WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 9, 84-89. Short Communication ISSN 2277– 7105

NEW IDENTIFICATION OF PHAGES IN WATER AND IN FISHES FROM LAGOON EBRIÉ AND THEIR APPLICATION FOR MULTIDRUG STRAINS IN ABIDJAN, WEST AFRICA

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Article Received on
28 June 2017,
Revised on 18 July 2017,
Accepted on 08 August 2017
DOI: 10.20959/wjpr20179-9006

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ABSTRACT

Phages are widely apply to screen, to detect and to eliminate bacterial in different applications. In Africa, the emergence of antibiotic resistance strains and the fecal contamination in the aquatic environment require to explore the phage therapy. In Abidjan, the Lagoon Ebrié is an important aquatic ecosystem and the increase of the microbial contamination represents a risk for the population and the fisheries activity. The aim of this study is to identify novel phages in water and in fish samples from the Lagoon and to test their virulence against multidrug resistant strains. Water samples from different lagoon sites were collected and analyzed in culture and phages test assay. Fresh fishes were collected from Lagoon's fish market in Abobodoumé/Locodjoro site. Water samples (*PWLO6, PWR7, PWAD8)* and intestinal solution were passed through 0.45 µm syringe filters and incubated in bacteria growth.. The enrichment of bacteria and phages were done in LB media at 37°C. We have isolated 5 phages from different types of fishes and 3 phages from Lagoon sites. The phages of fishes *PFSO1, PFMU2, PFCAR4 and PFCAP5* have positive virulence for all tested antibiotic multidrug resistant strains *Pseudomonas aeruginosa, Salmonella sp, Enterobacter aerogenes, Klebsiella pneumoniae and E. coli*. The

phage *PFMA3* has shown not effect against *Klebsiella pneumoniae* and *Enterobacter aerogenes*. 3 phages from water have identical virulence effect in all tested bacteria. This study report the first isolation of 8 novel phages in Lagoon Ebrié with virulent effect on multidrug resistant's strains and can be apply as a new approach for eliminating of pathogens.

KEYWORDS: Phages, aquatic ecosystem, fishes, Lagoon Ebrié, multidrug resistant strains, West Africa.

INTRODUCTION

Water borne diseases are emergent and represents over 80% of the human infection. In Africa several epidemics were associated with water contamination because the lacks of the surveillance in water systems and non-accessibility to the biosafety water distribution. The use of environmental water stays a major source for riverine populations and associated water activities like fishing, washing, and industrial releasing were frequently reported.[1,2] In Abidjan, the Lagoon Ebrié is an aquatic ecosystem and their distribution in several points has several impacts for riverine populations.[3] Recent studies have reported the incidence of fecal contamination in some gastroenteritis outbreaks. *E. coli* was been detected as fecal contamination indicator in Lagoon Ebrié in the past years.[4] Some studies have reported the circulation of multi-resistant's strains in humans and in Lagoon Ebrié.[5]

Bacteriophages are viruses that are significantly distributed in nature and specifically attack bacterial hosts.[6,7] Phage therapy or use of bacteriophages as therapeutic agents for eliminating bacterial infections was introduced by preliminary studies of Twort and D'Herelle at the first of the 20th century.[8,9]

Several studies have proved the usefulness of phage therapy for controlling infectious diseases caused by pathogenic bacteria such as *E. coli* in human children and adults[10], *E. coli* infections in mice and calves.[11] In this study, we propose to identify lytic phages in 2 aquatic matrices (water and fishes) of Lagoon Ebrié in Abidjan and to test their virulence on multidrug resistant strains.

METHODOLOGY

Bacterial strains

Multi-resistant strains were provided from the collection of Bacterial Multi-Resistant strains in the National Reference Center of Pasteur Institute, Abidjan. Positive strains *E.coli B* and

phage T4 were obtained from the phages'f collection of university Laval as a gift from Sylvain Moineau.

Samples

50ml of water samples were collected in 3 different sites (Adiopodoumé, River 17km, Locodjoro) of Lagoon Ebrié and the samples were transferred in cool packs. Fresh fishes were collected in the fish market in Lagoon site Abobodoumé/Locodjoro and transferred separately in sterile bags in freezer box. Intestinal tracts were isolated and incubated in sterile PBS for 20min. 10ml of water and 3 ml of intestinal solution were filtered by 0.45µm and the filtrate were incubated in LB media for amplification with 100µl of water or intestinal tract solution.

Culture of bacteria

Fresh colonies from bacteria were inoculated in 3ml LB media and incubated 37°C, overnight. Positive strains of *E.coli B* were inoculated in LB media or in LB agar and incubated in the same conditions. Multidrug resistant's strains were collected in Abidjan from BMR research group for multidrug resistant's strains and were inoculated in LB media after confirmation in bacteriological tests.

Isolation and Enrichment of phages from water and fish samples

The isolation and enrichment were done with the protocol described by Maal et al. (12) with minor modifications. Briefly, 50 ml water was centrifuged at 100000 rpm at 4°C, 20min, the supernatant were passed through 0.45µm syringe filters and store at 4°C. 100µl of filtrat were inoculated in 3ml LB with 100µl of bacteria and incubated at 37°C, overnight. The obtained culture was centrifuged and the supernatant was passed through 0.45µm syringe filters to separate phages from bacteria. The filtrate was inoculated in 3ml LB with fresh bacteria and incubated at 37°C, overnight. 5 cycles of purification were applied for the enrichment of pure phages.

Fresh fishes were placed on sterile hood and the dissection of intestinal organs was processed. The intestinal tract was added to 5ml of Phosphate Buffer Saline (1X). The solution was passed through 0.45µm syringe filters and store at 4°C. 100µl of fish 's filtrate were inoculated in LB containing bacteria 100µl bacteria in 3ml LB and incubated at 37°C, overnight. Several cycles were applied to purify phages and to isolate phages from fishes.

Tests of phages on bacteria strains

20µl of phage solution were tested on bacterial strains and incubated 37°C overnight, by using spot method. Positive strains of phage T4 were against *E.coli B* tested for the validation of the virulence tests.

RESULTS

From water samples collected in Lagoon Ebrié, we have identify 3 different *phages PWLO6, PWR7, PWAD8* in Abobodoumé/Locodjoro (site 1), Adiopodoumé River 17 (site 2) and in Adiopodoumé (site 3) respectively.

In Fishes samples from the site Abobodoumé/Locodjoro (site 1), we have identified 5 phages (*PFSO1, PFMU2, PFCA4, PFCAP5*) in different fish's types. The determination of genome types by enzymatic hydrolyze with restriction enzyme Xba has shown that 87.5 % of isolated phages have RNA genome (Table 1).

The virulence test of isolated phages shown positive effect on tested bacteria *E. coli B* (Figure 1 & 2) Only phage *PFMA3*, all phages have demonstrated virulence against all 5 tested strains *Pseudomonas aeruginosa, Salmonella sp, Enterobacter aerogenes, Klebsiella pneumoniae* and *E. coli*.

Table 1: List of isolated phages.

Phage	Source	Type/Origin	Genome types*	Bacteria host
Pfso1	Fish	*Pseudotholitus senegalensis*	RNA	*E. coli*
Pfmu2	Fish	*Mugil cephalus*	RNA	*E. coli*
Pfma3	Fish	*Chrysichthys nigrodigitatus*	RNA	*E. coli*
Pfca4	Fish	*Pomadasys jubelini*	RNA	*E. coli*
Pfcap5	Fish	*Lates niloticus*	RNA	*E. coli*
Pwloc6	Water	Lagoon Ebrié	RNA	*E. coli*
PwR7	Water	River km17	**DNA**	*E. coli*
Pwadi8	Water	Lagoon Ebrié	RNA	*E. coli*

*after digested by XbaI.

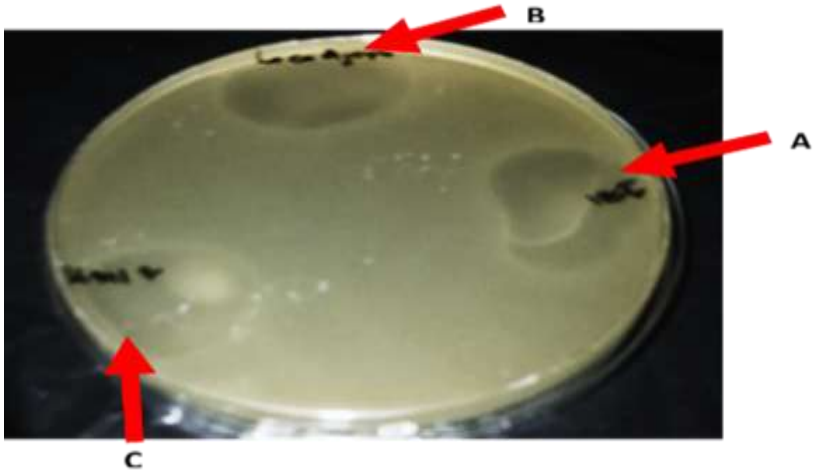

Figure 1: The spotting of isolated phages from Lagoon Ebrié against *E. coli B* after 24 hours incubation at 37°C. A: phage *PWAD8*; B: phage *PWLO6*; C: phage *PWR7*.

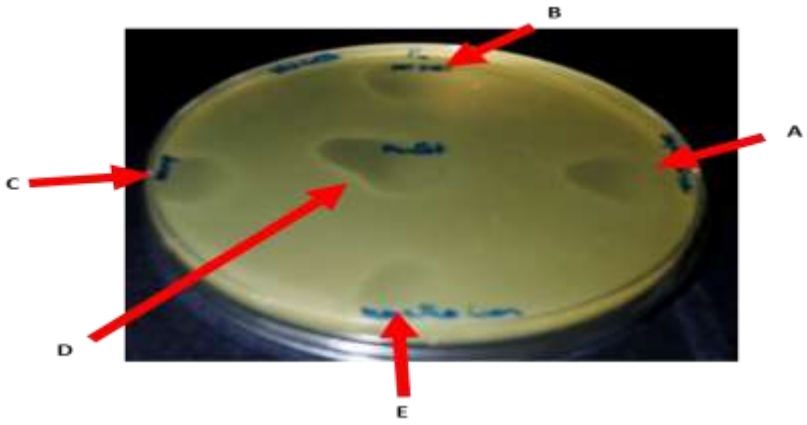

Figure 2: The spotting of isolated phages from fishes against *E. coli B* after 24 hours incubation at 37°C. A: phage *PFCAP5*; B: phage *PFSO1*; C: phage *PFCA4*; D: phage *PFMU2*; E: phage *PFMA3*.

CONCLUSION

This study report the first identification of 8 novel lytic phages in Lagoon Ebrié with virulent effect on multidrug resistant's strains and can be apply as a new approach for eliminating of pathogens in clinical use and in the environment. In West Africa, the needs of alternative biocontrol of bacteria can improve the public health by including low cost of the phage therapy for the population. We will explore the phage sequencing and characterization by the screening of more bacteria strains to complete the phage host spectrum.

ACKNOWLEDGMENTS

We thanks grateful Dr Sylvain Moineau for providing of positive control strains and Prof. Guessend KN for multidrug resistant's bacteria from different sources.

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