ended with a Gram stain showing the morphological characteristics (Gram, fine curved bacilli). Biochemical identification was carried out using pure cultures; a suspect colony was mixed in sterile distilled water under aseptic conditions to produce a bacterial suspension. Before performing the Api20E gallery required to confirm the species, we performed presumptive tests for Vibrio cholerae.

3. Results

We carried out a study on a sample of 78 samples, including 54 in Télimélé and 24 in Boké: 16 from boreholes, 20 from marshes, 19 from wells, 6 from taps, 10 from streams, 1 from bathing water, 3 from lakes and 3 from rivers. Of these samples, 3 were identified as positive, representing a percentage of 3.84.

Table 1 Results of water analysis in the Télimélé Prefecture.

N°	**Sources**	**Number of samples**	**Positive cases (Vibrio)**	**Percentage of positive cases**	**Other germs observed**
1	Drilling	12	0	0	*Salmonella spp (2)*
2	Marigots	19	0	0	*Entérocoques faecalis(2)*
3	Well	9	1	1.85	*V. parahaemolyticus*
4	Robinets	6	0	0	*Salmonella spp.(1)*
5	Streams	8	0	0	*Entérocoques faecalis(1)*
TOTAL		54	1	1.85	3

The results show that Vibrio spp. were not found, but *Vibrio parahaemolyticus* were found, and only in the wells. During our survey, we encountered two (2) other types of microorganisms: *Entérococcus faecalis* observed in ponds and streams, and Salmonella spp. found in taps and boreholes.

Table 2 Results of water analysis in Boké Prefecture.

N°	**Sources**	**Number of samples**	**Positive cases (Vibrio)**	**Percentage of positive cases**	**Other germs observed**
1	Drilling	4	0	-	_
2	Well	10	1	4,16	*V. alginolyticus Salmonella spp.*
3	Streams	2	0	-	_
4	Marigots	1	0	-	_
5	Rivers	3	0	-	_

6	Lakes	3	1	4,16	*V. alginolyticus*
7	Bathing Water	1	0	-	_
TOTAL		24	2	8,32	2

Observation and analysis of Table II reveals two positive cases in the sample as a whole, and both concern *Vibrio alginolyticus*, one found in wells and the other in lakes.

In this prefecture, we found a low rate of microorganism carriage, with most of the water sources analysed containing no germs indicative of pollution. Water sources containing germs other than *V. alginolyticus* were wells. In Boké, we detected one case of an association of germs between *V. alginolyticus* and Salmonella spp.

It should be noted that there are still a number of factors that can contribute to cholera epidemics, including: lack of access to drinking water, and poor sanitation that is not adapted to population growth.

4. Discussion

The results of the present study showed three (3) positive cases out of all the samples taken, with one case for *V. parahaemolitycus* and two cases for *V. alginolyticus*. These results differ from those of [14] M. Diallo et al. who worked in Conakry in 2017 and who showed that out of 54 samples taken, 05 samples were positive for Vibrio spp. i.e. 9.25%. Similarly, the results obtained by teams of IRBAG researchers in 2013 counted 27 cases, with a positive rate of 55.5% for the city of Conakry. Over the decade, we have seen a drastic drop in the prevalence of the Vibrio genus, although the species found differ (from 55.5% in 2013 to 9.25% in 2017 and 3.84% in 2022).

Compared to the studies carried out by researchers A. Aboulkacem1, et al; in 2007, in the waters of the valleys (Oueds Boufekrane and Ouislane) no cases of the *Vibrion* genus were found in the city of Meknès (Morocco), our results are superior to those reported by these researchers, who found no evidence of the presence of pathogenic Vibrio cholerae bacteria belonging to the O1 serological group, in the waters of the two oueds studied. In fact, non-O1 strains are more frequently isolated from river and estuary waters than O139 strains [15].

5. Conclusion

Research was carried out on a sample of 78 samples (16 from boreholes, 20 from marigots, 19 from wells, 6 from taps, 10 from streams, 1 from bathing water, 3 from lakes and 3 from rivers). The analysis revealed 3 positive cases, a percentage of 3.84. The bacterial germs detected were *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. This study did not reveal the presence of other species of the same genus. Two other types of microorganisms were encountered during the analysis: Salmonella spp. and *Entérococcus faecalis*.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that there are no conflicts of interest.

Authors' contributions

MD, being the principal author of this work, participated in all stages of the process.

The participation of the co-authors (MYB, BSSB, MGD, SD, AB, BK, TALB, NYL) was of paramount importance as they facilitated the correction and contributed to the follow-up and publication of this article.

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