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Full Length Research Paper

Multiple adaptations of bacteriophages in Lagoon Ebrie: Indicators of permanence microbiological pollution in the environment, Côte d'Ivoire, West Africa

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The Lagoon Ebrié in the city of Abidjan is a typical aquatic ecosystem that can be used to predict the existence and circulation of water pathogens. Phages are viruses that infect bacteria and can be used for the detection of their bacteria host. In this study, bacteria colonies were investigated in their natural habitat within the Lagoon by using phages as indicators. Their impact on pollution was also determined in different sites of the rural Lagoon in the city. Water samples were collected at different sites of the Lagoon for the detection of bacteria and phages. The virulence of phages was tested on bacteria host using diffusion on an agar plate. Our results showed three major bacterial strains; *Pseudomonas aeruginosa, Escherichia coli*, and *Enterobacter cloacae*. Twenty-four phages that infected five bacteria hosts were also isolated. The spectrum revealed that the phages have high virulence against *E. coli*, *P. aeruginosa* and *E. cloacae* by 82.5%, 71 and 70% respectively. The majority (83.3 %) of isolated phages produced lysis on *Enterobacteriaceae.* However, Vibrio phages were not detected in this study. These findings suggested the existence of multiple adaptations of phages and the persistence of diverse bacteria hosts in the Lagoon. The phages that were detected confirmed the bacterial presence in the Lagoon, this portray the ecological system in operation and possible microbiological risks for the riverine populations.

Keys words: Lagoon Ebrie, pollution, indicators, phages, adaptation, Abidjan.

INTRODUCTION

Bacteriophages, viruses that infect bacteria, are the most numerous of all viruses in the biosphere and are estimated to be globally more numerous than bacteria (Weinbauer, 2004; Williamson et al., 2005; Breitbart and Rohwer, 2005; Weitz and Wilhelm, 2012). This abundance plays an important role in the evolution of bacterial communities and may influence global biogeochemical cycles (Abedon, 2008; Harada et al., 2018). Detection of bacteriophage can be used as an indicator of water quality and wastewater treatment processes (Stewart et al., 2008). This is particularly important due to the difficulties that are often encountered with the direct detection of pathogens in water (Schmelcher and Loessner, 2014).

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Table 1. Sampling sites for phage isolation in this study.

Sites coding	Name of sites	GPS position
Ab	Abobodoume	5°18'36'N°4°2'06''W
Ad	Adiopodoume	5°20'05'N°4°7'37''W
Bo	Boribana	5°21'04'N°4°2'28''W
Ca	Carena	5°20'15'N°4°2'06''W
Lo	Locodjoro	5°19'28'N°4°2'06''W

Source: Authors

The potential presence of fecal pollution is typically assessed by studying the presence of Fecal Indicator Bacteria (FIB) which include, fecal coliforms, *Escherichia coli,* and *Enterococci* (Brian et al., 2017; Ashbolt et al., 2001).

Bacteriophage can be used as Microbial Source Tracking (MST) in the environment (Brian et al., 2017; Ezzat and Azzam, 2020). Several based-phage methods are developed to detect pathogenic bacteria using engineered phages that coding fluorescent, luminescent or colorimetric markers. Alternatively, labeled phage protein and phage DNA staining can allow direct detection of phages bound to host bacterial cells (Willford et al., 2011; Schofield et al., 2012; Jain et al., 2012; Vinay et al., 2015).

The poor management of household waste has been reported as one of the factors responsible for the deterioration of the living conditions and the natural environment in Abidjan (Adingra and Kouassi, 2011; Dongo et al., 2013; Tia, 2017).

P. aeruginosa, E. coli, *E. cloacae* and *S. aureus* have been reported as the major opportunistic bacteria responsible for nosocomial infections and diarrheal infections worldwide (Leski et al., 1998; Roman et al., 1997; Turnidge and Bell, 2000; Simor et al., 2001).

In this study, the presence of phages infecting *Enterobacter, E. coli*, *S. aureus, and V. alginolyticus* was investigated in the environmental water of the Lagoon Ebrie of the city of Abidjan.

MATERIALS AND METHODS

Water sampling

Five sampling sites on Lagoon Ebrie were used for this study. At each of the sites, 500 ml of water were collected and transported to the lab for microbiological analysis between September and December 2018. The specific sites were Locodjoro, Abobodoume, Boribana, Carena and Adiopodoume, with GPS locations presented in Table 1. Except for the Adiopodoume site, all the four other sites have the presence of human activities; pictures of these are presented in Figure 1.

Identification of bacteria

Three mL of water samples was inoculated in 10 mL of LB medium. The mixture was then incubated at 37 °C/ 24 h. Thereafter, colonies were identified and isolated. The selected colonies were diluted in distilled water for enrichment. They were then transferred to specific growth media for species identification. The strains were confirmed by biochemical tests, Malditoff and Antibiotic susceptibility tests (Koudou et al., 2021). *E. coli* stains B and C, Phage T4 that were used as the positive controls for virulence tests were provided from Dr Sylvain Moineau, University Laval. Canada (Addablah et al., 2021). Bacteria hosts and viral strains that were isolated in water from the lagoon for this study were stored at 20°C for subsequent analysis.

Phage isolation

A total of sixty-five water samples of 500 mL were collected from different sites and stored at 4 °C as the environmental water. Hundert (100) mL of the water was filtered through 0.45 µm. Tree (3) mL of the filtrate were inoculated in 3 mL LB Medium (DIFCO, Mexico) containing 100 µL of fresh bacteria host. Five major species were selected for bacteria hosts (*E. coli, P. aeruginosa, E. cloacae, S. aureus,* and *V. alginolyticus*). The enrichment solutions were incubated at 37 °C for 48 hours with gentle agitation. The solution was centrifuged at 6000 rpm for 10 min. The supernatant was filtered through a 0.45 µm membrane. The filtrate was inoculated in 3 mL of bacteria media twice times at 37 °C /24 h. After the centrifugation, the supernatant was collected and stored at 4 °C for phage' characterization. The phage T4 was the viral control strain for the lytic assay validation. Bacteria hosts were gifted from the collection of Phagetech project of Pasteur Institute Cote d'Ivoire (Addablah et al., 2021).

Virulence test assay

Twenty µL of filtrate were spotted on LB agar plate with enrichment bacteria to confirm phage presence. Lysates containing phages were tested in five bacteria species to determine host sensitivity to phages. Five (5) mL of fresh culture bacteria (OD = 0.3) were inoculated on agar and removed by sterile pipet. The plates were dried on a laminar flow hood. Twenty microliters of phage solution were spotted on the plate and incubated at 37 °C/ 24 h. Several phages were tested in the same bacteria plate. The method of Kakou-Ngazoa et al., 2020 was used for virulence test of phages.

RESULTS

Distribution of Lagoon phages

A total of twenty-four phages lysates were recovered in the Ebrie Lagoon. This is presented in Table 2. No phage lysate produced lysis on *V. alginolyticus, but* the highest percentage was detected in *E. coli.* A total of 83.3 % of isolated phages that produced lysis are *Enterobacteriaceae*.

Virulence spectrum of phages lysate

The distribution of virulence showed that enriched phages produced lysis on *E. coli*, *P. aeruginosa*, and *S. aureus* bacterial lawn. The study of the virulence spectrum of phages showed that a single enriched phage could lead to cell lysis in 2 or more bacterial species

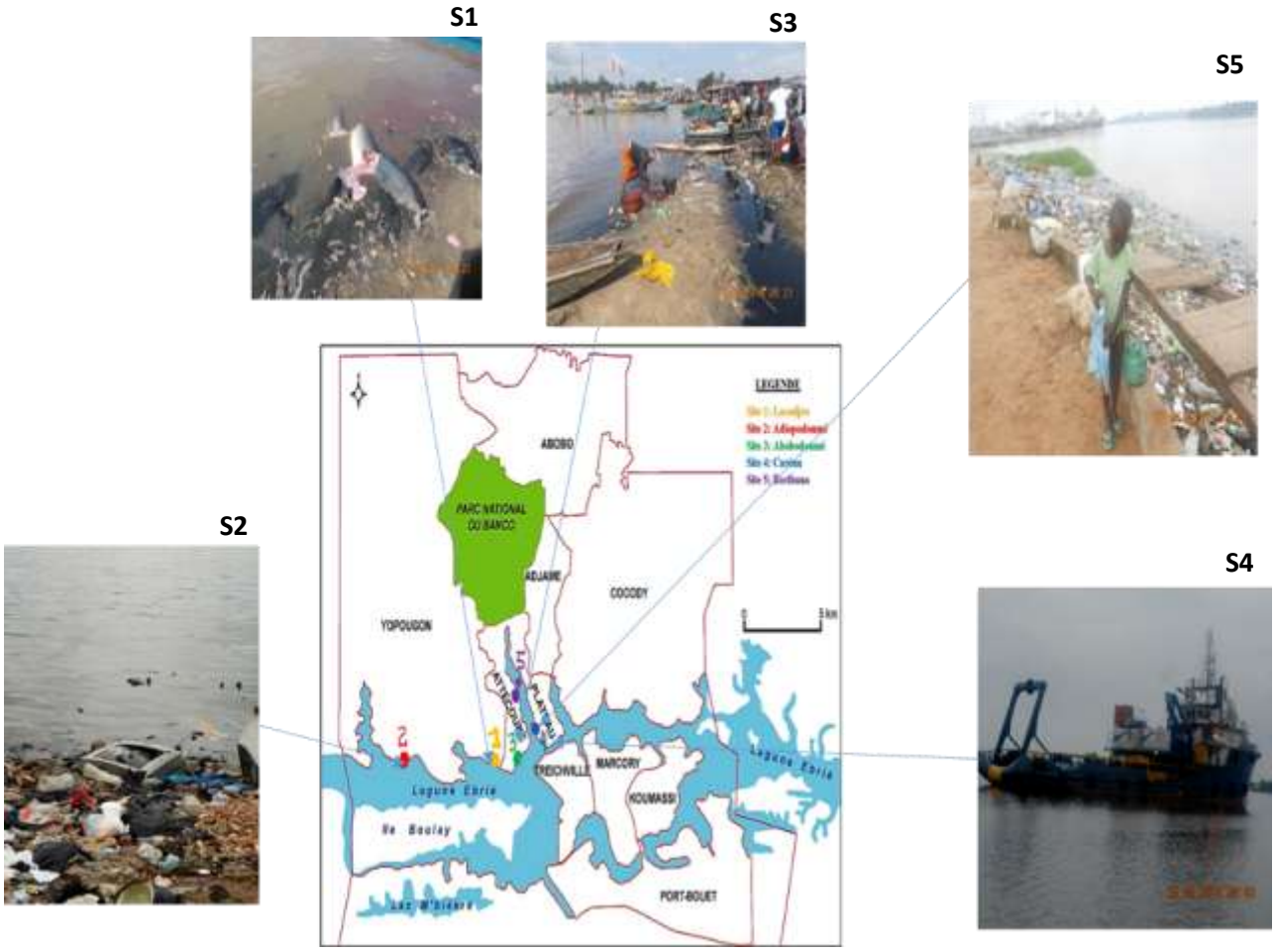

Figure 1. Sampling Sites of Lagoon Ebrie, Abidjan (2018). Sites Locodjo (S1, Site 1), Adiopodoume (S2, Site 2), Site Abobodoume (S3 , Site 3), Site Boribana (S5, Site 5), Site Carena (S4, Site 4).
Source: Pictures Koudou/Ivorycoast/2018 collection

Table 2. Distribution of phage according to their enrichment bacteria.

Bacteria hosts	Phages lysates (n)	Total (%)
Escherichia coli	10	41.67
Enterobacter cloacae	5	20.83
Pseudomonas aeruginosa	5	20.83
Staphylococcus aureus	4	16.67
Vibrio alginolyticus	0	0
Total (%)	24	100%

Source: Authors

(Table 3). Thus lysates such as those obtained after enrichment with the bacteria *P. aeruginosa*, *S. aureus*, and *E. coli* was all able in the vast majority (60 to 100 %) to infect all the bacterial species studied.

The spectrum revealed that the phages have high virulence against *E. coli*, *P. aeruginosa* and *E. cloacae* by 82.5 %, 71 % and 70 % respectively. Vibrio phages were not detected in the Lagoon Ebrie (Table 3).

Pollution of Lagoon Ebrié

By using the indicators of fecal contamination with bacteriophages, the different sites of Lagoon have different level of microbiological pollution. The level of contamination shows that all sites have high or very high pollution, by the presence of major bacteria hosts including *E. coli*, *P. aeruginosa*, *E. cloacae* and *S. aureus* in sites Boribana, Locodjoro and Abobodoume (Table 4). The sites Carena and Adiopoudoume have same pollution level. The lagoon is main contaminated permanent with plural phages and bacteria strains.

DISCUSSION

The surveillance of the ecosystems enhances the

Table 3. Percentage of virulence of enriched phages from Lagoon Ebrié.

Bacteria host/number of phages (n)	Virulence of bacteria strains (%)				
	E. coli B	***E. coli C***	***E. cloacae***	***P. aeruginosa***	***S. aureus***
E. coli /phages (10)	100	80	50	80	90
E. cloacae /phages (5)	60	20	100	0	0
P. aeruginosa /phages (5)	100	100	60	100	80
S. aureus/ phages (4)	100	100	75	100	100
Median virulence (%)	82.5		71	70	67.5

Source: Authors

Table 4. Distribution of bacteria hosts in the lagoon Ebrié.

Lagoon sites	Presence of bacteria	Level of pollution
Boribana	1, 2, 3, 4	Very high
Abobodoume	1, 2, 3	High
Locodjoro	1, 2, 3, 4	Very high
Adiopodoume	1, 2, 3, 4	Very High

1: *E.coli*; 2: *Pseudomonas aeruginosa*; 3: *Enterobacter cloacae*; 4: *Staphylococcus aureus.*
Source: Authors

monitoring of the distribution of bacterial pathogens and therefore, reduces the risk of transmission to humans (European Directive, 2006). These results showed the distribution of phages on the Lagoon Ebrie in Abidjan city. Three major phages were isolated in all sites. Regarding the level of virulence, our study shows that *E. coli, P. aeruginosa,* and *E. cloacae* infecting phages were dominant in the natural lagoon. These findings coincide with earlier results which recorded positive evidence for *E. coli, P. aeruginosa,* and *E. cloacae* infecting phages in all water samples with varying levels. These data have concerned sewage water and Ebrie lagoon which is subjected to sources of microbial pollution. These types of natural waters receiving discharges are known to represent a high public health threat of bacterial infections in various cities in Asia, Africa, and lateen America (Costa et al., 2014; Bonilla et al., 2007; Aka et al., 2017).

But in this study, we have evaluated for each water sample whether there was the possibility of recovering the phages infecting the targeted bacteria. Their presence could be correlated with the presence of their host in the study environment. Indeed, phages are known to be present where their host is located. According to the Kill the winner theory, their abundance is also related to that of their host (Koskella and Meaden, 2013). Since phages adapt to infect the most abundant bacterial population in the ecosystem. In fact, according to these results, it seems that *E. coli* and *P. aeruginosa* are the most abundant of the bacteria studied at the Ebrié lagoon. The presence of bacterial pathogens in tropical lagoons could directly be correlated with the presence of human activities and sewage discharge around/near the lagoon. The natural lagoon of Abidjan is a receptacle for several pollutions in all seasons (Tia, 2017). The studies of Addablah et al. (2021) linked the abundance of the enterophages to the abundance of their host in waste waters that are directly drained into the lagoon without treatment, thus causing pollution.

Regular phage isolation in Lagoon Ebrie has made it possible to monitor the quality of the distribution of phages in the lagoon. But also, this study offers a perspective on using phages as a detection agent to evaluate viable bacteria in the Lagoon Ebrie. The phage-based diagnostics have been successfully conducted for the detection of P. *aeruginosa* in water.

The value of a phage diagnostic assay is mainly dependent on the ability of the phage to specifically target its host species and to infect as many strains as possible. So, the future study will be focused on isolating the phages from these lysates and evaluating their ability to infect a variety of strains belonging to the same bacterial species.

Conclusion

This study shows coli phages and Enterococci phages in Lagoon Ebrie due to human fecal pollution by septic tanks, drain collector of homes in Abidjan city. Several sites of the lagoon have high level pollution. Plural phages were isolated in the lagoon and suggest

permanent pollution and adaptation of bacteriophages in this biotope. Future perspectives will characterize the biocollection of isolated phages, their genomic variation and the application to biocontrol of contaminated biotopes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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